Application of Cell Block Technique on Fine Needle Aspiration from Liver Disease in National Ribat University Hospital Khartoum state, Sudan 2014 - 2015

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B.sc in Histopathology and cytology, Shendi University (2008)

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Application of Cell Block Technique on Fine Needle Aspiration from Liver Disease in National Ribat University Hospital Khartoum state, Sudan 2014 - 2015

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Application of Cell Block Technique on Fine Needle Aspiration from Liver Disease in National Ribat University Hospital Khartoum state, Sudan 2014 - 2015

Jihad Ahmed Eltyeb Mohammed Elsheiakh

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Date: 23/8/2015
Authorization

I authorized that my dissertation Application of Cellblock technique in the Diagnosis Liver Disease” submitted by me, under the supervision of Dr. Rania Mahgoub Sid Ahmed and Dr. Ali Sid Ahmed for the partial fulfillment for award of Master degree in Medical Laboratory Science in Medical Histopathology and cytology. University of Gezira in Medical Laboratory Science of Medical Histopathology and cytology; Wad-Medani, Sudan and this is original and it was not submitted in part or in full, in any printed or electronic means, and is not being considered elsewhere for a warding any degree.

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Date: August / 2015
قال تعالى
بسم الله الرحمن الرحيم

(وفي أنفسكم أفلا تبصرون)

سورة الطوراء
الأية (٣١)
Dedication

To my . . .

Father . . .

Mother . . .

Brothers and sisters . . .

To my college . . .

And . . .

My great family . . .

For their endless support,

Love and encouragement
Acknowledgment

Deepest thanks to my supervisor Dr. Rania mahgoub for her efforts and support throughout this study.

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Deep thanks to staff of Alyaa Specialize Hospital for encouragement.

Many thanks to my great family specially my mother for their endless support, love and encouragement.
Cell block prepared from the residual tissue fluids and fine needle aspirations can be useful adjacent to smears for establishing a more definitive cytopathologic diagnosis. They can be particularly useful for categorization of tumors that otherwise may not be possible from smears themselves. The study was conducted in National Ribat University Hospital during the period (June 2015 - July 2015) to assess the application of cell block technique in the diagnosis of liver disease based on specimen adequacy and diagnostic accuracy. The objectives of the study to find out the age and gender of patient, to evaluate the level of background staining, cellularity, nuclear and cytoplasmic preservation in cell block section, to compare the differences between the diagnostic results of cell block slides and conventional smears and to explore the feasibility of the use of cell block preparation in routine cytology. Eighty study subjects were selected for the study, ultrasound guided fine needle aspiration was obtained from liver, then aspirate cells fixed in 40% formalin overnight, the supernatant fluid was decanted and settle cells warped in filter paper and then placed in a tissue cassette. All tissue cassette processed in an automatic tissue processor, the cell block were embedded in paraffin wax, and 4-6 micron were cut using standard rotary microtome. The sections were stained with Haematoxylin and Eosin (H&E) stain and cover with cover slip using DPX mounting media, and cytological smears was also stained with Haematoxylin and Eosin stain. We compared the cellularity for both methods cytology smears and cell block we found that the high score (+3) in smear 41 (51.3%) while in cell block 27 (33.8%). On the other hand, when compared the final architecture preservation which include nuclear and cytoplasmic preservation, the nuclear preservation were better by cytological smears than cell blocks, score (+3) in smears 43 (53.8%) while in cell block 37 (46.3%). In the comparison of cytoplasmic preservation between the two methods we found that the cytological smears was better, the high score (+3) 42 (52.5%) while in cell block 35 (43.8%). When comparing the background staining we found that the cell block has mild background staining 55 (68.8%) while smears 7 (8.8%). In this study smear preparation was better than cell block technique in preservation of cells, nucleus and cytoplasm of liver disease. It recommended that smears preparation for liver disease should be used routinely and modification cell block techniques should be done to achieve more preservation of cells.
تطبيق تقنية كتلة الخلية في تشخيص امراض الكبد في مستشفى الرباط القىمي الجامعي
ولاية الخرطوم، السودان (2004-2015)

جهاد أحمد الطيب محمد الشيخ

ملخص الدراسة

كتل الخلايا المحضرة من سواحل الأنسجة المنقحة وتطبيقات الابر الرفيعة يمكن أن تكون مفيدة بشكل خاص لتصنيف الأمراض التي على خلاف ذلك قد لا تكون ممكنة من المسحات. وقد أجريت الدراسة في مستشفى جامعي بالزراعة الوطني خلال الفترة (يونيو 2015 - يوليو 2015) لتطبيق تقنية كتلة الخلية في تشخيص أمراض الكبد على أساس كفاءة العينة ودقة التشخيص. أهداف الدراسة الوقف على سنجن المرض، تقييم مستوي الخلوية، وحصانات النواة والسائل الخلوي في تقنية كتلة الخلية، ومقارنة الاختلافات بين نتائج التشخيص من شرايين كتلة الخلايا والمساحات التقليدية واستخدام جدي استخدم إعداد كتلة الخلية في علم الخلايا الروتبي. وقد تم اختيار ثلاثين مفردا للدراسة، تم الحصول على الوجبات فوق الصوتية الموجهة بالأربه من الكبد، ثم وضع الخلايا المسحوبة في 40% من الفروماينات يوم كامل، وبعدها تم التخلص من السائل وتسوية الخلايا في ورق الترشيح ومن ثم وضعها في شريحة الأنسجة ومعالجتها في معالج الأنسجة التلقائي، ومن ثم وضع كتل الخلايا في شمع البارافين، وقطعت 8-4 أقسام ميكون باستخدام مشوار الدوار. تم صبح الأقسام بصفعة الهيماتوكسيلين والإيوزين. وغطيت باستخدام مادة دي بي إكس الدماغ. تمت مقارنة الخلية على حد سواء بين طريقة المسحات الخلوية وكتلة الخلية ووجدنا أن الدورة المرتفعة (3) في المسحات (34.3%)، في حين في الكتلة الخلوية (32.3%)، ارتفاع أخرى عند مقارنة الخلايا المعمارية النهائية التي تشمل المحافظة على النووية والسائل الخلوي، والحفاظ النووي كانت أفضل من خلال المسحات الخلوية من كتل الخلوية، والنماذج (3) في المسحات (38.8%)، في حين في كتلة الخلية (53.7%)، في المقارنة بين الخلايا السائل الخلوية بين الطرقتين وجدنا أن المسحات الخلوية كانت أفضل والدرجة العالية (3) في المسحات (52.9%) في حين في كتلة الخلية (35.4%)، عند مقارنة تلوين الخلية وجدنا أن الكتلة الخلوية لديها تلوين خفيف (35.4%)، بينما المسحات الخلوية لديها 7 (8.8%) في هذه الدراسة وجدنا أن المسحات الخلوية أفضل من كتلة الخلايا في المحافظة على الخلايا، النواة والسائل الخلوي في ارتفاع الكبد. فمن المستحسن أن تستخدم مسحات تشخيص الكبد بشكل روتبي. وينبغي أن يتم تعديل تقنية كتلة الخلية لتحقيق المزيد من الحفاظ على الخلايا.
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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Cell block cytology is a paraffin-embedded specimen derived from dried mucus, sputum, or debris found in fluids of pleural, pericardial, endobronchial and other sites that cannot be processed in usual fashion for cytological analysis (McGraw, 2002).

