Transplantation of Human Umbilical Cord Blood Stem Cells in Rabbits' Fibrotic Liver

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Abstract: Background: The prognosis of patients with liver fibrosis is poor as the therapeutic treatment for fibrosis remains inadequate. Liver transplantation is currently the only curative approach, but with the worldwide shortage of donor organs which is likely to increase over the coming decades, research into alternative methods of treatment to whole organ transplantation is essential. Liver cell transplantation and cellular-based therapies are evolving as viable clinical alternatives to whole organ transplantation. The aim of this work was to investigate whether human umbilical cord blood derived CD34+stem cells could improve liver fibrosis in an experimental model. **Materials and methods**: Liver fibrosis was induced in V-Line New Zealand male rabbits using allyl alcohol, then after induction (confirmed by pilot study) CD34+ magnetically purified stem cells derived from human umbilical cord were transplanted directly into the liver through portal vein infusion. All experimental animals were not subjected to any immune system attenuation through the whole study period. Two months later, they were sacrificed for assessment in comparison to a control group with induced liver fibrosis. Assessment was done through liver function tests (serum albumin, total protein, alanine transaminase and aspartate trasnaminase)and histopathological analysis. **Results**: results were statistically analyzed and showed significant improvement of liver functions in stem cell treated group in comparison to the control one. Also statistical analysis of the histopathology using measurements of fibrosis taken by image analysis (software-soft imaging system 2005- analysis life science serious) showed significant regression of fibrosis in stem cell treated group in comparison to the control group. All rabbits survived healthy through the whole study period after the xenogenic transplantation without immune suppression. **Conclusion**: human cord blood derived CD34+ stem cells can be effective as a cell based therapy in liver fibrosis.
[Radwa A. Mehanna, Nihal M. Habachy, Gihan M. Sharara, Mohamed Sharaan, Magda M. El Dakhakhny, Mona K. Marei. **Transplantation of Human Umbilical Cord Blood Stem Cells in Rabbits' Fibrotic Liver.** Journal of American Science 2012; 8(4):83-94]. (ISSN: 1545-1003). http://www.americanscience.org. 12

Key words: CD34+ hematopoietic stem cells, Umbilical cord blood, Liver fibrosis.

1. Introduction

Hepatic fibrosis is the result of the wound-healing response to repeated injury [1] . After an acute liver injury (e.g., viral hepatitis), parenchymal cells regenerate and replace the necrotic or apoptotic cells. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant ECM, including fibrillar collagen[2]. As fibrotic liver diseases advance, disease progresses from collagen bands to bridging fibrosis than to frank cirrhosis[3]. Accumulation of ECM results from both increased synthesis and decreased degradation [4], the latter is mainly due to decreased activity of ECM-removing matrix metalloproteinases (MMPs) due to an over expression of their specific inhibitors.

The regenerative capacity of the adult mammalian liver is immense [5]. Liver regeneration is a misnomer as the liver actually heals by deoxyribonucleic acid (DNA) synthesis and mitosis rather than regeneration in the true sense. The healing process in the liver is characterized by the proliferation of all existing cell lines within the liver, including hepatocytes, epithelial cells that line the canaliculi, endothelial cells, Kupffer and HSCs cells[6].

It is generally accepted that in addition to these cells, the liver contains "stem" cells or liver progenitor cells that can be activated by liver damage [7 ,8].Unique to the liver is that pre-existing mature cells constitute the primary option of response to injury, while progenitor cells function as a reserve compartment that is activated when the regenerative capacity of mature cells is compromised.

In liver diseases as acute zonal , massive hepatic necrosis or chronic liver injury, the ability of the liver to divide and replace dead or dying hepatocytes is reduced , the hepatic progenitor cells that are normally not involved in the regeneration process are activated [9] . It may take years for significant recovery to be achieved; the time varies depending on the underlying cause of the liver injury and its severity.

The prognosis of patients with liver fibrosis is poor as the therapeutic treatment for fibrosis remain

inadequate. Therapeutic efforts aim to diminish the fibrosis progression thus prevent the development of cirrhosis[1]. For patients with cirrhosis and clinical complications, liver transplantation is currently the only curative approach. With the worldwide shortage of donor organs which is likely to increase over the coming decades, research into alternative methods of treatment is essential. Liver cell transplantation and cellular-based therapies are evolving as viable clinical alternatives to whole organ transplantation [10].

Although liver cell transplants are safe and simple, there are not enough donor organs to spare for a procedure that is still experimental and has not been proven to be effective in the long-term. Compounding this to the problem of low cell yield from current liver cell isolation methodologies [11]. It would be of great value if an alternative cell source could be found for transplantation. Stem cells, whether adult or embryonic derived, offer just such a possibility [12] .

Stem cell therapy is an important new era of medicine with the potential of offering viable therapeutic options for debilitating degenerative disease and injury [13] .

