Histopathological markers of liver cirrhosis correlating with fat pads in cadavers

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INTRODUCTION

The liver is an organ with many functions in the body, including storage of essential nutrients, detoxification of blood, and digestion and metabolism. The major functional unit of the liver is the liver lobule (Figure 4), which contains the microanatomy of the liver, including hepatocytes, sinusoids, portal triads, and the central vein. The liver is a resilient organ that has the capability to regenerate up to two-thirds of its tissue. In cirrhosis, a condition in which collagen is produced and results in fibrotic tissue and causes normally functioning tissue to become scar tissue, the liver is no longer capable of its regenerative functions.

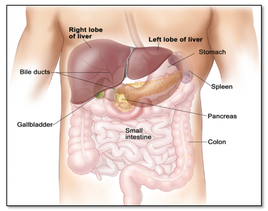


Figure 1. The liver is an organ located in the upper right quadrant of the abdomen.

QUESTIONS:

1. Is there a visibly and measurably increased presence of Kupffer cells (KC) and hepatic stellate cells (HSC) and a decreased presence in liver sinusoidal epithelial cells (LSEC) in samples from cadaver livers?

2: Is there a correlation between cellular and histological markers of cirrhosis, such as the degree of presence of Kupffer cells, hepatic stellate cells, and liver sinusoidal endothelial cells, and the size of substernal and pubic fat pads in cadavers?

HISTOPATHOLOGICAL MARKERS OF CIRRHOSIS

Biopsies are performed for almost all patients and will show varying degrees of fibrotic tissue and nodule formation, which are indicative of what stage of cirrhosis the patient is in.

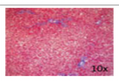
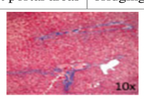
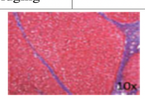
METAVIR score	**F0**	**F1**	**F2**		**F3**		**F4**
	No fibrosis	**Fibrosis expansion of portal zones**	**Fibrosis expansion of most portal zones, occasional bridging**		**Marked bridging, occasional modules**		**Cirrhosis**
Ishak Score	0	1	2	3	4	5	6
	No fibrosis	Fibrosis expansion of some portal areas	Fibrous expansion of most portal areas	Portal to portal bridging	Portal to central bridging	Occasional nodules	Cirrhosis
Representative histological photographs per scoring							

Figure 2. Liver cirrhosis is determined based on the METAVIR scale and Ishak score, which are distinguished through examining biopsies for the presence of fibrotic tissue and nodule formation. (Haep et. al., 2022)

Other hallmarks of cirrhosis include the degree of presence of specialized cells that contribute to liver function and cirrhotic progression.

Hepatic stellate cells (HSC) are fat storing cells in the liver, which are normally dormant but become activated upon damage or injury to the liver. Activated cells secrete transforming growth factor (TFG-β), which contributes to collagen and scar tissue production.

Kupffer cells (KC) are a specialized macrophage in the lining of the sinusoid, which are normally dormant, but can be activated by damage or injury to the liver. Activated cells destroy hepatocytes and also activate HSCs, making them a part of the initiation of fibrosis.

Liver sinusoidal epithelial cells (LSEC) make up the endothelial lining of the sinusoidal wall. They contribute to the fenestration of the sinusoidal walls and facilitate gas and particle exchange. In cirrhosis, the sinusoidal wall epithelium becomes defenestrated, which leads to a decrease in the presence of LSECs and can be an important step in the generation of fibrosis.

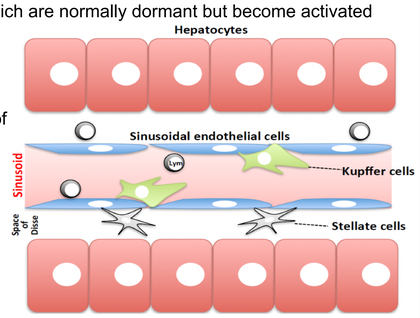


Figure 3. Diagrammatic depiction of HSCs, KCs, and LSECs in relation to the sinusoid spaces and hepatocytes of liver tissue (Tsutsui et. al., 2014)

MATERIALS AND METHODS

Multiple liver samples were obtained from cadavers at Misericordia University. Cadavers varied in cause of death and in health of the liver. Samples were preserved by fixating them in NBF solution before histological procedures commenced. The samples were then dehydrated by various solutions in tissue processing using the Autotechnicon automatic tissue processor. The tissues were embedded using paraffin wax using a Fisher Histo-Center apparatus. The embedded tissues were sectioned at a thickness of 12 mcm using a microtome. Slides were created by floating the sectioned tissue samples in a hot water bath, allowing them to expand the tissue to normal tissue dimensions. Prepared microscope slides were placed on a preheated warming plate for 20 hours in order for the tissues to be better affixed to the slide. Samples were then stained using hematoxylin and eosin stains and imaged under a microscope with a camera attachment.

RESULTS

Normal Liver Tissue Sample Images

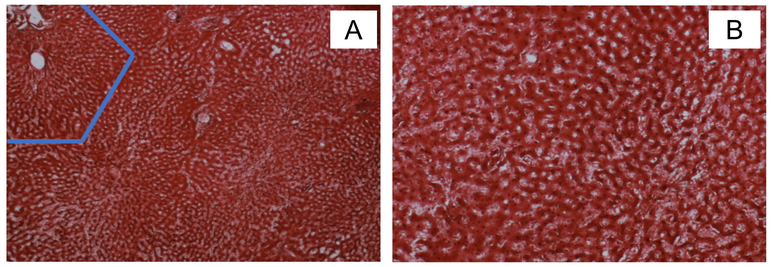


Figure 4. (A) Normal liver tissue at 4x magnification with a visible liver lobule and central vein intact (outlined with blue line). **Figure 5.** (B) Normal liver tissue at 10x magnification. Hepatocytes and sinusoids are visible.