Cell blocks prepared from residual tissue fluids and fine-needle aspirations can be useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis. They can be particularly useful for categorization of tumors that otherwise may not be possible from smears themselves (Zito FA, et al; 1995).

The paraffin blocks that prepared from any suspension of cells or fluids are suitable for sectioning, staining, and microscopic study; cells are concentrated by centrifugation or filtering techniques, and the resulting aggregation is processed as if it were a solid specimen of tissue (Farlex Partner, 2012).

In routine cytological practice, the cell morphologic changes in smears are not always obvious. Sometimes judgment is difficult; therefore the application of cell block may give a better presentation of detailed cytological architectural features. Also applying histochemical or immunocytochemical staining has been a useful adjunct for establishing a more definitive cytopathological diagnosis. Furthermore, cell block is also a source of archival material for cytological research (Ceyhan, et al, 2006).

Sometimes cytology does not provide sufficient information and the risk of false negative or undetermined diagnosis exist (kulkarni, et al, 2000). The cell block technique increases the chance to achieve a reliable result and also to help demonstrate better architectural patterns which could be of great help in the routine for approaching correct diagnosis (mansy, et al, 2006).
1.2 History:

The cell block preparation for microscopic evaluation was first introduced by Bahrenburg in 1896. The cell block technique has been in use since 1900 but has been overshadowed by smear technique which has gained considerable momentum since generalized acceptance of papanicolaou's methods. With the development of excellent cell preparation techniques, the cell block technique has been abandoned by many laboratories and this neglect is not justified (ShailajaPrabha, et al, 2014).

1.3 Justification

Cell block techniques are widely used in diagnostic cytopathology. It is a valuable tool for evaluation of various cytological specimens most importantly, in addition to architectural details of the specimens, Despite of all cell block has not yet established in the diagnostic histopathology and cytology central laboratories in Gezira state, so we are seeking to apply this technique.

1.4 Objectives

1.4.1 General objective:
- To assess application of cell block technique in the diagnosis of liver biopsy based on specimen adequacy and diagnostic accuracy.

1.4.2 Specific objectives:
- To evaluate the level of background, cellularity, nuclear preservation, and cytoplasmic preservation in cell block sections.
- To compare the differences between the diagnostic results of cell blocks slides and conventional cytology smears.
- To explore the feasibility of the use of cell block preparation in routine cytology.
- To find out the age and gender of the patients
CHAPTER TWO

Literature review

In spite of years of research progress, the diagnosis of many diseases, especially cancer, still requires light microscopic evaluation of a sample of cells. To make a diagnosis, pathologists look for alterations in cell structure, and for changes in the composition and organization of tissues (Fischer AH, et al, 2010).

It is obviously an advantage to be able to make a diagnosis, or to guide therapy, based on the smallest possible biopsy sample; the smaller biopsy has fewer risks and complications for the patient. (Fischer AH, et al, 2010).

2.1 Cytology:

Is the field that uses the smallest possible "micro biopsies" for diagnosis. The appeal of cytology is that it can provide a small but diagnostic sample. By minimizing risks and complications for detecting certain diseases, cytology can be used for the screening of disease. When cellular level alterations alone are sufficient for a diagnosis, a minimal sample size is acceptable. For many diagnoses, however, it may be necessary to be able to recognize the larger-scale alterations in tissue architecture, or to study biochemical and molecular characteristics of the cells; cell blocks fulfill this need. (Fischer AH, et al, 2010).

2.2 Haematoxylin and Eosin stain (H&E stain or HE stain):

Is one of the principal stains in histology. It is the most widely used stain in medical diagnosis and is often the gold standard; for example when a pathologist looks at a biopsy of a suspected cancer, the histological section is likely to be stained with H&E and termed "H&E section", "H+E section", or "HE section" (Wikipedia, the free encyclopedia, 2013).

The staining method involves application of hemalum, a complex formed from aluminum ions and hematein (an oxidation product of haematoxylin). Hemalum colors nuclei of cells (and a few other objects, such as keratohyalin granules and calcified material) blue. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colors eosinophilic structures in various shades of red, pink and orange. The staining of nuclei by hemalum is ordinarily due to binding of the dye-metal complex to DNA, but nuclear staining can be obtained after extraction of DNA from tissue sections. The mechanism is different from that of nuclear staining by basic (cationic)
dyes such as thionine or toluidine blue. Staining by basic dyes occurs only from solutions that are less acidic than hemalum, and it is prevented by prior chemical or enzymatic extraction of nucleic acids. There is evidence to indicate that coordinate bonds, similar to those that hold aluminum and hematein together, bind the hemalum complex to DNA and to carboxy groups of proteins in the nuclear chromatin. (Wikipedia, the free encyclopedia, 2013).

The eosinophilic structures are generally composed of intracellular or extracellular protein. The Lewy bodies and Mallory bodies are examples of eosinophilic structures. Most of the cytoplasm is eosinophilic. Red blood cells are stained intensely red (Wikipedia, the free encyclopedia, 2013).

The structures do not have to be acidic or basic to be called basophilic and eosinophilic; the terminology is based on the affinity of cellular components for the dyes. (Wikipedia, the free encyclopedia, 2013).

2.3 Cell blocks:

Cell blocks are micro biopsies embedded in paraffin. A standard histologic section, measuring four or five microns in thickness, shows the organization and cellular composition of a micro biopsy fragment. Generally, diagnoses can be made with the most confidence when combinations of cellular and tissue level morphology are present. (Fischer AH, et al, 2010).

Cell blocks are micro biopsies embedded in paraffin. They broaden the diagnostic value of cytology specimens and are complementary to cytology preparations. A combination of cytology preparations and cell blocks are therefore synergistic, and provide this confidence. Cell blocks are the link between cytology and surgical pathology, allowing the strengths of each approach. (Fischer AH, et al, 2010)

2.4 Immunohistochemistry and cell block:

Immunohistochemistry (IHC) allows disease-specific antigens, or combinations of antigens, to be detected. Immunohistochemistry can be performed on cytology preparations of micro biopsy samples, however there are many advantages in using cell blocks. IHC on cytology can trap antibodies or reagents in large tissue fragments, giving the impression of a positive staining reaction. Paraffin sections allow each part of a micro biopsy to have equal access to IHC reagents. Another advantage of using cell blocks for IHC is the relative ease of scoring or quantifying positivity on a per cell basis. Cell blocks are the ideal platform for IHC and molecular diagnostic ancillary studies. IHC on cell blocks also allows the
staining reaction to be correlated with larger-scale tissue architecture. For example, the finding of hepatocytes growing more than a few cells away from the CD31 positive endothelial cells within a tissue fragment is virtually diagnostic of hepatocellular carcinoma. Serial sections that can be cut from cell blocks allow multiple IHC reactions to be studied in the same sample. One tissue fragment 100 microns in diameter gauge fine needle) could be used to study 20 IHC reactions in five micron serial sections, but it can only be studied in one immunohistochemical staining reaction in a cytology preparation. Cell blocks are a convenient and stable means for archiving biopsies at room temperature, with many advantages over freezing, storing glass slides, or storage of liquid fixatives. Defining individually optimal therapies is becoming an essential duty for pathologists with the advent of new molecular-based treatments. Paraffin embedded tissue has emerged as the standard platform to achieve this goal of "personalized medicine'' (Fischer AH, et al, 2010).