Stem cells (SCs) are cells that have two important characteristics which distinguish them from other cell types . First, they are undifferentiated cells that renew themselves for long periods through cell division , resulting in considerable amplification of stem cell numbers Second under certain physiologic or experimental conditions, they can be induced to become cells with special functions such as cardiomyocytes , pancreatic cells hepatocyte etc. [14]

Cord blood contains several types of stem cells including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and multilineage progenitor cells (MLPC) [15]. Stem cells present in cord blood are mainly hematopoietic, they are present in the blood of the umbilical cord during and shortly after delivery, as they migrate from the liver and spleen , where blood-formation takes place during fetal life, to the bone marrow(BM), where blood is synthesized after birth[16] . This process is characterized by the migration and selective homing of HSCs. At birth, cord blood (CB) contains a large number of HSCs their concentration in cord blood per milliliter is at least 100-fold greater than is found in the peripheral blood of a normal adult.

The hematopoietic tissue contains cells with long term, short term regeneration capacities and committed multipotent, oligopotent and unipotent progenitors [17] .

The most primitive HSC is called Extended long term culture initiating cells (ELTC-ICs) which give rise to another primitive cells designated Long term culture initiating cells (LTC-ICs) , both populations are pluripotent ,capable of self renewal and lack colony forming activity . On the other hand, they are the precursors of the colony forming units (lin) which give the Common lymphoid progenitors (CLPs) and the Common myloid progenitors (CMPs). HUCB stem/progenitors have higher proliferative potential, reportedly up to eightfold greater than similar cells in BM, a higher cell-cycle rate and a relatively long telomere length [18] . HUCB stem cells have very long telomeres as they undergo a limited number of cell divisions prior to birth. The length of HUCB cell telomeres is 11-13 kbp, compared with 8-9 kbp in BM cells of 16-59 year old donors [19]. Moreover, HUCB stem cells contain telomerase, the enzyme which elongates telomeres. Thus, telomere-shortening caused by cell replication is further decreased in cells from HUCB [19].

Identification of cells typically relies on use of cell surface markers – cluster of differentiation (CD) antigens - that denote the expression of particular proteins associated with genomic activity related to a particular differentiation state of the cell[20] . The stem cells and early progenitors express the CD34 surface membrane protein, a complex stage-specific molecule. It is an integral membrane glycoprotein of 90-120 kD, and is the hallmark of HSPCs[21]. $CD34^+$ cells present in UCB constitute a very heterogeneous cell population. The vast majority of $CD34^+$ cells express both HLA-DR(a major histocompatibility complex class II) and CD38(a surface antigen present on many leukocytes) antigens [22]. It has been previously demonstrated that the most primitive HSPC lack the expression of HLA-DR and CD38. It is noteworthy that the frequencies of $CD34^+HLA\text{-}DR^-$ and $CD34^+CD38^-$ cells in UCB are higher than in adult BM (e.g., $CD34^+CD38^-$ cells in UCB account for 4% of the $CD34^+$ fraction, compared with only 1% in BM) [23]. This supports the notion that UCB possess a higher proportion of immature HPCs than adult BM.

In this study , human umbilical cord blood CD34 derived hematopoietic stem cells are examined as cell based therapy in experimental model of induced liver fibrosis.

2. Material and Methods:

This study was held on forty New Zealand white male rabbits weighed from 2.2 kg to 3.3 kg purchased from Faculty of Agriculture, Alexandria University. The rabbits were housed in separate cages in a windowed rabbitary. They were fed with a commercial pelleted food in which the minimum content of crude protein was18% while the maximum crude fiber was 14%. The rabbits were fed a

restricted amount of food (140g/day) to keep them in a good condition and not overweight. A minimum temperature of 10°C in winter and maximum of 35°C in the summer were maintained. A period of 12-14 hours of daylight was provided . All experimental procedures were carried out based on the NIH guidelines for care and use of laboratory animals[24].

I- Induction of animal model liver fibrosis

Induction of liver fibrosis was through the intraperitoneal injection of 0.62 mmol/kg b.wt allyl alcohol twice weekly for 8 weeks [25] . A pilot study was done on five rabbits to insure that the given dose of allyl alcohol can induce liver fibrosis in them. Then after induction period the occurrence of fibrosis was tested by liver function tests and histopathological examination and the results were compared to another five normal sacrificed rabbits of the same age, weight and sex.

After proving its success the same protocol of induction was applied to thirty rabbits that were divided after the period of induction into the two following groups:

Group 1: Control group

The control group composed of 15 rabbit (n=15) , which received infusion of 1 ml of stem cell free media, (Iscove's Modified Dulbecco's Media) IMDM in the portal vein over 5 minutes .

Group 2: Stem cell treated group

The stem cell treated group composed of 15 rabbit (n=15), which received infusion of 1 ml of $CD34^+$ cells loaded media (IMDM) in the portal vein over 5 minutes.

2- Collection of human umbilical cord blood:

Human umbilical cord blood was driven from full term pregnant women immediately after their normal vaginal delivery and before the placental separation[26], in Alexandria university "Shatby" hospital.

