Aberrant Metaplastic Biliary Phenotype Acquired by the Transplanted Hepatocytes in Bilharzial and non Bilharzial Hamsters Spleens

Mona M Magdy[1], Mona N Moharib [2] and Soad A Sherif [2]

Departments of Pathology[1]and Biochemistry & Molecular Biology[2]. Theodor Bilharz Research Institute (TBRI). El Nile St. Warrak ElHadar P.O. Box 30 Imbaba Giza, 12411 Egypt
monaamagdi@gmail.com monamoharib@hotmail.com

Abstract: Background: Hepatocytes transplantation (HCTx) is an alternative method to orthotopic liver transplantation used in treatment of several liver injuries. It can be used either fresh or as encapsulated hepatocytes. Expression of Hep Par 1, CK7 and Albumin were performed to identify and analyze the transplanted isolated adult hepatocytes in hamsters spleens. **Aim:** This work aimed to study the expression of Hep Par1, CK7 and Albumin in intraspelinically transplanted hepatocytes. **Methods**: The study was performed on histopathological archival paraffin sections hamsters spleens having two models of liver injury (CCl_4 induced hepatotoxicity and *Schistosoma mansoni* infection). Histopathological examination achieved. Immunohistochemical expression of Hep Par1, CK7 and Albumin evaluated in intraspelinically transplanted hepatocytes. **Results:** Regarding the phenotype, the intrasplenically transplanted hepatocytes aberrantly expressed the biliary marker CK7 alongside with the hepatocytic marker Hep Par 1. Their expression values were the highest on the 10th day post transplantation with a subsequent gradual decrease along the remaining duration, reaching the least value on the 45th day post transplantation. Regarding the function, all the transplanted hepatocytes kept their hepatocytic function and expressed Albumin. **Conclusion**: Intrasplenically transplanted hepatocytes approved aberrant "metaplastic" biliary phenotype through expression of CK7 without losing its essential hepatocytic phenotype or function by keeping expression of Hep Par1 and Albumin.

[Mona M Magdy, Mona N Moharib and Soad A Sherif. **Aberrant Metaplastic Biliary Phenotype Acquired by the Transplanted Hepatocytes in Bilharzial and non Bilharzial Hamsters Spleens.** *Life Sci J* 2014;11(10):1202-1210]. (ISSN:1097-8135). http://www.lifesciencesite.com. 179

Key words: Hepatocytes, Transplantation, CK7, Hep Par1, Albumin, Aberrant, Metaplastic, Biliary.

1. Introduction:

Transplantation of the whole or part of the liver is currently the only well-established treatment of a spectrum of life threatening liver diseases, including acute liver failure, hepatic cirrhosis, and several inherited metabolic diseases that result from an absence or deficiency of hepatocyte-derived gene products (1). However the chronic shortage of donor livers and parallel growth of transplant waiting list mean that substantial proportion of patients die while waiting for donor graft (2). This situation motivated the development of various alternative methods to conventional liver transplantation such as treatment to alleviate chronic or terminal liver illnesses at least as a temporary procedure until organ procurement is possible (3). The most satisfactory results are obtained from hepatic cell transplantation by using a graft of already differentiated cells such as hepatocytes which means transferring of normal hepatocytes into diseased liver to allow the retention of hepatic functions until procuring an organ and in some cases can achieve hepatic regeneration (4). Hepatocyte transplantation in animals can reconstitute defective hepatic enzymes in metabolic models, improve survival rate in acute hepatic failure and improve liver function in cirrhosis (2). Hepatocytes transplanted in the liver or ectopic sites such as spleen can support liver function in times of hepatic insufficiency (5).The main obstacle for wider usage of hepatocytes transplantation is their rapid elimination by recipient macrophage (6). The microencapsulating technique as a preliminary step toward allogeneic hepatocyte transplantation without immunosuppression initiated by Lim and Sun (7) brought new hopes for artificial liver and hepatocyte transplantation as result of the potential to immunoisolation of these cells retained in a semi permeable membrane that both protects them from immune system and maintain their survival and metabolic function (3).

The immunohistochemistry technique is used in the search for cell or tissue antigens ranging from amino acids and proteins to infectious agents and specific cellular populations. Immunostaining for cell markers represents a way to “talk with cells”, because it allows not only the histological origin of the cell to be identified but also indicates its function *in vivo*, when duly investigated with the correct antibodies (8). One of immunohistochemical parameters used to ensure hepatocyte transplantation is **Hep**atocyte **Par**affin **1** (Hep Par1) which recognizes an epitope localized in hepatocyte mitochondria (9). It is normally positively expressed in hepatocytes and