2.5 Liver:
The liver is a vital organ of vertebrates and some other animal (Abdel-Misih, et al, 2010). In the human it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemical necessary for digestion (Canadian Cancer Society, 2015). The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification (Canadian Cancer Society, 2015). It is an accessory digestive gland and produces bile, alkaline compound which aids in digestion via the emulsification of lipids. The gallbladder, a small pouch that sits just under the liver, stores bile produced by the liver (Gerard J, et al, 2015). The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton, et al, 1993). Estimates regarding the organ's total number of functions vary, but textbooks generally cite it being around 500 (David, et al, 2002).

Terminology related to the liver often starts inhepar- orhepat- from the Greek word (Etymology online hepatic, 2013). There is currently no way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term. Liver transplantation is the only option for complete liver failure (Emedicine.medscape.com, 2015).
2.5.1 Structure of liver:
The liver is a reddish brown wedge-shaped organ with four lobes of unequal size and shape. A human liver normally weighs 1.44–1.66 kg (3.2–3.7 lb) (Cotran, et al, 2005). It is both the largest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlying the gallbladder (Gerard J. Tortora and Bryan H. Derrickson, 2015). It is connected to two large blood vessels, the hepatic artery and the portal vein, the hepatic artery carries oxygen-rich blood from the aorta, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas, these blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to a lobule (Emedicine.medscape.com, 2015). Lobules are the functional units of the liver and each lobule is made up of millions of hepatic cells (hepatocytes) which are the basic metabolic cells. The lobules are held together by fine areolar tissue which extends into the structure of the liver, by accompanying the vessels (veins and arteries) ducts and nerves through the hepatic portal, as a fibrous capsule called Glisson's capsule (Dorland's, 2012). The whole surface of the liver is covered in a serous coat derived from peritoneum and this has an inner fibrous coat (Glisson's capsule) to which it is firmly adhered. The fibrous coat is of areolar tissue and follows the vessels and ducts to support them (Dorland's, 2012).

2.5.2 Microscopic anatomy of liver:
Microscopically, each liver lobe is seen to be made up of hepatic lobules. The lobules are roughly hexagonal, and consist of plates of hepatocytes radiating from a central vein, the central vein joins to the hepatic vein to carry blood out from the liver (Marieb, et al, 2012). A distinctive component of a lobule is the portal triad, which can be found running along each of the lobule's corners. The portal triad, misleadingly named, consists of five structures: a branch of the hepatic artery, a branch of the hepatic portal vein, and a bile duct, as well as lymphatic vessels and a branch of the vagus nerve (Marieb, et al, 2012). Between the hepatocyte plates are liver sinusoids, which are enlarged capillaries through which blood from the hepatic portal vein and hepatic artery enters via the portal triads, then drains to the central vein (Marieb, et al, 2012). The study of microscopic anatomy, shows two major types of liver cell: parenchymal cells: 70–85% of the liver volume is occupied by parenchymal hepatocytes (Kmieć Z, 2001): non-parenchymal: constitute 40% of the total number of liver cells but only 6.5% of its volume (Kmieć Z, 2001). The liver sinusoids are lined with two types of cell, sinusoidal endothelial cells, and phagocytic Kupffer cells (Pocock, Gillian, 2006). Hepatic stellate
cells are non-parenchymal cells found in the space of Disse, between a sinusoid and a hepatocyte (Kmieć Z, 2001). Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen (Kmieć Z, 2001).

2.5.3 Physiology of liver:
The various functions of the liver are carried out by the liver cells or hepatocytes. The liver is thought to be responsible for up to 500 separate functions, usually in combination with other systems and organs. Currently, there is no artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure (Gerard J, et al, 2015).

2.5.3.1 The function of liver includes:

- **Blood supply**

  The liver receives a dual blood supply from the hepatic portal vein and hepatic arteries (Shneider, et al, 2008)

- **Biliary flow**

  The biliary tree is derived from the branches of the bile ducts. The biliary tree, or biliary tract, is the path by which bile is secreted by the liver then transported to the first part of the small intestine, the duodenum. The bile produced in the liver is collected in bile canaliculi, small grooves between the faces of adjacent hepatocytes (Marieb, et al, 2012).

- **Synthesis**

  The liver plays a major role in carbohydrate, protein, amino acid, and lipid metabolism. The liver plays a key role in digestion, as it produces and excretes bile (yellowish liquid) required for emulsifying fats and help the absorption of vitamin K from the diet. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder (Marieb, et al, 2012).

  The liver also produces insulin-like growth factor 1 (IGF-1), a polypeptide protein hormone that plays an important role in childhood growth and continues to have anabolic effects in adults (Marieb, et al, 2012).
• **Breakdown**

The liver is responsible for the breakdown of insulin and other hormones. The liver breaks down bilirubin via glucuronidation, facilitating its excretion into bile.

The liver is responsible for the breakdown and excretion of many waste products. It plays a key role in breaking down or modifying toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism (Marieb, *et al*., 2012).

• **Other functions**

The liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin B12 (1–3 years' supply), vitamin K, iron, and copper (Marieb, *et al*., 2012).

The liver is responsible for immunological effects—the reticuloendothelial system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.

The liver produces albumin, the most abundant protein in blood serum. It is essential in the maintenance of oncotic pressure, and acts as a transport for fatty acids and steroid hormones. The liver synthesizes angiotensinogen, a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure (Marieb, *et al*., 2012).

### 2.5.4 Common Liver Diseases and Prevention

**Hepatitis A:** A liver disease caused by the hepatitis A virus (HAV). HAV can cause the liver to swell and not work well. **Prevention:** Hepatitis A vaccination is the best way to prevent HAV. Other ways to stop the spread of HAV are always washing your hands with soap and warm water immediately after using the bathroom or changing a diaper, always washing your hands with soap and warm water before preparing or eating food.

**Hepatitis B** is a liver disease caused by the hepatitis B virus (HBV). HBV can cause the liver to swell and can lead to cirrhosis and liver cancer. **Prevention:** Hepatitis B vaccination is the best way to prevent HBV. Other ways to stop the spread of HBV are: Not sharing needles, Practicing safe sex, Not sharing razors, toothbrushes or other personal items, Using only clean needles for tattoos and body piercings.

**Hepatitis C:** A liver disease caused by the hepatitis C virus (HCV). HCV can cause the liver to swell and can lead to cirrhosis and liver cancer. **Prevention:** There is no vaccine to prevent HCV. The only
way to prevent HCV is to avoid direct contact with infected blood. Other ways to stop the spread of HCV are: Not sharing needles, Practicing safe sex, Not sharing razors, toothbrushes, or other personal items, Using only clean needles for tattoos and body piercings and getting medical care if you are exposed to blood or needle sticks at work.

