HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF HEPATIC STELLATE CELLS PRE AND POST-TRANSPLANTED HUMAN LIVER

By

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ABSTRACT

Background: Hepatic stellate cells (HSCs) regulate vitamin A metabolism and play a vital role during the activation of the immune response. During liver damage, HSCs transform to myofibroblast-like cells, leading to loss of their lipid content and synthesis of extracellular matrix (ECM) inducing liver fibrosis.

Objective: The present study aimed to establish the correlation between HSCs activity within different areas of hepatic tissue and the degree of liver fibrosis in individuals with normal liver, hepatitis C virus infected patients and post-transplanted liver.

Patients and methods: The study involved thirty four cases from the international medical center classified into three groups: **group 1** included ten healthy individuals as control, **group 2** included twelve patients with chronic hepatitis C virus and liver cirrhosis and **group 3** included twelve patients with post-transplanted liver due to liver previous hepatitis C virus infection and hepatic cirrhosis subdivided into **group 3a** five patients who received antihepatitis C treatment, and **group 3b** seven patients who didn't receive antihepatitis C treatment. We used H&E and Masson trichrome stains, immunohistochemical detection of á-smooth muscle actin (á-SMA), Glial Fibrillary acidic protein (GFAP) and transmission electron microscopy (TEM).

Results: H&E stain revealed hepatic tissue with preserved architecture in group 1, disrupted architecture and areas of feathery degeneration in group 2, normal tissue histology in group 3a and moderate cellular infiltration in group 3b. Masson trichrome stain revealed normal collagen fibers distribution in group1, advanced fibrosis in group 2, no fibrotic changes in group 3a and few foci of bridging fibrosis in group 3b. Immunohistochemical analysis revealed increased expression of α-SMA in group 2 and group 3b in comparison to group 1 and group 3a. Immunohistochemical analysis revealed increased expression of GFAP in group 3b in comparison to group 3a, group 2 and group 1. TEM showed increase in the fibrous tissue and degeneration in the ultrastructure of the hepatocyte with few lipid droplets in HSCs of group 2 compared to group 1.

Conclusion: The hepatic stellate cells play an important role in the fibrosis of the liver damaged by hepatitis C virus and in the post-transplanted liver which not treated from HCV.

Keywords: HSCs – HCV – fibrosis – post-transplanted liver.

INTRODUCTION

HSCs are cells characterized by the presence of cytoplasmic droplets of fat which contain vitamin A *(Juan et al., 2019)*. Acute and chronic liver cell damage leads to activation and transformation of HSCs into myofibroblast-like cells which are responsible for synthesis of ECM and their loss of intracellular lipid droplets *(Kasztelan-Szczerbińska et al., 2010* and *Ionescu et al., 2013)*.

Activation of HSCs consists of two major phases, i.e. initiation and perpetuation, followed by a resolution phase if the liver injury subsides *(Friedman and Lee, 2011)*. During resolution, loss of activated stellate cells occurs by apoptosis or it becomes senescent or reverts to a quiescent phenotype *(Kisseleva et al., 2012* and *Troeger et al., 2012)*. The apoptosis and necrosis of the parenchymal cells may contribute to the activation of HSCs as well as liver macrophages. Activated HSCs and liver resident macrophages engage other immune cells such as circulating monocytes, T cells and neutrophils by secretion of pro-inflammatory mediators *(Pellicoro et al., 2014)*.

The majority of patients with chronic HCV infection develop hepatic cirrhosis, failure or hepatocellular carcinoma *(Bhatia et al., 2014)*. The history of infection and the disease progression is influenced by various factors such as level of HCV infection, age of onset, sex, co-infection with hepatitis B virus and duration of infection *(Barakat et al., 2013)*. Once the causative agent of damage is removed, the progression of liver disease is attenuated and recovery is a possible scenario even if the patient has state of fibrosis *(Salas Villalobos et al., 2017)*.