small intestinal epithelium and negatively expressed in bile duct adenoma and hepatoid adenocarcinoma (10). It is used by pathologists to determine hepatocellular origin, particularly in panel with other markers to differentiate hepatocellular carcinoma from cholangiocarcinoma or metastases to liver (11). Cytokeratins are proteins of keratin containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue (12). The subsets of cytokeratins which an epithelial cell expresses depends mainly on the type of epithelium, the moment in the course of terminal differentiation and the stage of development. Thus this specific cytokeratin fingerprint allows the classification of all epithelia upon their cytokeratin expression profile (13). Keratin, type II cytoskeletal 7 (cytokeratin-7 "CK-7") is a protein that in humans is encoded by the KRT7 gene. It is specifically expressed in the simple epithelia lining the cavities of the internal organs and in the gland ducts (14). CK7 is expressed normally in hepatic progenitor cells (HPCs) and in cholangiocytes but not in normal hepatocytes (15). On the other hand, CK7 expression in hepatocytes may represent activation of hepatic progenitor cells or a regenerative phenomenon of hepatocytes themselves (16) it's expression is believed to reflect a metaplastic change in the setting of cholestasis and loss of hepatocyte contact with the biliary tree (17).

Albumin is synthesized in the liver as pre-Albumin. It has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. Its expression by the mature hepatocytes indicates functioning hepatocytes (18). It confirming that functioning hepatic cells are capable of expressing of Albumin as protein characteristics feature for hepatocytes (19).

2. Material and methods:

This work was done using archival paraffin blocks of hamsters spleens transplanted with hepatocytes, 11 weeks post *Schistosoma mansoni* infection and post hepatotoxicity induced by CCl_4.

1. Hepatocyte isolation, microencapsulation and transplantation:

Fresh hepatocytes were isolated from hamster's liver using Seglen's *in situ* collagenase perfusion technique (20). After isolation of hepatocytes, count and test of viability of yield cells was performed by using 0.4% trypan blue exclusion dye test (21). Isolated cells were microencapsulated in sodium alginate according to the modification of Lim and Sun (7) and Fritschy *et al.* (22) methods. Fresh and microencaosulated hepatocytes were suspended in sterile normal saline in aseptic conditions. Transplantation of one million-cell suspension of each was injected slowly into hamsters spleens (23).

2. Animals:

Male Syrian golden hamsters, average weight 110 gm ± 20, were bred and maintained at the Schistosome Biological Supply Center (SBSC) of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Animals were housed in a controlled temperature and light environment, and were given water and commercial chow *ad libitum*. The animal experiments were conducted at the animal unit according to the international ethical guidelines for the care and use of animals for research purposes.

3. Experimental design:

Two strategies of hepatocytes transplantation:

***A) Fresh hepatocytes transplantation (HCTx)*:** Two models of liver injuries were performed:

1) ***Schistosoma mansoni* infected group**: Cercariae of *S. mansoni* (Egyptian strain) were obtained from infected intermediate host snails maintained at SBSC. Hamsters were infected intraperitoneally with a single dose of 50 ± 5 cercariae / hamster (24). One hundred and five male Syrian golden hamsters, average weight 110 gm ± 20 were divided into 3 main groups: donor group (10 hamsters), normal group (5 hamsters) and schistosome infected group (90 hamsters). This group was subdivided into six subgroups each containing fifteen hamsters at the start of the experiment, one was untreated for 11 weeks. One million of isolated hepatocytes were intrasplenicly transplanted 11 weeks post infection in each hamster after treated with PZQ 7 weeks PI and sacrificed: 7 Days (**G A1-I**), 10 days (**G A1-II**), 15 days (G **A1-III**), 25 days (**GA1-IV**), and 45 days (G **A1-V**) post intrasplenic hepatocytes transplantation.

2) **Hepatotoxicity induced by CCl_4 group**: Forty Hamsters, as model of liver cirrhosis using CCl_4 toxic dose were intraperitoneally injected with 0.02 ml of 10% CCl_4 per 100 gm of body weight for each to induce hepatotoxicity (25, 30). Hamsters were sacrificed 10 days post intrasplenic hepatocyte transplantation (**G A2**). One million of isolated hepatocytes were transplanted 24 hours post CCl_4 injection.

In fresh hepatocytes transplantation, hamsters were treated orally with immunosuppressive drug “cyclosporine” 24 hours before intrasplenic injection.

B) Microencapsulated hepatocyte transplantation (HCTx):

The same schedule was applied to hamsters with the two models of liver injury [*Schistosoma mansoni* infected group (**GB1**) contain 15 hamsters and CCl_4 hepatotoxicity induced group (**GB2**),contain 15 hamsters] using microencapsulated hepatocyte transplantation instead of fresh cells. Isolated hepatocytes were microencapsulated in 4% sodium alginate (ALG) according to Lim and Sun (7) and

Fritschy *et al.* (22).

4. Immunohistochemical assessment:

Immunohistochemical staining was performed on 5-µm, formalin-fixed, paraffin-embedded sections using the Hep Par1, CK7 & Albumin antibodies at 1:50 dilution (DAKO, Carpinteria, CA). Antigen retrieval was performed in all cases by steam heating the slides in a 1-mmol/L solution of EDTA (pH 8.0) for 30 minutes. After blocking of endogenous biotin, staining was performed using an automated immunostainer (DAKO) followed by detection by using a streptavidin-biotin detection system (DAKO). Positive and negative control sections were used for each assay. The mean number of expressed Hep Par1, CK7 and Albumin of transplanted hepatocytes per five high power fields (5HPFs) were calculated for each case (26).