Cirrhotic Liver Tissue Sample Images

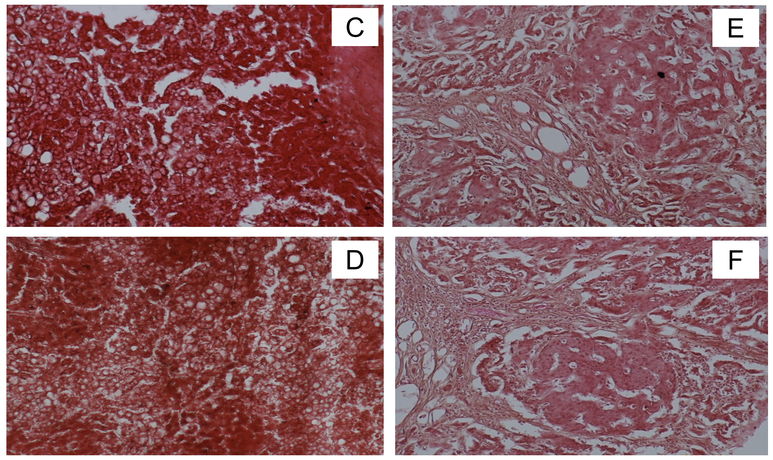


Figure 6. (C) Liver tissue exhibits presence of fat cells and lack of liver lobule distinction at 10x magnification. **Figure 7.** (D) Liver tissue from same patient as Figure 4. Tissue exhibits fat cell presence and lack of liver lobule distinction at 10x magnification. **Figure 8.** (E) Liver tissue exhibits clear fibrotic bridging between nodules at 10x magnification. **Figure 9.** (F) Liver tissue from same patient as Figure 6. Liver tissue exhibits clear fibrotic bridging between distinct nodule.

Correlation Between Cirrhosis and Size of Fat Pads

	Estimated % Cirrhosis	**Pubic Fat Pad (cm)**	**Substernal Fat Pad (cm)**
Sample 3	10	2.3	1.0
Sample 4	20	4.0	2.5
Sample 7	45	5.0	1.9
Sample 2	90	2.7	1.9

Table 1. Estimations for the percentage of cirrhosis present in cadaver patients were taken based on the images collected. Size of pubic fat pads and substernal fat pads were obtained in the cadaver lab in centimeters.

Correlation between Est. % Cirrhosis and **Pubic** Fat Pads	**0.232262**	**Slight Positive**
Correlation between Est. % Cirrhosis and **Substernal** Fat Pads	**-0.0491496**	**No Correlation**

Table 2. Correlations between estimated percentage of cirrhosis present in the images and the size of pubic fat pads and the size of substernal fat pads was calculated. A slight positive correlation was found between the estimated percentage of cirrhosis and the size of pubic fat pads, while there was no correlation between estimated percentage of cirrhosis and size of substernal fat pads.

DISCUSSION AND FUTURE RESEARCH

1: Is there a visibly and measurably increased presence of KCs and HSCs and a decreased presence in LSECs in samples from cadaver livers?

Images could not be obtained at a magnification above 10x, so KCs, HSCs, and LSECs could not be discerned.

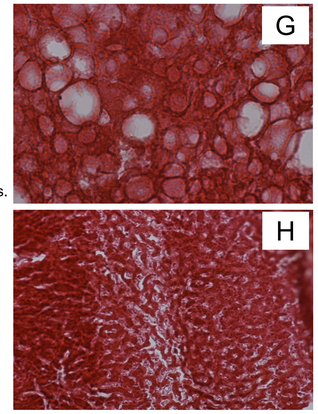


Figure 10. (G) Image taken at 40x and image could not be focused on a monolayer of cells at this magnification, not allowing for the distinction of cell types. **Figure 11.** (H) Image taken at 10x and image could not completely focus on the entire section visible under the microscope.

2. Is there a correlation between cellular and histological markers of cirrhosis, such as the degree of presence of KCs, HSCs, and LSECs, and the size of substernal and pubic fat pads in cadavers?

Due to the inability to identify the different cell types proposed to be investigated, an estimated percentage of cirrhosis was found using the viable images instead. A correlation between estimated percentage of cirrhosis present and fat pad size was completed. This led to the finding that there is a slight correlation between estimated percentage of cirrhosis present and pubic fat pad size (Table 2). There was found to be no correlation between percentage of cirrhosis present and substernal fat pad size (Table 2).

Future Research:

The sample size for this study was exceptionally small with only four viable participants. A correlation can better be understood if the sample size is increased. Because samples came from the Gross Anatomy Lab at Misericordia University, the sample size is limited yearly, so this study would be best carried out over an extended period of time. Modifying the histological techniques and procedures used could lead to more favorable outcomes as this could increase the viability of tissues and microscope slide specimens.

AUTHOR CONTRIBUTIONS

This project was co-designed by Victoria Wrobleski and Dr. Anthony Serino, Ph.D. Data collection, histological processing, sectioning, staining, and preparation of microscope slides, imaging of samples, and data summary tables were done by VW. Correlations were interpreted by VW and AS.

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