**Fatty Liver Disease:** Fatty liver disease is the buildup of fat in liver cells. It can cause the liver to swell and can lead to cirrhosis. **Prevention:** Ways to prevent fatty liver disease are: Eating a healthy diet, maintaining a healthy weight, Exercising regularly, limiting the amount of alcohol you drink, maintaining a normal cholesterol level.

**NASH (Nonalcoholic Steatohepatitis):** is a type of fatty liver disease. Its causes the liver to swell and become damaged due to reasons unrelated to alcohol. **Prevention:** Ways to prevent NASH are: Eating a healthy diet, Maintaining a healthy weight, Exercising regularly, Limiting the amount of alcohol you drink, Maintaining a normal cholesterol level.

**Alcohol-Related Liver Disease:** Alcohol-related liver disease is caused by drinking too much alcohol. It can cause the liver to swell and can lead to cirrhosis. **Prevention:** The best way to prevent alcohol-related liver disease is to not drink more alcohol than what your doctor recommends.

### 2.6 Liver biopsy

Histological assessment of the liver, and thus, liver biopsy, is a cornerstone in the evaluation and management of patients with liver disease and has long been considered to be an integral component of the clinician’s diagnostic armamentarium. (Rockey DC, et al, 2006)

Although sensitive and relatively accurate blood tests used to detect and diagnose liver disease have now become widely available, it is likely that liver biopsy will remain a valuable diagnostic tool. (Rockey DC, et al, 2006).

Although histological evaluation of the liver has become important in assessing prognosis and in tailoring treatment, non-invasive techniques (i.e., imaging, blood tests) may replace use of liver histology in this setting, particularly with regard to assessment of the severity of liver fibrosis (Rockey DC, et al, 2006).
Several techniques may be used to obtain liver tissue, all liver biopsy techniques require specific training so as to ensure appropriate-sized specimen retrieval and the lowest rate of complications. (Rockey DC, 2008).

Although liver biopsy is often essential in the management of patients with liver disease, physicians and patients may find it to be a difficult undertaking because of the associated risks. (Rockey DC, 2008).

### 2.6.1 Liver Biopsy Terminology:

#### Table 2.1 Liver Biopsy Terminology

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Liver biopsy</td>
<td>Any type of liver biopsy</td>
</tr>
<tr>
<td>Transthoracic palpation/percussion-guided</td>
<td>The most appropriate biopsy site is guided transcutaneous determined on the basis of clinical examination. Traditionally used in practice.</td>
</tr>
<tr>
<td>Transthoracic, image-guided</td>
<td>The most appropriate biopsy site is determined or confirmed usually by ultrasound (US) imaging before the biopsy</td>
</tr>
<tr>
<td>Transthoracic, real-time image-guided</td>
<td>The most appropriate biopsy site is determined by US (or CT) imaging. Image guidance is used in real-time for tissue procurement</td>
</tr>
<tr>
<td>Subcostal, real-time image-guided</td>
<td>This biopsy is accomplished in almost identical fashion as above, except that the approach is subcostal rather than transthoracic</td>
</tr>
<tr>
<td>Transvenous or Transjugular</td>
<td>Biopsy is accomplished through a jugular or femoral venous approach under fluoroscopic guidance</td>
</tr>
</tbody>
</table>

### 2.6.2 Indication for liver biopsy:

Historically, liver biopsy was used almost exclusively as a diagnostic tool (Sherlock S, 1945).

However, as the result not only of new natural history data and the introduction of many new therapies for patients with liver disease, liver biopsy and histological assessment of the liver has now taken an important role in clinical management (Reuben A, 2003).
2.6.2.1 Liver biopsy currently has three major roles

(1) Diagnosis:
Diagnosis for many diseases, clinical and/or blood-based tests suffice to establish a diagnosis (typical examples include hepatitis B [HBV] or hepatitis C virus [HCV] infection). Nonetheless, liver biopsy is often a critical component in establishing the diagnosis of many (other) forms of liver disease. Although histological assessment alone may be able to make a diagnosis on occasion (i.e., a florid duct lesion in primary biliary cirrhosis [PBC]), liver histology is typically and most appropriately considered in conjunction with the full gamut of clinical and laboratory data. Acute and chronic hepatitis, cholestatic disorders, fatty liver disease, vascular diseases, infiltrative or storage diseases, some infectious and granulomatous diseases, and other disorders may be associated with characteristic histological abnormalities that are helpful in diagnosis (Czaja AJ, et al, 2007). Liver biopsy is particularly useful in patients with atypical clinical features. For example, liver histology can help distinguish between autoimmune hepatitis (AIH) and nonalcoholic fatty liver disease (NAFLD) in an obese patient with elevated levels of alanine aminotransferase (ALT), raised immunoglobulin G concentration (IgG) and/or a positive antinuclear antibody (ANA) titer (Powell EE, et al, 2005). Liver biopsy may also be very helpful in patients with coexisting disorders such as steatosis and HCV or hemochromatosis (Powell EE, et al, 2005) or an “overlap” syndrome of PBC with AIH (Zein CO, et al, 2003). It is likely that liver biopsy will always play a role in the management of the patient with a diagnostic dilemma. This includes the patient with abnormal liver tests of unknown etiology or the patient in whom a specific liver disease has been considered, but has not yet been confirmed. Examples include patients with a variety of possible diseases, including, but not limited to hereditary disorders such as Wilson disease, alpha-1-antitrypsin disease, glycogen storage diseases, tyrosinemia, Niemann-Pick disease, amyloidosis, and others (Dahlin DC, et al, 1950). Liver histology may also be helpful diagnostically in patients with apparent systemic diseases in which the liver appears to be involved (Chahal P, et al, 2008). Liver histology may provide important diagnostic information in patients with acute liver failure (ALF) (Polson J, Lee WM, 2005). Liver biopsy is helpful in making a specific diagnosis in specific settings (e.g., herpes virus infection, Wilson disease, AIH, and malignancy), which in turn may guide more specific therapy (Miraglia R, et al, 2006). Liver histology in patients with hepatomegaly or apparent diffuse disease may help establish a diagnosis, but whether it is clinically useful or cost effective is unknown (Volwiler W, et
Examples of diffuse diseases include amyloidosis granulomatous hepatitis caused by any number of processes, and a host of other miscellaneous disorders (Reuben A, 2004).

(2) **Assessment of prognosis (disease staging):**

A further important use of liver biopsy is in assessing disease severity, not ably fibrosis, which, as a precursor to cirrhosis, may predict the emergence of complications of portal hypertension and also liver-related morbidity and mortality. Evidence in the area of HCV emphasizes the role of fibrosis assessment in determining prognosis (Powell EE, et al, 2005). For example, alcohol consumption, increased hepatic iron concentration, and/or hepatic steatosis, all of which are associated with more rapid fibrosis progression in patients with chronic HCV (Hourigan LF, et al, 1999), are currently assessed best by histology (Younossi ZM, et al, 2004) Further, specific evidence links fibrosis and prognosis; an example of this logical relationship is that in patients with HCV infection after liver transplantation, mortality was increased in those with advanced compared to minimal fibrosis (Blasco A, et al, 2006).

NAFLD and eventual liver-related mortality appear to also, progression be related to the initial fibrosis stage (Ekstedt M, et al, 2006).