Statistical Analysis

Data were expressed as mean values ± SD by the statistical software package SPSS (version 16.0). Continuous variables are presented, and frequencies with their respective percentages are given for categorical variables. Comparisons between 2 groups were done using the Student's t-test. *P*-value equal to or less than 0.05 was considered significant.

3. Results:

The mean number of viability of isolated hepatocytes that used either in fresh or microencapsulated transplantation was a about 92.97± 1.2%.

A- Assessment of the phenotype of the transplanted hepatocytes:

The transplanted hepatocytes were found in the red pulps in the parafollicular / interfollicular areas as well as in the splenic subcapsular area; all among an epithelioid granulomatous reaction.

Hep Par1, CK7 and Albumin were expressed in cytoplasm of transplanted hepatocytes. The mean number of expressed Hep Par, CK7 and albumin showed in table (1).

Table (1): Albumin, CK7 & Hep Par 1 expression in hamsters spleens in five high power fields.

Groups	Name	Albumin	CK7	Hep Par 1
G A1-I	7 days post (HCTx)	40	38	36
G A1-II	10 days post (HCTx)	45	44	32
G A1-III	15 days post (HCTx)	30	29	20
G A1-IV	25 days post (HCTx)	30	28	19
G A1-V	45 days post (HCTx)	19	17	14
G A2	Hamsters with CCL4 induced hepatotoxicity (0.02 mg/kg) having fresh (HCTx) 10 days post transplantation	35	31	21
G B1.	Shistosoma mansoni infected hamsters with microencapsulated HCTx 10 days post transplantation	24	23	17.2
G B2.	Hamsters with CCL4 induced hepatotoxicity with microencapsulated HCTx 10 days post transplantation	25	23	22

I. The expression of Hep Par1 and CK7 in transplanted fresh hepatocytes into spleens of *S.mansoni* infected hamsters according to the duration of transplant:

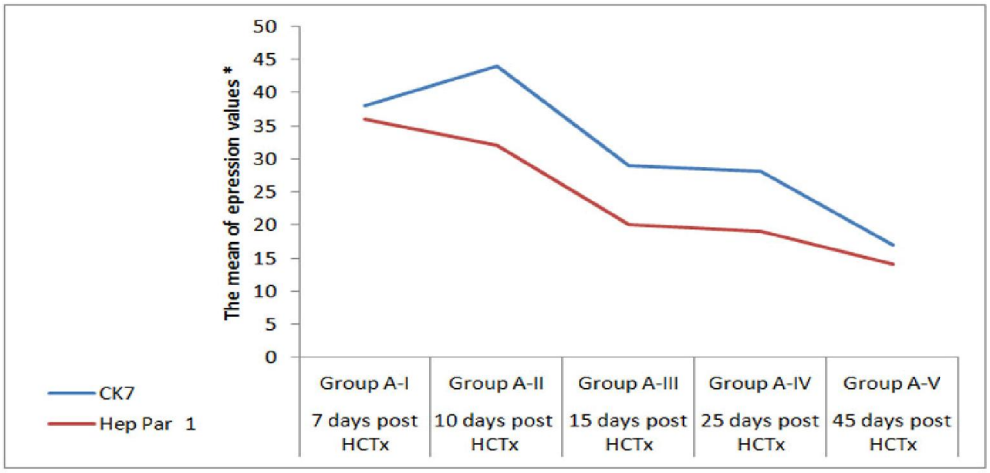

Chart (1): The expression values of both Hep Par 1 and CK7 post different durations of fresh (HCTx) into *S.mansoni* infected hamsters.

* expressed as number in five high power fields (5HPF).

The expression of Hep Par1 and CK7 after fresh hepatocytes transplantation in *Schistosoma mansoni* infected group is presented in chart (1). It represents the relation between the mean number of expression

with the duration of time after hepatocytes transplantation. Their expression values were inversely correlated with post transplantation duration. The CK7 expression values come in conjunction with Hep Par1 expression but in higher values (chart 1& figure 1). Moreover, the expression values of Hep Par 1 & CK7 in *Schistosoma mansoni* infected group are higher than those of CCl_4 induced hepatotoxicity group (chart 2, figure 1).