End stage liver disease due to hepatitis C virus (HCV) infection remains a leading indication for liver transplantation (LT) worldwide. Recurrent HCV infection is associated with more rapid fibrosis progression leading to higher rates of graft loss and patient mortality compared to patients transplanted for non-HCV etiologies. Re-transplantation for allograft cirrhosis due to recurrent HCV remains controversial *(deLemos et al., 2014)*.

The aim of this study was to establish a correlation between hepatic stellate cells (HSCs) activity and the degree of liver fibrosis in individuals with healthy liver, hepatitis C infected patients, and post-transplanted liver.

PATIENTS AND METHODS

Patients were selected from the international medical center with ages ranged from 20-65 years in the period of March 2017 to April 2019. Liver biopsy specimens were obtained percutaneously, using an automatic Autovac gun with Tru-Cut® needle with a diameter of 18-G. Only samples longer than 20 mm with more than eight portal tracts were included in this study. Informed consent from all patients and controls was obtained in accordance with the local ethical committee.

Thirty four cases were included in this study and classified into 3 groups: group 1 included ten healthy individuals (donor for liver transplantation) serve as a control, group 2 included twelve patients with chronic hepatitis C and liver fibrosis

and group 3 included twelve patients with post-transplanted liver divided into group 3a five patients who received anti-hepatitis C treatment and group 3b seven patients who did not receive anti-hepatitis C treatment.

We used H&E stain for morphological examination, Masson trichrome stain to detect fibrosis, immunohistochemistry of α-SMA, GFAP to detect HSCs activation. We also used a transmission electron microscope to detect the ultrastructure of the hepatocytes and HSCs content. The quantification of hepatic stellate cells was performed in liver biopsies of all groups using Optimas image analysis software. SPSS program (version 16) was used to evaluate the results and assessment of immunoreactivity using the semi quantitative method by determining the percentage of positive cells by Olympus BX50F4 microscope.

Statistical analysis:

Data were collected, revised coded and entered to the statistical package for social sciences (SPSS), version 16. Qualitative data were presented as number and percentages, while quantitative data were presented as mean, standard deviations, median and interquartile range. The comparison between four groups with qualitative data were done using Fisher exact test. The comparison between four groups regarding quantitative data with parametric distribution was done using ANOVA test followed by post hoc analysis for multiple comparison and as regarding quantitative data with non-parametric distribution was done using Kruskal-Wallis test. P-value was considered significant when P was < 0.05.

RESULTS

Hematoxylin and eosin sections:
Group 1: Showed normal liver architecture (figure 1a, b).
Group2: Liver tissues showed cirrhotic nodules and disrupted architecture and areas of feathery degeneration. The portal tracts revealed biliary epithelial dysplastic changes and moderate cellular infiltration (figure 1c). Other tissue samples showed steatosis infiltrated by malignant epithelial cells (figure 1d).
Group 3a: Showed preserved architecture with mild cellular infiltration (figure 1e).
Group 3b: Revealed areas of centrilobular degeneration in the form of marked diffuse macrovesicular and microvesicular steatosis and portal cellular infiltration (figure 1f).
Masson's trichrome sections:
Group 1: Reveled normal collagen fibers distribution (figure 2a).
Group 2: Reveled advanced fibrosis around the central vein and periportal fibrosis (figure 2b).
Group 3a: Reveled no significant fibrosis (figure 2c).
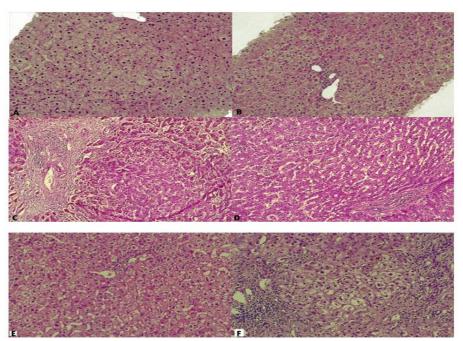Group 3b: Reveled few foci of fibrosis (figure 2d).