(3) **To assist in making therapeutic management**

Currently, liver biopsy is used more than ever to develop treatment strategies. As previously emphasized, this has evolved because of the many new therapies available for patients with a variety of liver diseases. (Dienstag JL, et al, 2006).

Not only can a treatment plan be instituted in a patient after a specific diagnosis is made (i.e., steroids in the setting of AIH), but among those with established liver disease, treatment may be predicated on the specific histological lesion. In the latter circumstance, therapy is usually directed at the patient with a more advanced histological stage. For example, histological analysis of the liver in patients with HCV provides information about the grade (degree of inflammation), which in turn presumably reflects to what extent the liver disease injury remains ongoing (Dienstag JL, et al, 2006).

**2.7 Preparation for Liver Biopsy**

The general approach to liver biopsy has changed substantially over the past to 10-20 years. Currently, liver biopsy is typically undertaken on an outpatient or “same day” basis. Most often, the patient will have been seen in the clinic or office within the preceding month where a discussion about the indications for, benefits, and risks of liver biopsy will have occurred.
it is well-appreciated that many patients undergoing liver biopsy experience significant anxiety about the procedure, the following practical points should also be discussed before the procedure:

1. By whom and where the biopsy will be performed
2. Whether sedation of any sort may be taken prior to the procedure, or will be available immediately beforehand.
3. What degree of pain may be anticipated during and after the procedure, and the measures available that might help minimize and/or attenuate it.
4. When the patient may return to their usual level of activity, and to work outside the home if applicable
5. When the result will be known, and by what means this information will be communicated to the patient.

Being clear and precise about these pragmatic issues are important to facilitate performance of the procedure and instill in the patient a sense of confidence.

Written informed consent, including risks, benefits, and alternatives, should be obtained prior to liver biopsy.

2.8 Prebiopsy Testing:

Common practice includes measurement of the complete blood count, including platelet count, prothrombin time (PT)/international normalized ratio (INR), in some institutions the activated partial thromboplastin time, and/or cutaneous bleeding time at a suitable juncture prior to the biopsy. Some experts recommend having a specimen of blood typed, so that blood could be made available at short notice in case of bleeding. Previously documented abnormalities in laboratory tests Patients with. May require these to be repeated closer to the time of biopsy; the time frame will vary depending on the specific clinical scenario and local policies (Segand Dzik WH, 2005).

The utility of these tests in predicting bleeding risk is uncertain. However, generally not supported by the available literature (. Tripodi A and Mannucci PM, 2007). Moreover, the prevalence of more complex hemostatic defects in patients undergoing biopsy, such as hyper fibrinolysis, which are undetectable by conventional tests, is unknown, although some 10%-15% of hospitalized patients with cirrhosis appear to have this particular problem. (Leebeek FW, et al, 1991). Hyper fibrinolysis should be suspected when there is late (hours) post procedure bleeding, consistent with initial clot formation and premature clot dissolution thereafter. (Hittelet A and Deviere J, 2003). Additional studies are needed to assess the pre procedure utility of more global measures of hemostasis such as thrombo-
elastography, this test assesses indices of hyperfibrinolysis and platelet function.
Additionally, imaging reports should be reviewed to ensure that:

(1) no focal lesion exists in the right hepatic lobe, e.g., hemangioma
(2) that biliary dilation is not present. Either of these conditions might give rise to an otherwise unsuspected (and avoidable) complication.

2.9 The Liver Biopsy Procedure
The liver biopsy should be performed in a dedicated area, with adequate space for the operator(s), assistants, emergency equipment if necessary, or for family members during recovery (Caldwell SH, 2001).
Use of oral or intravenous anxiolytic therapy or conscious sedation is variable; available data indicate that it is safe when used (Eisenberg E, et al., 2003). If such medications indicate that it is safe when used (Eisenberg E, et al. 2003). If such medications are or may be utilized, then any substantial oral are or may be utilized, then any substantial oral intake should be avoided prior to the procedure. (Caldwell SH, 2001). Routine placement of an intravenous cannula prior to the procedure there be significant pain and/or bleeding after the procedure, but the cost/risk benefit of this approach is unknown. (Eisenberg E, et al. 2003).

2.10 Liver Biopsy Method
1. Percutaneous Biopsy
This method may be undertaken in one of three ways, namely palpation/percussion-guided, image-guided, and real-time image-guided.
A palpation/percussion-guided transthoracic approach, after infiltration of local anesthesia, is the classic percutaneous method. Although the subcostal approach has been performed in patients with hepatomegaly that extends well below the right costal margin it is not recommended in routine practice without image guidance. (Perrault J, et al., 1978).
2. Trans venous (Trans jugular or Trans femoral) Biopsy
A number of specific situations warrant consideration of this approach. Patients with clinically demonstrable ascites; a known or suspected hemostatic defect; a small, hard, cirrhotic liver; morbid obesity with a difficult-to-identify flank site; or those in whom free and wedged hepatic vein pressure measurements are additionally being sought should be considered candidates to undergo liver biopsy by the trans venous route. (Sherlock S, et al., 1985). The technique has been well described in the literature
and should be considered standard. (Lebrec D, et al, 1982). Expense and availability of local expertise are also important variables when considering trans venous biopsy. (Bull HJ, et al, 1983).

3. Surgical/Laparoscopic Biopsy:

In many circumstances, a surgical or laparoscopic approach is utilized because the liver is noted to be abnormal in appearance prior to planned surgery or at the time of surgery. (Orlando R, et al, 1990). Biopsy in this situation is performed either with typical needle devices or by wedge resection. (Orlando R, et al, 1990). Notably, the latter has been criticized as producing overestimates of fibrosis due to its proximity to the capsule. (Orlando R, et al, 1990). Laparoscopic liver biopsy allows adequate tissue sampling under direct vision, with direct (and immediate) control of bleeding. (Orlando R, et al, 1990). It is generally performed by those with special expertise, typically under general anesthesia. (Orlando R, et al, 1990). It should be noted that creation of a pneumoperitoneum (with nitrous oxide) is highly reliable and allowsthe use of conscious sedation and performance of the procedure in specialized areas within an endoscopy unit. (Poniachik J, et al, 1996). Most studies that have compared laparoscopic biopsy to transthoracic percutaneous biopsy have demonstrated greater accuracy in diagnosing cirrhosis with the former approach, probably because of the added benefit of peritoneal inspection. (Denzer U, et al, 2007). Complications of this method include general anesthesia, local abdominal wall or intraperitoneal trauma, and bleeding. Expense and the requirement for special expertise have limited its use. (Steele K, et al, 2008).

New laparoscopic techniques may facilitate laparoscopic liver biopsy, and could theoretically be performed safely at low cost. An exciting possibility is that techniques extending from natural orifice transluminal endoscopic surgery could be used to perform liver biopsy. (Steele K, et al, 2008). In one study, transgastric flexible endoscopic peritoneoscopy allowed systematic visualization of the liver with subsequent liver biopsy (and adequate tissue samples for histologic examination) in a small number of obese patients for whom percutaneous biopsy would have been technically difficult or associated with unacceptably high risk of complication. (Steele K, et al, 2008).