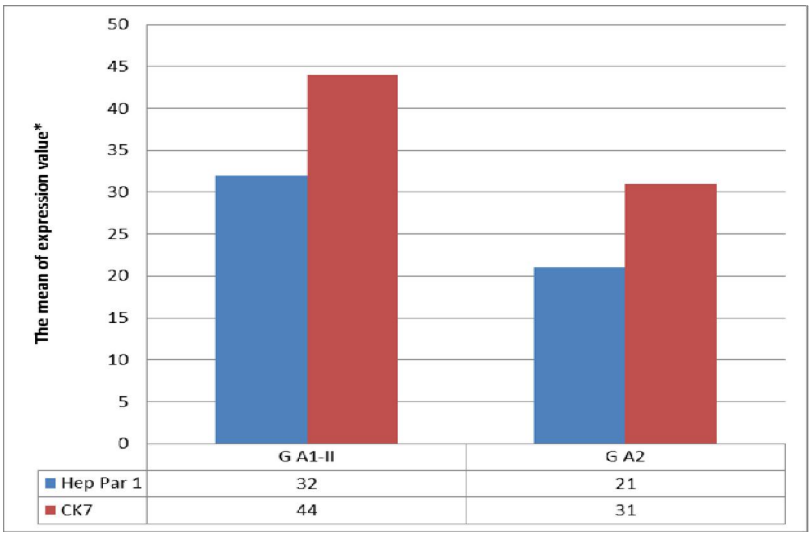

Chart (2): The expression values of both Hep Par 1 and CK7 10 days post fresh (HCTx) in two models of liver injury.
* expressed as number in five high power fields (5HPF).

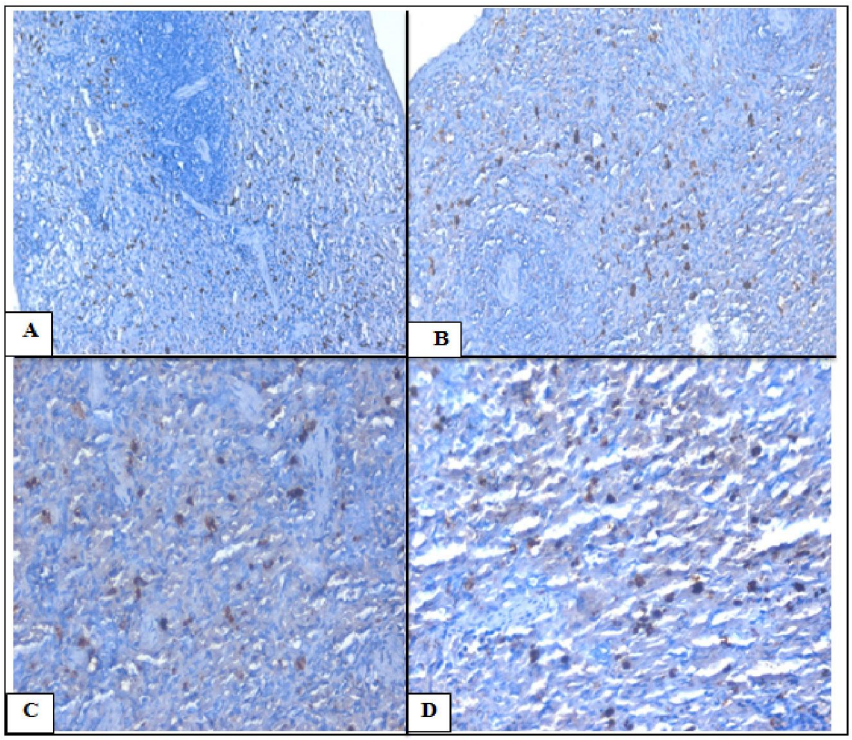

Figure (1): Spleen sections of hamsters transplanted with fresh hepatocytes. **A&C- Expression of CK7** in CCl_4(x200) and *schistosome* (x400) groups respectively. **B&D-Expression of Hep Par 1** in CCl_4 and *schistosome* groups(x200) respectively.

II- The expression of Hep Par1 and CK7 in microencapsulated hepatocytes transplantation in two models of liver injury:

The expression of Hep Par1 and CK7 of two models of liver injury transplanted with microencapsulated hepatocytes for 10 days is nearly equal in both groups, that showed in chart (3) and figure (2).

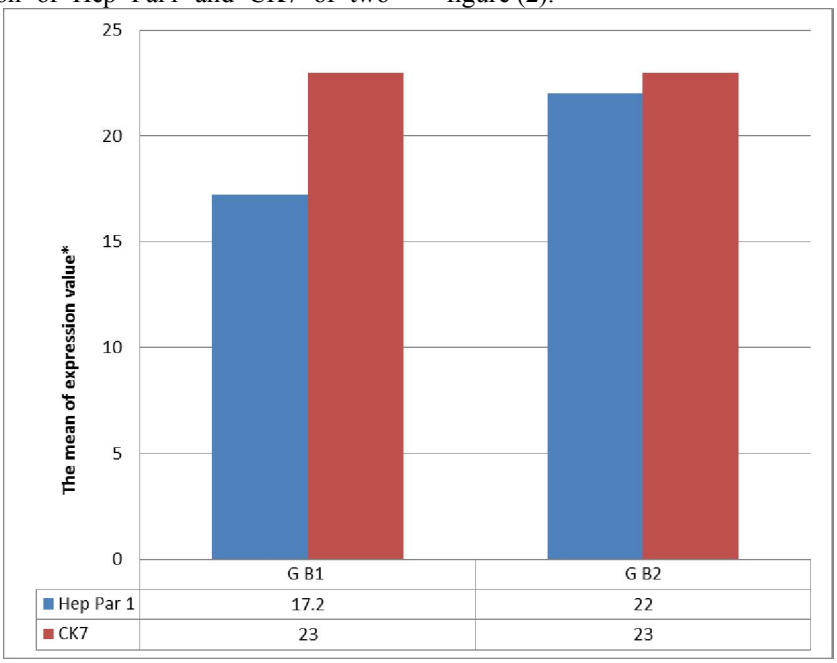

Chart (3): The expression values of both Hep Par 1 & CK7 10 days post microencapsulated (HCTx) in two models of liver injury.