Figure (1): Section in the liver showed a, b) Group1showed normal architechire of liver tissue (H and E X 200). c, d) Group2 showed disrupted architecture of liver tissue. The portal tracts reveal biliary epithelial dysplastic changes and mild cellular infiltration (H and E X 200). e) Group3a showed hepatocytes with preserved architecture with microvesicular Steatosis (H and E X 20). f) Group3b showed diffuse macrovesicular and microvesicular steatosis, portal inflammation with cellular infiltration and widening of portal tracts (H and E X 200).
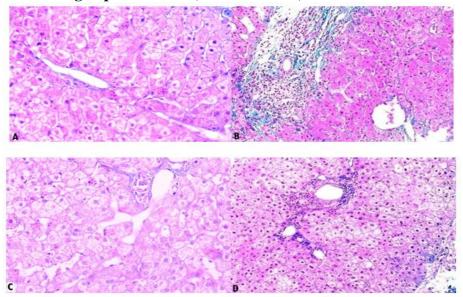

Figure (2): Section in the liver showed a) Normal collagen fibers distrubution in group1 (Masson trichrome stain X 200). b) Massive periportal fibrosis and surrounding central vein in group2 (Masson trichrome stain X 200). c) No fibrotic changes in group3a (Masson trichrome stain (X 200). d) Portal tract with foci of bridging fibrosis in group3b (Masson trichrome stain X 200).

Immunohistochemical results:

Control positive tissue: Tissue samples from surgically excised uterus after hysterectomy were fixed, processed and embedded in the same manner as the liver biopsies and used as positive control for immunohistochemical detection of monoclonal α-SMA (figure 3a). Tissue samples from surgically excised human astrocytoma were fixed, processed and embedded in the same manner as the liver biopsies and used as positive control for the GFAP (figure 3b).

Control negative tissue: Tissue samples from surgically excised lipoma were fixed, processed and embedded in the same manner as the liver biopsies and used as negative control for the monoclonal α-SMA (figure 3c). Tissue sections from liver biopsy were cut and processed by immunohistochemistry without adding GFAP antibodies were used as a negative control (figure 3d).

Alpha Smooth muscle actin:

Group1: Showed negative expression to α-SMA antibody (figure 4a).

Group 2: Considerable expression of α-SMA antibody reflecting fibrosis in tissue samples (figure 4b).

Group3a: Negative expression to α-SMA (figure 4c).

Group3b: Positive expression to α-SMA antibody (figure 4d).

Glial Fibrillary Acidic Protein:

Group1: Showed mild expression to GFAP (figure 5a).

Group2: There was no significant expression (figure 5b).

Group3a: Mild expression of GFAP in (figure 5c).

Group3b: Revealed the highest percentage of GFAP-positive hepatic stellate cells indicating activation of HSCs (figure 5d).

Transmission electron microscopy:

Group1: Ultrathin sections of liver cells revealed normal hepatic architecture (figures 6a and 6b).

Group 2: Showed marked alteration in the normal structure of the hepatocyte. The nuclei of liver cells were irregular in shape with heterochromatin. The cytoplasm was highly vacuolated with a large lipid droplet, the rough endoplasmic reticulum appeared swollen and destructed, the mitochondria were swollen with destructed cristae (figure 7 a). HSCs surrounded by fibrous tissue with a few fat droplets in its cytoplasm. The space of Disse contained collagen bundles and the blood sinusoids (SD) contain Kupffer cell (K) with intended nucleus (figure 7 b).
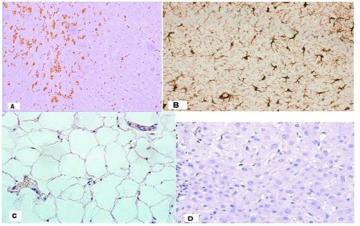

