Chapter Three

Materials and Methods

3. Materials and Methods:

3.1 Materials:

Archive tissue blocks previously diagnosed as liver hepatocelluler carcinoma adenocarcinoma and metastatic adenocarcinoma were used in this study.

3.2 Methods:

3.2.1 Study design:

This is hospital based descriptive cross sectional study aimed to study the role of Hepar-1 antibody as marker for differntial diagnosis of liver carcinomas.

3.2.2 Study samples:

Forty tissue formalin fixed paraffin blocks of liver carcinomas were obtained from tissues previously diagnosed as hepatocelluler carcinoma (20 samples) and adenocarcinoma (10 samples) and metastatic carcinoma(10 samples) during the period from January 2011 to October 2018. Patient age and sex and diagnosis were obtained from patient`s file.

3.2.3 Sample processing:

Section of 3µ in thickness was obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome, mounted into positively slides (Thermo) then dewaxed in oven.

3.2.4Staining method:

3.2.4.1Immunohistochemical staining:

Paraffin sections were immunostained using avidin biotin technique. Sections were put in oven and cleared in two change of xylene for two

minutes, then rehydrated through descending concentration of ethanol (100%, 90%, 70% and 50%) and water two minutes for each, then antigen retrieved by water path for forty minutes, then treated with 3% hydrogen peroxide and methanol solution for fifteen minutes, then washed in phosphate buffer saline (pH7.4) for five minutes. Then treated with Hepar-1 for thirty minutes, then rinsed in phosphate buffer saline then binding of antibody detected by incubating for twenty minutes with biotin followed by fifteen minutes with streptoavidin, then the sections were washed in three changes of phosphate buffer saline, then treated with substrate and 3,3-diaminobenzidene tetra hydrochloride (DAB) chromogen for three minutes, then washed in phosphate buffer saline, then counterstained in Mayer's haematoxylin for one minute, then washed and blued in running tap water,let to dry , then cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn, 2008).

3.2.5. Result interpretation:

All quality control measures were adopted during sample staining for immunohistochemical results assessment. Positive and negative controls were used to confirm the location of positivity of hepar-1 expression that was confirmed by five cells per field.

3.2.6. Statistical analysis:

Data were analyzed using SPSS version 20 computer program. Frequencies, means and Chi-square test values were calculated.

3.2.7. Ethical considerations:

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