* expressed as number in five high power fields (5HPF).

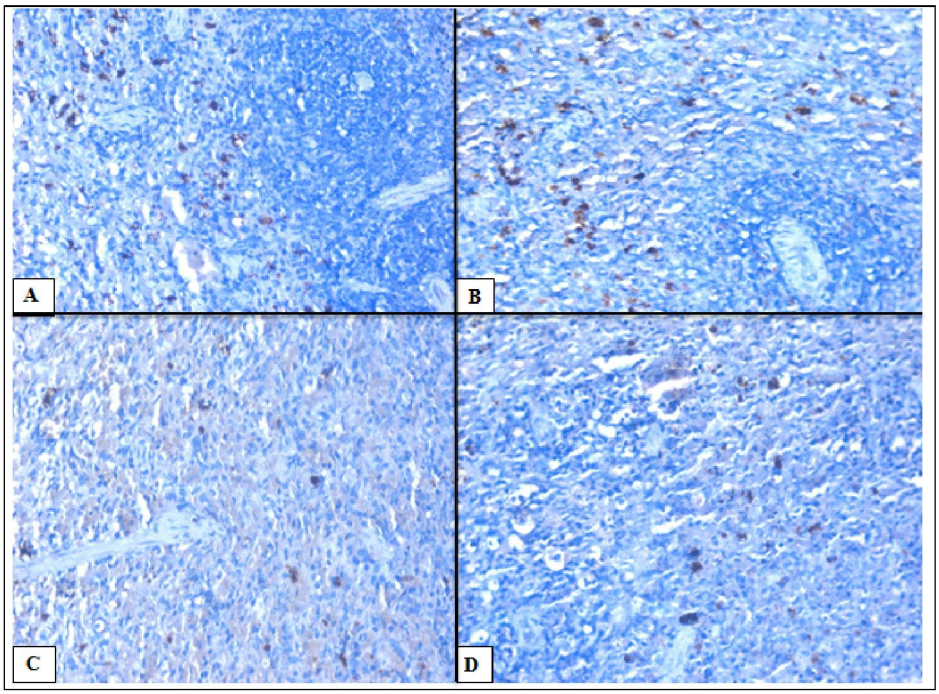

Figure (2): Spleen sections of hamsters transplanted with microencapsulated hepatocytes. **A&C- Expression of CK7** in CCl_4 and *schistosome* groups (x400) respectively. **B&D-Expression of Hep Par 1** in CCl_4 and *schistosome* groups (x400) respectively.

B – Assessment of the function of the transplanted hepatocytes:

The transplanted hepatocytes in all groups either with fresh or microencapsulated transplanted hepatocytes exhibited a positive cytoplasmic expression of Albumin represented in table (1), chart (4) & figure (3).

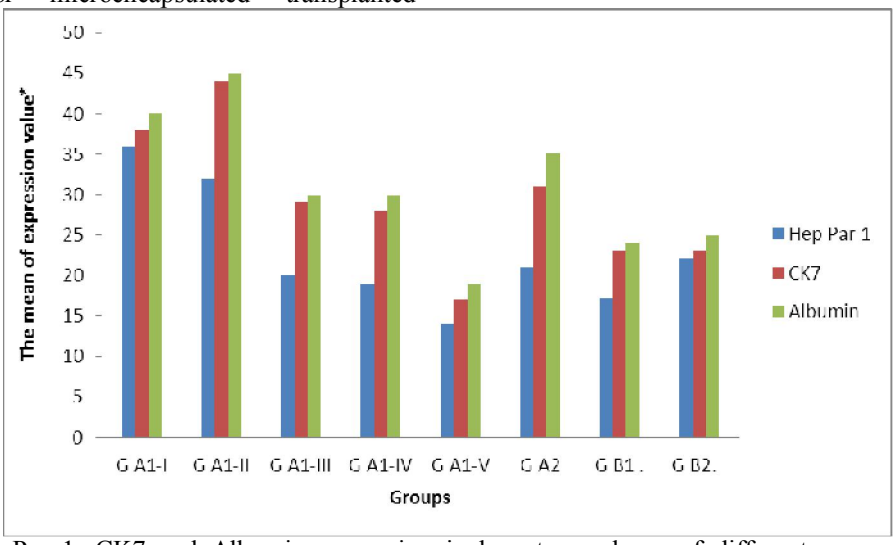

Chart (4): Hep Par 1, CK7 and Albumin expression in hamsters spleens of different groups either fresh or microencapsulated transplanted with hepatocytes.
* expressed as number in five high power fields (5HPF).