Figure (3): (a) Control positive uterus for α-SMA (X 200). (b) Control positive human astrocytoma tissue to GFAP (x200). (c) Control negative lipoma to α-SMA (x400). (d) Control negative liver tissue to GFAP without adding the GFAP primary monoclonal antibody (x400).
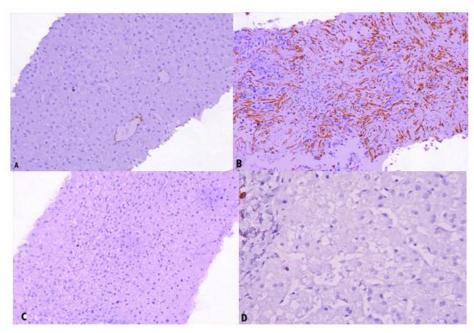
Figure (4): Sections stained by α-SMA antibody showed a) Negative immunoreactivity to α-SMA antibody in group1 (X200). b) Positive staining was distributed widely within the lobule in group 2 (X 200). c) Negative expression to α-SMA in group 3a d) Positive staining distributed in the periphery and within portal tract in group 3b.
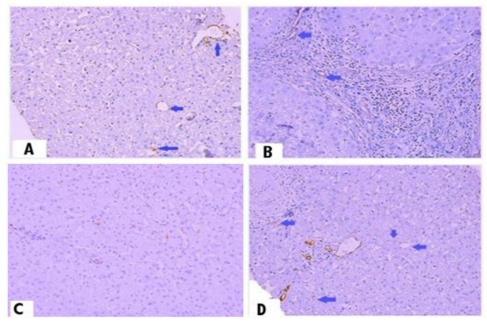

Figure (5): Section stained by GFAP antibody showed a) Mild positive staining (blue arrow) in group1 (X 200). b) Mild positive staining (blue arrow) in group2 (X200). C) Mild positive staining in group3a (X 200). d) Positive staining near to portal tract and along hepatic lobule in group3b (blue arrow) (X200).
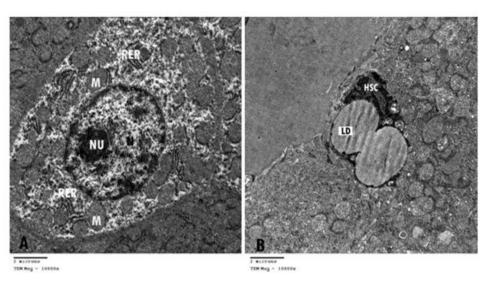
Figure (6): TEM of liver section in group 1 showed a) Normal hepatocyte (H) with the rounded euchromatic nucleus and crowded cytoplasm with cell organelles, mitochondria (M), (RER) and glycogen granules (X10000). b) Stellate cells adherent to the outer surface of the endothelium. The cytoplasm filed with lipid droplets and the nucleus is indented (X10000).

Figure (7): TEM of group 2 showing a) Cytoplasm with a large fat droplet (F). rER appeared swollen destructed. The mitochondria were swollen with destructed cristae. (X10000). b) The cytoplasm appeared pale and highly vacuolated. HSC surrounded by fibrous tissue showing few fat droplets in the cytoplasm and the space of Disse contained many collagen bundles. (K) Kupffer cell with the intended nucleus. (X2500).

Characteristics and age of the studied groups:

The obtained results showed that The mean age of the studied groups among the control was 30.7 years versus (59.25, 55.14& 55.6) years among hepatitis C virus patients, post-transplant without HCV treatment patients and post-transplant with HCV treatment patients respectively. There was a statistical significant difference between the studied groups regard age (table 1).