4. Plugged Biopsy:

The plugged biopsy has been proposed as being potentially safer than standard percutaneous biopsy among certain patients (i.e., those believed to be at high risk for bleeding such as those with coagulopathy and/or thrombocytopenia or a small cirrhotic liver). (Riley SA, et al, 1984). The plugged biopsy is a modification of the percutaneous method in which the biopsy tracks is plugged with collagen or thrombin (or other materials) as the cutting needle is removed from a
sheath, while the breath is still being held. (Tobin MV and Gilmore IT, 1989). In one study, the approach was both well-tolerated and safe. (Fandrich CA, et al, 1996). In another study, this technique was compared to trans jugular liver biopsy among patients with prolonged PT and reduced platelet counts. (Sawyerr AM, et al, 1993). The plugged- percutaneous liver biopsy technique was quicker and yielded specimens of significantly longer length than the trans jugular approach, but was complicated by hemorrhage that required blood transfusion in 2 of 56 (3.5%) of plugged biopsy patients, compared with 0 of 44 (0%) undergoing trans venous biopsy. (Sawyerr AM, et al, 1993).

2.11 Liver Biopsy Devices:
Liver biopsy devices originated in the late 1800s and proliferated in the early 20th Century. The liver biopsy devices used most widely today for diagnosis and management of patients with parenchymal liver disease are the core-aspiration needles (Menghini, Jamshidi, or Klatskin-style) and sheathed cutting needles (either manual or spring-loaded, often referred to as a “Trucut-style” in reference to one of the earliest cutting devices). Newer automated versions of this latter type have recently emerged, allowing variable pitch and specimen length. (Sherman KE, et al, 2007). The cutting needle devices generally pass into the liver parenchyma using a troughed needle before an outer sheath or hood slides over this to secure a core of tissue. (Sherman KE, et al, 2007). This is especially helpful among patients with suspected or established cirrhosis because it limits the tendency for the specimen to shatter or fragment. (Sherman KE, et al, 2007). In general, cutting needles have been shown to produce more reliable specimens in advanced fibrosis, although studies so far have not included the newer variable pitch automated core device. (Sherman KE, et al, 2007). The caliber of (most) current cutting needles is about 16 gauge (1.6 mm) and the trough length is usually 1.6-1.8 cm, thereby limiting the overall dimensions of the specimen that may be retrieved, and thus the number of portal tracts that may be available for analysis. (Alexander JA and Smith BJ, 1993). Conversely, the traditional core-aspiration technique relies on suction generated via a syringe in conjunction with a flat or a beveled (Menghini or Klatskin) needle tip to procure a core of liver tissue. (Alexander JA and Smith BJ, 1993). The pressure of suction may cause some specimens, particularly those from cirrhotic livers, to fragment more easily and should be an important consideration in the choice of device. (Castera L, et al, 2001). Newer automated core needle devices have recently emerged, these utilize a tiny inflection of the cannula at its tip, which serves to trap the specimen and obviates the need for suction. Thus, longer cores may be obtained without fragmentation. (Castera L, et al, 2001).
2.12 Liver Biopsy Procedure

Standard transthoracic percutaneous liver biopsy is performed with the patient placed supine in a comfortable position, the right arm and hand should be placed gently behind the head, also in a comfortable, neutral, position. Selective use of sedative medications during liver biopsy may alleviate anxiety and appear to reduce postprocedure pain; their use should be considered to be a matter of local preference and expertise. The skin is prepped and draped, then anesthetized with a local anesthetic agent, typically lidocaine, 1%. The area from the skin to the peritoneum is also anesthetized using care to advance only above the appropriate rib (intercostal arteries generally run below the rib so that intercostal arterial injury can be avoided by inserting the needle over the cephalad rather than the caudal aspect of the rib) and ensuring that anesthesia is not injected into a vascular structure (typically the anesthesia plunger is withdrawn slightly to see that blood does not return, before injection of local anesthetic). Pain with insertion of the biopsy needle indicates inadequate local anesthesia. The liver capsule itself may be anesthetized using a small, 23-gauge or 25-gauge “finding” needle, but whether this practice is beneficial in the absence of real time image guidance is unknown (because it is unlikely that the specific portion of the liver anesthetized would subsequently be biopsied). If used, application of the local anesthetic is facilitated by observing the patient’s respiratory cycle and instilling the agent during a brief breath hold. Care should then be taken to perform the biopsy at the same point in the respiratory cycle (usually at a full but not forced expiration) to insure piercing the peritoneum and liver capsule at exactly the same point as application of the local anesthetic agent previously administered. It is well recognized that the liver physically moves during breathing. Further, because there is concern that the liver may be lacerated if it is moving, breath-holding is often advocated and used during the actual passage of the biopsy needle in the routine transthoracic approach. (Grant A and Neuberger J, 1999). It is believed to both reduce the risk of capsule laceration and to facilitate biopsy at the site of local (liver) anesthetic if used. Although many techniques have been utilized (i.e., performing biopsy at the end of deep expiration, during simple breath-holding, etc.) and some perform liver biopsy without formal breath holding, no study has addressed the use of a breath-hold or which technique is best. (Sherlock S, et al, 1985). Once the liver biopsy has been accomplished, the patient then rests quietly and is carefully observed by experienced nursing staff. (Hyun CB and Beutel VJ, 2005)

Immediately after the biopsy, vital signs are typically obtained at least every 15 minutes for the first hour, and every 30 minutes during the second hour. The patient is often placed in the right lateral
decubitus position (presumably to allow the liver to rest against the lateral abdominal wall and thereby limit bleeding), although this is largely performed as a result of longstanding clinical practice. (Hyun CB and Beutel VJ, 2005). It is recommended that patients simply recover in a quiet, comfortable, setting. (Hyun CB and Beutel VJ, 2005). The risk of bleeding is greatest initially after liver biopsy; thus, it is recommended that patients be observed carefully over the first several hours after biopsy. (Firpi RJ, et al, 2005). Although the optimal length of observation after the liver biopsy has not been firmly established, it appears that an observation period of 2-3 hours is most appropriate. (Firpi RJ, et al, 2005)

### 2.12.1 Ultrasound Guidance (Under Radiological Considerations)

Ultrasound guidance helps direct the liver biopsy needle away from the gallbladder, large vascular structures, colon, and lung, and thus has the potential to reduce complication rate. (Manolakopoulos-S, et al, 2007). Nonetheless, there is controversy about the use of US. It has been used either in real-time or via a prebiopsy marking technique where the patient subsequently has liver biopsy performed at the marked site. (Stone MA and Mayberry JF, 1996).

### 2.13 Contraindications

Specifying contraindications to liver biopsy is fraught with difficulty given the scarcity of data in this area. Additionally, many of the older studies may not be applicable to practice in the modern era. It should also be emphasized that contraindications will vary depending on the physician operator and available local expertise; for this reason, most of the listed contraindications are considered to be relative. In daily clinical practice, the considerations that are often of the greatest concern to the care provider include an uncooperative patient, one in whom there is increased potential for bleeding, and the morbidly obese patient.

Many studies have compared the value of cell block with smears. Kulkarni et al, 2000 studied 27 FNA biopsies and found that cell block sections add more information to cytological smears in 12 cases, gave similar results in 14 cases while in conclusion in one case. It was concluded that cell block technique is very useful to utilize available material when re aspiration is difficult.