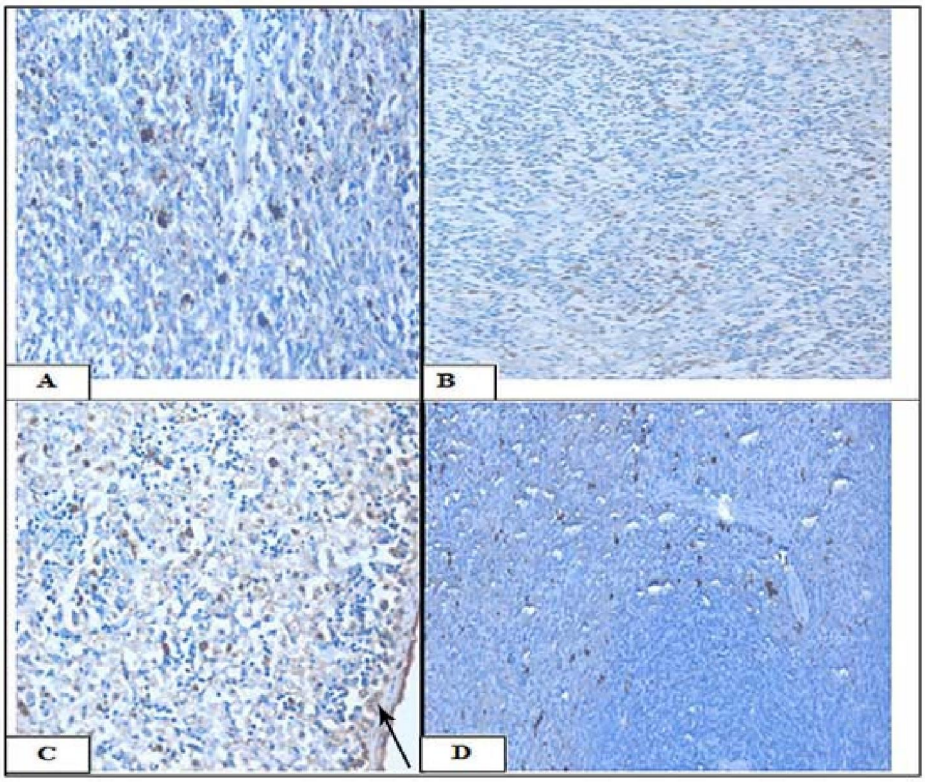

Figure (3): Albumin expression in hamsters spleens 10 days post hepatocyte transplantation. **A &B:** Fresh hepatocytes transplanted in *Schistosma mansoni infected* and CCl_4 induced hepatotoxicity groups respectively (x400). **C&D**: Microencapsulated hepatocytes transplanted in *Schistosma mansoni infected* and CCl_4 induced hepatotoxicity groups respectively (x400). **C:** The transplanted microencapsulated transplanted hepatocytes located in the subcapsular area (arrow).

4. Discussion:

Hepatocytes transplantation is a potential therapeutic modality for overcoming the shortage of liver donors. There are two major problems with allogenic hepatocyte transplantation: protection of transplanted hepatocytes from rejection and stimulation of the rapid proliferation of surviving cells without immunosuppression (27). It alleviates acute and chronic hepatic injury in many studies when they transplanted particularly in spleens (28, 29). These data demonstrate the feasibility and efficacy of fresh or microencapsulated hepatocyte transplantation in the spleen (30).

Spleen is the most appropriate site for transplantation as it provides a rich blood supply to the transplanted cells as well as good access to the liver through the portal circulation without the fear of massive embolization after intraportal injection (31,32). From this perspective, hamsters spleens were the ideal model organ for our study.

Microencapsulation of hepatocytes by Na-alginate provides a three dimensional microenvironment for cellular attachment and proliferation. It separates the external environment by an unidirectional semipermeable membrane preventing immunologic tissue rejection of transplanting hepatocytes. This showed improvement of rats' acute fulminant hepatitis by transplantation of hepatocytes from golden hamsters after treatment either with sodium alginate or with ultraviolet irradiation (33). Accordingly, the intrasplenic transplantation of microencapsulated hepatocytes could provide a temporary liver support and reduce the mortality in *S. mansoni*-infected animals. Its mechanism is not only related to the immunosuppressive and substitution effects of the transplanted cells, but also is associated with liver repair promoted by the transplanted cells (33). Despite of this, our study showed that there is no significant role for the microencapsulation in protection and viability of the transplanted hepatocytes in hamsters spleens.

Hep Par-1 is a hepatocytic marker normally not expressed in spleen (9).

CK7 is a biliary marker usually not expressed by normal non neoplastic hepatocytes or in spleen as well (17). From this point, Hep Par 1 & CK7 were used for phenotypic evaluation. Albumin is considered one of hepatocyte functional products which is not expressed normally in spleen. In this study, Albumin was used for functional evaluation of the transplanted hepatocytes in hamsters' spleens (34).