Table (1): Characteristics and age of the studied groups

Items \ Groups	Control	HCV	Post-transplant without HCV treatment	Post-transplant with HCV treatment	P
Number of patients / group	n=10	n=12	n=7	n = 5	**<0.01***
Age (years) (Mean ± SD)	30.7 ± 7.5	59.25 ± 3.8	55.14 ± 8.7	55.6 ± 4.9	
	P₁value < **0.01*** P₄value= 0.2		P₂value < **0.01*** P₅value=0.9	P ₃value < **0.01*** P ₆value=0.1	

P1 == represent the statistical difference between control and HCV cases

P2 = represent the statistical difference between control and post-transplant without HCV treatment cases.

P3= represent the statistical difference between control and post-transplant with HCV treatment cases.

P4= represent the statistical difference between post-transplant with HCV treatment cases and chronic HCV cases.

P5= represent the statistical difference between post-transplant with HCV treatment cases and post-transplant without HCV treatment cases.

P6= represent the statistical difference between post-transplant without HCV treatment cases and chronic HCV cases.

The obtained results showed that there was a statistical significant difference between means and frequencies of the studied groups regarding fibrosis scale and fibrosis stage respectively (table 2).

Table (2): Difference between studied groups as regard fibrosis

Parameters \ Groups	Control (N=10)		HCV (N=12)		Post-transplant without HCV treatment (N=7)		Post-transplant with HCV treatment (N=5)		P
Fibrosis scale -Mean± S.D. -Median -Interquartile range	0.04 ± 0.05 0.00 (0 - 0.1)		2.4 ± 1.01 2 (1.6 – 3.3)		0.9 ± 0.8 1 (0 – 1.5)		1.2 ± 0.6 1 (0.7 – 1.7)		< **0.01***
	No	%	No	%	No	%	No	%	< **0.01***
Fibrosis stage: No Mild to moderate Sever	10 0 0	100 0 0	0 7 5	0 58.3 41.7	3 4 0	42.9 57.1 0	1 4 0	20 80 0	

The obtained results showed statistically significant increase in area stained by α smooth muscle actin (α SMA) by (40% & 51.6%) in HCV group and post-transplant without HCV treatment group respectively in comparison to the control group.

However, there was a statistically significant decrease in area stained by α smooth muscle actin (α SMA) by 9.8% in post-transplant with HCV treatment group in comparison to the control group (table 3).

Table (3): Immunohistochemical staining of α smooth muscle actin

Groups / Parameter	Control (n=10)	HCV (n=12)	Post-transplant without HCV treatment (n=7)	Post-transplant with HCV treatment (n=5)	P
Area stained by α smooth muscle actin (α SMA)					**< 0.03***
-Mean± S.D.	10.02 ± 5.11	14.1 ± 4.2	9.1 ± 3.1	15.2 ± 3.8	
-Median	11.05	9.7	8.2	14	
-Interquartile range	(5.08 – 13.8)	(10.2 – 18.4)	(7.9 – 12.9)	(12.1 – 18.8)	

The obtained results showed a statistically significant increase in area stained by Glial Fibrillary acidic protein (GFAP) by (12.5% , 87.5% &12.5%) in HCV group, post-transplant without HCV treatment group and post-transplant with HCV treatment group respectively in comparison to the control group (table 4).

Table (4): Immunohistochemical staining of Glial Fibrillary Acidic Protein

Groups / Parameter	Control (n=10)	HCV (n=12)	Post-transplant without HCV treatment (n=7)	Post-transplant with HCV treatment (n=5)	P
Area stained by Glial Fibrillary Acidic Protein (GFAP)					**< 0.01***
-Mean± S.D.	0.8 ± 0.3	0.9 ± 0.2	1.5 ± 0.8	0.9 ± 0.05	
-Median	0.81	0.9	1.1	0.85	
-Interquartile range	(0.4 - 1)	(0.7 – 1)	(1.05 – 1.6)	(0.7 – 0.9)	

DISCUSSION

Chronic hepatitis C leading to end-stage liver disease and one of the most important indications for liver transplantation *(Lin et al., 2014)*. Liver lesions in the hepatitis C virus is attributed to the interaction of several pathways in the host immune response, disruption of pathogen associated pattern and interference with cellular immune-regulation and subversion of natural killer (NK) cell activity *(Kaplan, 2015)*.