Also Kulkarni et al, 2000 reported that the main problem with the cell block technique is the losing the cellular material during preparation. Therefore, a critical point during preparation of cell block is the collection of sediments without loss of material.

Kern and Habar, 2008, studied 393 cases of cell block preparation in 237 (60.3%) the finding were confirmatory, and in 103 cases (26.2%) cell block provided additional information for diagnosis.
Wojcik and Selaggi, 2006, showed that 84% of the cases had identical results on both smears and cell blocks.

Shehnaz Khan, et al, 2012, determined the effectiveness of cell block technique by comparing cytomorphological preservation on paired cell block and conventional fine needle aspiration (FNA) samples. In this study material of both glass slides and cell blocks were collected simultaneously during fine needle aspirates from 47 samples of lung masses, grading of cellularity, morphological preservation, architectural preservation and presence of background staining on paired FNA smears and cell block samples were compared, cellularity were agreement between the two methods of sample preparation, morphological preservation in FNA samples were 100%, compared to 94% in cell block samples, architectural preservation in FNA was 100%, compared to only 47% in cell block sample.

Raafat Awad Hegazy, et al, 2014, evaluated the importance of the combined use of fine needle aspiration cytology (FNAC) and cell block in the diagnosis of different breast lesions, in this study 310 cases (301 females and 9 males) with breast swelling coming to cytopathology unit, FNA and cell block were performed, the study showed that combining FNA with core biopsies has been increase diagnostic accuracy, this study suggests that combining a smear preparation of breast FNA with the cell block can also combine the advantages of both approaches, the sensitivity was 94%, specificity was 98%.

Leung and Bedard (2005), found that all cases with adequate material could be diagnosed on a cell block preparation.
CHAPTER THREE

Material and Method

3.1 Materials:

3.1.1 Study design:
This was prospective and comparative study.

3.1.2 Study area:
study was carried out in Khartoum state, the capital of Sudan, it is one of the eighteen states of Sudan. Although it is the smallest state by area (22,142 km2), it is the most populous (5,274,321 in 2008 census). It contains the country's largest city by population, Omdurman, and the city of Khartoum, which is the capital of the state as well as the national capital of Sudan. It is surrounded by River Nile State in the north-east, in the north-west by the Northern State, in the east and southeast by the states of Kassala, Gedaref and Gezira, and in the west by North Kurdufan.

3.1.3 Study Duration:
during the period (June 2015 – July 2015).

3.1.4 Study setting:
National Ribat University Teaching Hospital (NRUTH) is one of the leading teaching hospitals in the Sudan that is located in Khartoum State - Khartoum/Burri. The hospital was made initially for providing health care and services for policemen who were retired and those who still work in police. Latter it grew up to receive patients from all over Sudan. The hospital is rated as one of the best in Khartoum state as it includes all major medical departments and higher specializations like neurosurgery and pediatric surgery. Most of the consultants working in the hospital are broadly recognized.

3.1.5 Study population:
All liver disease patients attending National Ribat University Hospital for ultrasound guide fine needle aspiration.

3.1.6 Inclusion criteria:
All liver disease patients.

3.1.7 Sample size:
80 liver ultrasound guide fine needle aspiration, each sample divided into two, one for cell block and the other for routine cytology smears, then compared together.

3.1.8 Type of samples:
Liver ultrasound guided FNA.

3.2 Methods:
3.2.1 Basic EUS-guided Sampling Technique:
Endoscopic ultrasound guided fine needle aspiration is a valuable and safe tool to obtain cellular material for cytological examination. The advent of (euS) accessories like core biopsy devices facilitated obtaining histological materials from solid lesions. In experienced hands, (euS-Fna) or core biopsy of lesions and lymph nodes above and below the diaphragm has been demonstrated to be extremely safe when compared with other tissue sampling modalities, with a risk profile similar to that of conventional endoscopy.

(EuS) guided tissue acquisition starts by appropriately identifying the lesion of interest and its characteristics including shape, size, and relationship to surrounding structures. The lesion is then interrogated using the linear array echoendoscope to determine an appropriate puncture site. The ultrasound transducer on the distal tip of the echoendoscope permits needle advancement into the lesion under real-time guidance. euS-Fna and core biopsies are performed after Doppler assessment to avoid puncturing intervening blood vessels.

Once the gut wall is punctured and the needle enters the target lesion, the sty let is withdrawn. Whether or not to apply suction during FNA remains controversial but should be tailored to the specimen’s volume and the amount of blood noted on the first pass performed with suction. The needle is then moved back and forth through the lesion for at least 30 seconds or until deemed adequate by the endosonographer. The needle is then withdrawn back into the sheath, the assembly is removed, and the sample is processed per institutional protocol.

3.2.2 Liver ultrasound guided fine needle aspiration cytology technique procedure:

3.2.2.1 Preprocedural evaluation:

- Patient consent and preprocedure targeted physical exam
• If ultrasound is being used as the imaging guidance, a preprocedure targeted ultrasound exam is commonly performed.

3.2.2.2 Equipment:

The "fine needle" in an FNA varies depending on the system being biopsied and the nature of the lesion, but is typically a 25 gauge to 27 gauge needle with a stylet. For ultrasound-guided procedures, the transducer may have a needle guide.

3.2.2.3 Technique:

The FNA technique will vary depending on the system being targeted and the nature of the lesion. Techniques common to all procedures include:

• local anesthesia with 2% buffered lidocaine
• advancing the fine needle under imaging guidance until the tip is in the intended area of biopsy
• removal of the stylet
• multiple short passes through the lesion (filling the needle with cells)
• removal of the needle with appropriate disposition of the cells (e.g. slide for smear, container for cell block)
• multiple samples are usually obtained in a session

3.3 Sample processing:

3.3.1 Smearing technique:

The ultrasound guided fine needle aspiration specimens were obtained and 2-4 smears were prepared from each sample using coated – slides and immediately fixed in 95% ethanol then H&E stained.

3.3.2 Cell blocking:

Cell blocks are prepared from residual material obtained from FNA after smears are prepared and are useful adjuncts for establishing a more definitive cytopathological diagnosis. Although cell blocks
are almost routinely obtained during (euS-Fna) using excess material after smear preparation, they
can be particularly useful for the diagnosis and classification of lesions that otherwise may not be
possible from smears only. in such cases, it is important to obtain at least 2 additional passes
dedicated for cell block (i.e., no smears are prepared from these passes) to ensure adequate material
is provided. The specimens are typically submitted in a special preservative solution, we used
40%-formalin. The then aspirate cells fixed in 40% formalin overnight, the supernatant fluid was
decanted and settle cells warped in filter paper and then placed in a tissue cassette. All cassettes
processed in an automatic tissue processor, the cell blocks were embedded in paraffin wax, and 4-6
micron sections were cut using standard rotary microtome. The sections were stained with
Haematoxylin and eosin(H&E) stain and covered with cover slips using DPX mounting media, and
the cytological smears were also stained with Haematoxylin and eosin stain.