In the current study, the transplanted hepatocytes were founded in the parafollicular and interfollicular areas as well as in the splenic sub-capsular area; all among an epithelioid granulomatous reaction. This comes align with Mito M and Kusano M findings (35). Moreover, transplanted hepatocytes in spleen expressed high values of Hep Par1 ten days post transplantation. Their numbers decreased gradually by time to reach half of its value 45 days post transplantation. This comes in agreement with Fujikura *et al.*, (36) who stated that when fetal hepatocytes transplanted heterotopically, a mass consisting of hepatocyted and bile duct-like structures founded 7 weeks after transplantation. Isolated adult and fetal hepatocytes were able to survive in the spleen for various periods. After transplantation, these cells might be functionally equivalent to hepatocytes (37).

Furthermore, our study showed that transplanted hepatocytes in the spleen expressed high value of CK7 ten days post transplantation and gradually lowered till half value on the 45th day post transplantation. Watanabe *et al.*; (37) interpreted this by the fact that transplanted hepatocytes can assume the biliary phenotype by transdifferentiation / metaplasia. The positive association between hepatocyte CK7 expression and liver disease stage suggests that the increased extracellular matrix present in severe fibrosis and cirrhosis may produce a survival or maturation factor (38,39).

Regarding the function of the transplanted hepatocytes in hamsters spleens they kept their function which approved by Albumin expression in all groups and in values similar to CK7 expression values. This result agrees with the results of David (2001) (34) who mentioned that serum Albumin production was observed up to 32-49 days post transplantation. In the same context, the Albumin expression was noticed regardless presence or absence of the microencapsulation which comes along with Volpes *et al.* (40) and Wen *et al.* (41) data.

Conclusion:

Intrasplenic transplanted hepatocytes approved aberrant metaplastic cholangiocytic phenotype by expression the biliary marker CK7 in hamsters spleens. Nevertheless, they didn't lose their essential hepatocytic functional expression of Albumin or its Hep Par 1 positive hepatocytic phenotype.

Footnotes:

List of abbreviation

CCl_4	Carbon Tetrachloride
CK7	Cytokeratin 7
HCTx	Hepatocytes transplantation
HepPar1	Hepatocyte Paraffin 1
SBSC	Schistosome Biological Supply Center
TBRI	Theodor Bilharz Research Institute

Acknowledgments:

We acknowledge Fatma Hassan (assistant researcher) at Department of Biochemistry & Molecular Biology. Theodor Bilharz Research Institute (TBRI) for the help in manuscript arrangements.

References:

1. Ohashi K, Park F and Kay MA. Hepatocyte transplantation: Clinical and experimental application. J Mol Med 2001; 79: 617-30.
2. Akhter J, Johnson LA, Gunasegaram A, *et al.* Hepatocytes transplantation: A review of laboratory techniques and clinical experiences, Surgeon 2007; 5: 155-64.
3. Yang YG and Sykes M. Xenotransplantation: Current status and a perspective on the future. Nat Rev Immunol. 2007; 7: 519-531.
4. Eugenia P, Amparo M, Miriam C, *et al.* Hepatic cell transplantation: Technical and methodological aspects. Cir. ESP. 2010; 8787:139-47.
5. Strom S and Fisher R. Hepatocyte transplantation: New possibilities for therapy. Gastroenterology 2003; 124: 568-71.
6. Gewartowska M and Olszewski W. Hepatocyte transplantation biology and application. Ann.transplant, 12: 27-36.
7. Lim F and Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. Science 1980; 210: 908-910.
8. Brandtzaeg P. The increasing power of immunohistochemistry and immunocytochemistry. J Immunol Methods. 1998; 216:49–67.
9. Wennerberg AE, Nalesnik MA and Coleman WB. Hepatocyte paraffin 1: a monoclonal antibody that reacts with hepatocytes and can be used for differential diagnosis of hepatic tumors. Am J Pathol. 1993; 143:1050-1054.
10. http://www.pathologyoutlines.com/topic/stainsheppar1.html.
11. Shiran MS, Isa MR, Sherina MS, Rampal L, Hairuszah I and Sabariah AR. The utility of hepatocyte paraffin 1 antibody in the immunohistological distinction of hepatocellular carcinoma from cholangiocarcinoma and metastatic carcinoma. Malays J Pathol.2006; 28:87-92.
12. Franke WW, Schmid E, Osborn M and Weber K. Intermediate-sized filaments of human endothelial cells. J Cell Biol. 1979; 81: 570–580.
13. Walid MS, Osborne TJ and Robinson JS. Primary brain sarcoma or metastatic carcinoma? Indian J Cancer 2009; 46: 174–175.
14. Leong, Anthony S-Y, Cooper, *et al.* Manual of Diagnostic Cytology (2 ed.) 2013; Greenwich Medical Media, Ltd. p.173.
15. Durnez A, Verslype C, Nevens F, *et al.* The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. Histopathology 2006; Aug; 49(2):138-51.
16. Matsukuma S, Takeo H, Kono T, *et al.* Aberrant cytokeratin 7 expression of centrilobular hepatocytes: a clinicopathological study. Histopathology 2012; 61:857-862.
17. Andrew MB, Robin D, LeGallo, *et al.* Hepatocyte cytokeratin 7 expression in chronic allograft rejection. Am J ClinPathol.2011; 135:238-244.
18. Zhang Z, Gong Y, Guo Y, *et al.* Direct transdifferentiation of spermatogonial stem cells to morphological, phenotypic and functional hepatocyte-like cells via the ERK1/2 and Smad2/3 signaling pathways and the inactivation of cyclin A, cyclin B and cyclin E. Cell Commun Signal. 2013; 11:67.
19. Touboul T, Nicholas R. F, Corbineau S, *et al.* Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology 2010; 51(5): 1754–1765.
20. Seglen PO. Preparation of isolated rat liver cells. Meth Cell Biol. 1979; 13:29.
21. Freshney R. Culture of Animal Cells: A manual of basic techniques. Alan R. LissInc 1987; p. 117.
22. Fritschy WM, Wolters GH and Schilfgaarde RV. Effect of alginate- poly lysine-alginate microencapsulation on in vitro insulin release from rat pancreatic islets. Diabetes 1991; 40: 37-43.
23. Gürden G, Didem K, Banu B, *et al.* Intrasplenic hepatocyte transplantation in rats: A preliminary report. The Turkish Journal of Gastroenterology 2001; 12:1-5.
24. Liang Y, Bruce J and Boyd D. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. Proceedings of the first Sino-American Symposium 1987; 1:34-48.
25. Kumata H, Wakui K and Suzuki H. Glutathione reductase activity in serum and liver tissue of human and rat with hepatic damage.Tohoku J Exp Med 1975; 116:127-132.
26. Sanjay K, Trudie M, Linda MM, *et al.* Immunoreactivity of Hep Par 1 in hepatic and extrahepatic tumors and its correlation with albumin in situ hybridization in hepatocellular carcinoma. Am J ClinPathol. 2003; 119:361-366.