In our study, we found a strong relationship between the number of activated HSCs and liver fibrosis in chronic HCV group which was in agreement with most studies that demonstrate the role of HSCs in the development of liver fibrosis *(Săndulescu et al., 2011, Ionescu et al., 2013* and *Bakiera et al., 2016).*

In the present study, significant numbers of α-SMA positive HSCs were detected in patients with chronic HCV infection group, but in normal donors group α-SMA positive HSCs were not detected, this finding was in agreement with *Zakaria et al. (2010)* and *Hirabaru et al. (2014)* whose postulated that the activity of HSCs stimulates the expression of tissue inhibitors of matrix metalloproteinase (TIMP-1 & TIMP-2). This effect leads to the alteration in the balance between the production and degradation of ECM components and its accumulation in the hepatic tissue *(Ionescu A.G. et al., 2013).* We found in our study that α-SMA showed a well-known and reliable mesenchymal marker of HSCs activation this was in correspondence to *Olsen et al. (2011).*

In the present study, GFAP-positive HSCs in post-transplant HCV recurrence positively correlated with fibrosis progression. Moreover, GFAP immunostaining seemed to identify HSCs detectable in liver fibrosis mainly in the early stages compared to α-SMA in fact, the closer to transplantation recurrence was observed, the more diffusely GFAP expression was found this was in agreement with *Busletta et al. (2011).*

Our results for GFAP expression identified resting hepatic stellate cells and there was a high expression in the post transplanted liver not received treatment indicating recurrence of hepatitis C, while in the chronic injury low expression was detected. This result was in agreement with Hassan et al. (2014). Our observation of strong association of GFAP with the gold standard immunohistochemical marker α-SMA suggested that GFAP could be a useful indicator of early HSCs activation in post transplanted liver patients.

HSCs, portal fibroblast, and myofibroblasts have been reported to comprise > 90% of the collagen expressing cells suggesting that they were the major source of collagen expressing cells in the fibrotic liver. HSCs are generally accepted as major contributors to liver fibrosis that give rise to hepatic myofibroblasts in response to liver injury *(Iwaisako et al., 2014).*

In our study, the preportal fibrosis associated with chronic hepatitis C increase number of HSCs as fibrous tissue increased compared with the typical HSCs observed in normal liver. The cells encountered in this pathological condition have fewer lipid droplets and an elongated indented nucleus. This was in agreement with *Nafady et al. (2017)* who have postulated that activation of fat storing cells evidenced by transforming to fibroblast-like cell producing extraordinary amounts of collagen fibers in chronic stages. These fibers were seen around them in space of Disse, and in between the hepatic cells.

In our study, liver transplanted patients without hepatitis C treatment showed an increased amount of activated HSCs compared to patients with liver

transplanted patients with hepatitis C treatment, and normal livers. These data strongly suggest a role for HSCs in the pathogenesis of graft fibrosis and graft loss in hepatitis C recurrence this observation was in agreement with *Terrault (2012)*. The most effective means of preventing HCV recurrence is eradicating HCV prior to liver transplantion.

The newer direct antiviral agents (DAAs) for patients with HCV infection are highly effective, resulting in sustained virological response (SVR) in greater than 90% of patients *(Van der Meer et al., 2012)*. Our study demonstrated that treatment of post-transplanted patients with antihepatitis C treatment played an important role in the prevention of recurrent HCV infection this was in accordance with *Curry et al. (2015)* whose postulated that pre-transplantation treatment of HCV prevents HCV recurrence. Another study by *Ferrarese et al. (2018)* supported our results who have suggested that Hepatitis C virus-related cirrhosis decreased since the introduction of direct-acting antivirals agents.