3.3.3 Staining:
Routine H&E (Harris H&E) staining will be used on all cell block sections and smears.

H & E staining method:
- Deparaffinize section, hydrate through graded alcohols to water
- Remove fixation pigment if necessary
- Stain Harris Haematoxylin for 5 minutes
- Wash well in running tap water until section( blue) for 5 minutes or less
- Differentiate in 1% acid alcohol (1% HCL in 70% alcohol) for 5 second
- Wash in tap water until sections are again (blue) (10 – 15 minutes), or blue by dipping in an
  alkaline solution (e.g ammonia water), followed by 5 minutes tap water wash
- Stain in 1% eosin (y) for 10 minutes
- Wash in running tap water for 1 – 10 minute
- Dehydrate through alcohol, clear, and mount

3.4 Results interpretation:
All Haematoxylin and eosin stained smears and blocks sections will be reviewed by a
cytopathologist according to four parameters: background, cellularity, nuclear preservation,
cytoplasmic preservation.
The final diagnosis of the cell block sections and the cytological smears will be compared.
3.5 Statistical analysis:
All the data will be statistically analysis using SPSS program version 16.

3.6 Ethical approval:
was taken from the university ethical research board (ERB) and the hospital administration. Consent was taken from the Head department of histopathology lab in NRUTH, Consent was taken from patients to take samples after agreement of the participant name of patients were not taken but symbolized by numbers instead to guarantee confidentiality.
Chapter four

Result and Discussions

4.1 Result:

In this study 80 samples of liver biopsies were used, and the comparison between routine cytology and cell block technique was done including cellularity, nuclear and cytoplasmic preservation and background staining.

![Age Distribution Chart]

**Fig (4.1) Age of the patients**
Agewas classified into age groups; the high age group was 61-70 years 19 (23.8%).
4.2 Gender of the patients

The gender as the following, males were 45(56%) and females were 35(44%)

**Fig (4.2): Gender for patients**
Table (4.1) Comparison of Cellularity betweensmears and cell block

<table>
<thead>
<tr>
<th>Technique</th>
<th>Cellularity</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+ (0-10%)</td>
<td>2+</td>
<td>3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear</td>
<td>9 (11.3%)</td>
<td>30</td>
<td>41</td>
<td>80</td>
<td>100%</td>
</tr>
<tr>
<td>Cell block</td>
<td>35 (43.8%)</td>
<td>18</td>
<td>27</td>
<td>80</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>44 (27.5%)</td>
<td>48</td>
<td>68</td>
<td>160</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The high score (+3) in smear 41 (51.3%) while in cell block 27(33.8%), the moderate score (+2 ) in smear 30(37.5%) while in cell block 18 (22.5%), the low score (+1) in smear 9(11.3%) while in cell block 35(43.8%).

Assessment of agreement of cellularity between the two method of sample preparation the following were obtained :
P value of high score (+3) 0.000 ( highly significant) because most of cell block samples loss their cells during preparation in cell block this agree with Kulkarni et al, 2000, reported that the main problem in cell block technique is risk of losing the cellular material during preparation it’s important to mention that cell block component may be missed during sectioning due to low cellularity.
Table (4.2): Comparison between smears and cell block in nuclear preservation

<table>
<thead>
<tr>
<th>Technique</th>
<th>Nuclear Preservation</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+ (0-10%)</td>
<td>2+ (11-50%)</td>
<td>3+ More than 50%</td>
<td></td>
</tr>
<tr>
<td>Smear</td>
<td>4 (5%)</td>
<td>33 (41.3%)</td>
<td>43 (53.8%)</td>
<td>80 (100%)</td>
</tr>
<tr>
<td>cell block</td>
<td>8 (10%)</td>
<td>35 (43.8%)</td>
<td>37 (46.3%)</td>
<td>80 (100%)</td>
</tr>
</tbody>
</table>

The high score (+3) in smear 43 (53.8%) while in cell block 37(46.3%), the moderate score (+2) in smear 33(41.3%) while in cell block 35(43.8%), the low score +1 in smear 4(5%) while in cell block 8(10%).

P value of high score between two method of preparation were 0.003

The nuclear and cytoplasmic preservation in smears better than cell block, this may be due to many steps in processing of cell blocks which affect in to the cell agree to Shehnaz Khan, *et al*, 2012 reported that the agreement architectural preservation in FNA was 100%, compared to only 47% in cell block sample.
Table (4.3): comparison between smears and cell block in cytoplasmic preservation

<table>
<thead>
<tr>
<th>Technique</th>
<th>cytoplasmic preservation</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>Smear</td>
<td>2 (2.5%)</td>
<td>36 (45%)</td>
<td>42 (52.5%)</td>
<td>80 (100%)</td>
</tr>
<tr>
<td>cell block</td>
<td>11(13.8%)</td>
<td>34 (42.5%)</td>
<td>35 (43.8%)</td>
<td>80 (100%)</td>
</tr>
</tbody>
</table>

The high score +3 in smear 42 (52.5%) while in cell block 35(43.8%), the moderate score +2 in smear 36(45%) while in cell block 34(42.5%), the low score +1 in smear 2(2.5%) while in cell block 11(13.8%).

P value of high score between two method of preparation were 0.005

The cytoplasmic preservation in smears better than cell block, this may be due to many steps in processing of cell blocks which affect into cells agree to Shehnaz Khan et al, 2012 reported that the agreement architectural preservation in FNA was 100% , compared to only 47% in cell block sample.
Table (4.4): Comparison between smears and cell block in background staining

<table>
<thead>
<tr>
<th>Technique</th>
<th>1+ Mild background staining</th>
<th>2+ Moderate background staining</th>
<th>3+ Sever background staining</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>7 (8.8%)</td>
<td>31 (38.8%)</td>
<td>42 (52.5%)</td>
<td>80 (100%)</td>
</tr>
<tr>
<td>cell block</td>
<td>55 (68.8%)</td>
<td>15 (18.8%)</td>
<td>10 (12.5%)</td>
<td>80 (100%)</td>
</tr>
</tbody>
</table>

The sever background staining +3 in smear 42 (52.5%) while in cell block 10 (12.5%), the moderate background staining +2 in smear 31 (38.8%) while in cell block 15 (18.8%), the mild background staining +1 in smear 7 (8.8%) while in cell block 55 (68.8%).

P value of high score is between two method of preparation were 0.008.

Cell block give less background staining than smears, this agree to the study of Raafat Awad Hegazy, et al, 2014 reported that evaluated the importance of the combined use of fine needle aspiration cytology (FNAC) and cell block in the diagnosis of different breast lesions, in this study 310 cases (301 females and 9 males) with breast swelling coming to cytopathology unit, FNA and cell block were performed, the study showed that combining FNA with core biopsies has been increase diagnostic accuracy, this study suggests that combining a smear preparation of breast FNA with the cell block can also combine the advantages of both approaches, the sensitivity was 94%, specificity was 98%.
Chapter five
Conclusion and Recommendations

5.1 Conclusion:
Smear preparation was better than cell block technique in preservation of cells, nucleus and cytoplasm of liver biopsy specimens.
Cell block technique give less background staining than smear preparation.
Most of patients between the age 61-70 years old, and most of them were males.

5.2 Recommendations:
1. Smear preparation of the liver biopsies should be used routinely.
2. Modification cell block technique should be done to achieve more preservation of cells.
3. Cytological smears were used once, but cell blocks can be preserved for further use.
4. Further studies should be done using different types of specimens.
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