27. Dixit V, Darvasi R, Anthur M, *et al.* Restoration of liver function in gunn rats without immunosuppression using transplanted microencapsulated hepatocytes. Hepatology 1990; 12:1342.
28. Eguchi S, Kamelot A, Ljjubimova J, *et al.* Fulminant hepatic failure in rats: Survival and effect on blood chemistry and liver regeneration. hepatology 1996; 24:1452.
29. Khan AA, Parveen N, Mahaboob VS, *et al.* Management of hyperbilirubinemia in biliary atresia by hepatic progenitor cell transplantation through hepatic artery: A case report. Transplant Proc. 2008; 40:1153-5.
30. Moharib MN, Hammam OA, Salman FH, *et al.* Transplantation of modified and fresh hepatocyte reduces hepatotoxicity induced by Carbon tetrachloride. Life science journal 2014; 11: 641-652.
31. Ponder KP, Gupta S, Leland F, *et al.* Mouse hepatocyte migrate to liver parenchyma and function indefinitely after intrasplenic transplantation. Proc Nat Acad Sci 1991; 88:1217.
32. Gäbelein G, Nüssler AK, Morgott F, *et al.* Intrasplenic or subperitoneal hepatocyte transplantation to increase survival after surgically induced hepatic failure? Eur Surg Res. 2008; 41:253-259.
33. Saber MA, Sherif SA and Nosseir MN. Experimental trial of immunoisolated immunomodulated hepatocyte transplantation in acute fulminant hepatitis. The 6 th annual international congress 2001. The Egyptian society of tropical medicine infectious and parasitic diseases (ESTIP).
34. David P, Alexandre E, Audet M, *et al.* Engraftment and albumin production of intrasplenically transplanted rat hepatocytes (Sprague-Dawley), freshly isolated versus cryopreserved, into Nagase analbuminemic rats (NAR). Cell Transplant. 2001; 10(1):67-80.
35. Mito M and Kusano M. Transplantation of hepatocytes in spleen. Artificial liver support 1992; 296-312.
36. Fujikura Y, Kuniki H, Sawada T, *et al.* Membrane antigen expression of syngeneically but heterotopically transplanted hepatocytes in rats. Cell transplant. 1994; 3:23.
37. Watanabe H, Hata M, Terada N, *et al.* Transdifferentiation into biliary ductular cells of hepatocytes transplanted into the spleen. Pathology. 2008 Apr;40(3):272-6. doi: 10.1080/00313020801911546.
38. Eleazar JA, Memeo L, Jhang JS, *et al.* Progenitor cell expansion: an important source of hepatocyte regeneration in chronic hepatitis. Journal of Hepatology 2004; 41(6): 983- 991.
39. Fotiadu A, Tzioufa V, Vrettou E, *et al.* Progenitor cell activation in chronic viral hepatitis. Liver International 2004; 24(3): 268-274.
40. Volpes R, Van-den-Ord J and Desmet V. Can hepatocytes serve as "activated" immunomodulating cells in the immune response. J. Hepatol. 1992; 16:228.
41. Wen L, Calmus Y, Honiger J, *et al.* Encapsulated xenogeneic hepatocytes remain functional after peritoneal implantation despite immunization of the host.J.Hepatol. 1998; 29:960.

10/12/2014