LIMITATIONS

Limited availability of post transplanted hepatic tissue for TEM because assessment of graft fibrosis in patients with recurrent hepatitis C after liver transplantation was done now by transient elastography and it's considered now good alternative to liver biopsy in post-liver transplantation.

CONCLUSION

The hepatic stellate cells play an important role in the fibrosis of liver damaged by the hepatitis C virus and in the post-transplanted liver which was not

treated from HCV. So, antiviral treatment led to improvement cases of post-transplantion.

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دراسة هستولوجية و هستوكيميائية مناعية للخلايا النجمية الكبدية قبل وبعد زراعة الكبد البشرى

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خلفيــة البحـث: الخلايـا النجميـة الكبديـة هـى التـى تخـزن الـدهون وتـنظم التمثيـل الغـذائى لفيتـامين (أ) وتلعـب دور هـام اثنـاء النشـاط المنـاعى عنـد حـدوث اى ضـررلخلايا للكبـد، ممـا يـؤدى الـى زيـادة فـى نشـاطها ونقـص فـى وظائفهـا الفسـيولوجية وتتحـول الـى خلايـا تشـبه الخلايـا الليفيـة العضـلية التـى تبـدأ فـى تخليـق الانسجة الليفية مما يؤدى الى تليف الكبد.

الهـدف مـن البحـث: الكشـف عـن العلاقـة بـين نشـاط الخلايـا النجميـة الكبديـة ودرجـة تليـف الكبـد فـى المرضـى المصـابين بفيـروس الكبـد الوبـائى سـي و بعـد عمليـة زراعـة الكبد.

مـواد وطـرق البحـث: اشـتمل البحـث علـى اربعـة وثلاثـين حالـة تـم اخـذ عينـات الكبـد منهم من المركز الطبى العالمى تم تقسيمهم الى ثلاثة مجموعات:

المجموعـة الاولـى عشـرة عينـات كبديـة لأشـخاص طبيعـين مـن المتبـرعين بالكبـد ولا يعـانون مـن أى امـراض، والمجموعـة الثانيـة اثنتـا عشـرة عينـة كبديـة لاشـخاص يعـانون مـن التهـاب الكبـدى الوبـائى سـي وتليـف فـى الكبـد والمجموعـة الثالثـة اثنتـا عشـرة عينـة كبديـة ممـن خضـعوا لعمليـة زراعـة الكبـد، وقـد تـم تقسيمهم الـى مجمـوعتين فـرعيتين مجموعـة اخـذت علـاج الكبـد الوبـائى سـي ومجموعـة اخـرى لم تاخذ العلاج .

وقـد تـم أخـذ العينـات مـن اكبـاد الاشـخاص الـذين أجـرى علـيهم البحـث لتحضـيرها وصـباغتها بواسـطة الهيماتوكسـيلين والأيوسـين للكشـف الظـاهرى علـى انسجة الكبـد وخلايـاه وصـبغة الماسـون ترايكـوروم للكشـف علـى التليـف داخـل انسـجة الكبـد. كمـا تـم صـبغ عينـات أخـرى بواسـطة الصـبغات المناعيـة الخاصـة بهـا وهـى

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(الاكتـــين العضـــلى الامـــلس الفـــا والبـــروتين الحمضـــى الليفــى) للكشــف علــى الخلايــا النجميــة الكبديـــة. كمـــا تـــم تحضـــير عينـــات اخـــرى للفحـــص بالميكروســكوب الاليكترونـــى للكشـــف علـــى البنيـــة الدقيقـــة للخلايـــا الكبديـــة. تـــم فحـــص الشـــرائح وتصـــويرها وعمـــل الاحصائيات الحسابية اللازمة لها.

نتـــائج البحـــث: أظهـــرت صـــبغة الهيماتوكســـلين والأيوســـين فـــى المجموعـــه الأولـــى بنـــاء نســـيجى طبيعـــى للكبـــد، أمـــا فـــى المجموعـــه الثانيـــة فقـــد لـــوحظ تغيـــراً وخلـــلا فـــى شـــكل وتركيـــب خلايـــا الكبـــد، بينمـــا فـــى المجموعـــة الثالثـــة لـــوحظ التهابـــات بســـيطة إلـــى متوسطة بالقنـــاة البابيـــة فـــى الاشخاص الـــذين لـــم ياخذوا علاج الكبد الوبائى سي بينمـــا ألاشـــخاص الـــذين اخـــذوا العـــلاج كانـــت خلايـــا الكبـــد طبيعيـــة كمـــا أظهـــرت صـــبغة الماســـون ترايكـــوروم عـــن وجـــود اكبـــر نســـبة تليـــف فـــى المرضـــى المصـــابين بتليـــف الكبـــدى الوبـــائى ســـي وذلـــك بالمقارنـــة بالاشـــخاص الـــذين قامـــوا بزراعـــة الكبـــد ولـــم ياخذوا علاج الكبدى الوبائى سي او الاشخاص الطبيعيين.

وقـــد أظهـــر التحليـــل الاحصـــائى المنـــاعى لعينـــات الكبـــد زيـــادة فـــى مســـتوى المنـــاطق الموجبـــة للصـــبغة المناعيـــة (الاكتـــين العضـــلى الامـــلس الفـــا) للمجموعـــة المصـــابة بفيـــروس إلتهـــاب الكبـــدى الوبـــائى ســـي ويعـــانون مـــن تليـــف فـــى الكبـــد عـــن المجموعـــة التـــى قامـــت بزراعـــة الكبـــد ولـــم ياخـــذوا عـــلاج الالتهـــاب الكبـــدى الوبـــائى ســـي بينمـــا عينـــات الأشـــخاص الطبيعيـــن لـــم يســـجلوا اى منـــاطق موجبـــة للصـــبغة. وكـــذلك أظهـــر التحليـــل المنـــاعى لعينـــات الكبـــد (للبـــروتين الحمضـــى الليفـــى) زيـــادة فـــى مســـتوى المنـــاطق الموجبـــة للصـــبغة المناعيـــة للمجموعـــة الثانيـــة التـــى قامـــت بزراعـــة الكبـــد وكـــذلك لـــبعض الاشـــخاص الطبيعيـــن مـــن المجموعـــة الاولـــى بينمـــا انخفضـــت تـــدريجيا فـــى المجموعـــة المصـــابة بالتهـــاب الكبـــدى الوبـــائى ســـي مـــع وجـــود تليفـــات وتشـــمع بالكبـــد ممـــا يؤكـــد ان هـــذه الصـــبغة المناعيـــة الخاصـــة تعتبـــر مؤشـــر جيـــد للكشـــف الاولـــى عـــن نشاط الخلايا النجمية الكبدية.

وقـــد لـــوحظ بالميكروســـكوب ألاليكترونـــى أن الخلايـــا النجميـــة الكبديـــة يحـــدث بهـــا زيـــادة فـــى الأنســـجة الليفيـــة وانخفـــاض بنســـبة الـــدهون الموجـــودة بهـــا مـــع زيـــادة نشاطها فى الكبد المصاب بفيروس سي عن الكبد السليم.

الاستنتاج: الخلايـا النجميـة الكبديـة لهـا دور محـورى واساسـى فـى عمليـة تليـف الكبـد وذلـك بسـبب التغيـرات التـى تحـدث بهـا وزيـادة نشـاطها كمـا انهـا تلعـب دور مهـم فـى التغيـرات التـى تحـدث بعـد عمليـة زراعـة الكبـد وعـلاج فيـروس الكبـدى الوبـائى سـي قبـل عمليـة زراعـة الكبـد يـؤدى إلـى قلـة نشـاط الخلايـا النجميـة الكبديـة وتجنـب تخليـق نسيج ليفى فى الكبد مما يؤدى الى زيادة عمر الكبد المزروع.