Every participant in the study received full explanation for the cord blood collection procedure and signed a written informed consent. Participants were considered eligible for the study according to the following exclusion and inclusion criteria [27] .

Exclusion criteria:

1. Family history of gene based disorders.
2. Maternal fever during labour.

Inclusion criterion:

Delivery occurring less than 24 hrs after rupture of membranes

The umbilical cord was double clamped one inch or less apart at the infant's abdomen as soon as possible after the delivery and prior to the expulsion of the placenta. Umbilical cord was wiped with 70% alcohol followed by betadine at the needle insertion site to ensure sterility. The needle insertion site was just above the clamp that remains on the cord. Blood was allowed to flow as much as possible. The collection normally took about 3-5 minutes.

3-Separation of mononuclear cell layer of cord blood

The mononuclear cell layer was separated from whole blood using the density gradient technique with Ficoll- Paque PLUS solution[28,29] (*Biochrome*). Cell were counted using a "Neubauer haemocytometer" tested for viability using the "Trypan blue exclusion method".

4- Purification of $CD34^+$cells:

CD34+ hematopoietic stem cells were purified by their positive selection by the magnetic cell sorting technique [30,31] using the "AutoMACS" (magnatic cell sorter) (*Miltenyi Biotec, Germany*). 5-

Immunophenotyping of umbilical cord blood derived CD34+ cells:

Confirming and testing the success of the purification , and assessing the purified cells viability were done by the flowcytometric immunophenotyping technique [32].Using this technique , an absolute CD34+ viable cells could be determined in order to calculate the needed number of viable CD34+ cells for each transplantation.

6-Characterization of cell morphology:

Monitoring the cells and viewing their morphology were done under the inverted phase contrast light microscope and digital micrographs were taken.(Tissue Engineering laboratories , Faculty of Dentistry, Alexandria University)

7- *In vivo* animal study.

The stem cell treated group received about 5 x 10^5 HCB derived CD 34+ cells in one ml media injection over 5 minutes into the portal vein of rabbits fibrotic liver The portal vein was exposed by an open surgery performed carefully in a sterile environment . Rabbits were kept in intensive care unit after surgery until regaining their consciousness.

The control group was sham operated. No systemic antibiotics nor analgesics were given to the rabbits , only simple disinfection of the incision area with betadine until removing the sutures after seven days. No immunosuppressant was given to the rabbits by any route throughout the study period.

Eight weeks after CD34+ cells transplantation the rabbits were sacrificed for assessment that was held through :

Biochemical analysis

Alanine transaminase,[33] aspartate transaminase,[33] serum albumin,[34] and total protein,[35] (Diamond Diagnostic *,dp International)* levels were measured .

Histopathological analysis

Liver of sacrificed animals was taken and fixed in 10% buffered formalin, embedded in paraffin , sectioned for staining with hematoxylin-eosin (HE)

[36] and Masson Trichrom (MT) to assess fibrosis[37]. Sections were sliced from around the liver helium in all animals study, the section thickness was 5µm. Histopathological analysis was done using image analysis system (Olympus light microscope equipped with the spot digital camera and soft imaging system 2005- analysis life science serious) . All images were studied on a workstation in the Medical Research Institute, Alexandria University.

3. Results:

1- Results of isolation of CD34+ cells from human umbilical cord blood:

About 20 cord blood samples were collected throughout the study period. The average sample volume was 128 ml(range 90-150ml) containing an average 121 x 10^6 MNCs after separation by Ficoll – Paque density gradient centrifugation technique . This revealed an average 3.5x 10^6 CD34+ cells after purification using the magnetic cell sorting technique (auto MACS).

The purity and viability of the CD34+ fraction of all working samples were measured using the flow cytometer which revealed an average of 77% (range 60-84%) pure CD34+ viable cells (Figures1-4).

2- Results of the *in vivo* animal study:

The biochemical and histological results (image analysis) were statistically analyzed The statistical analyses were calculated with the software SPSS (statistical package for social sciences) version 13 and Excel. The statistical significance was set at a 5% (P< 0.05). Descriptive statistics were calculated which included: arithmetic mean [38], standard deviation[39] and median [40] using Mann Whitney test.

a) Biochemical Analysis:

Serum Albumin:

Serum albumin was estimated in all experimental groups. In control group (group 1), its level ranged between 1.6 and 3.0g/dl with mean of 2.3± 0.38 and median 2.4. While in stem cell treated rabbits (group 2) it ranged from 3.0 and 5.1 with mean 4.0± 0.65 and median 4.1.There is significant difference between the two groups as serum albumin in group 2 was significantly increased compared to group 1 (p= 0.0001, Z= 4.65)

Total Protein:

Total protein level was estimated in all experimental groups. In group 1, total protein ranged between 3.6 and 5.6 g/dl with mean of 4.7 ± 0.55 and median 4.8. On the other hand its level in group 2 ranged from 5.8 and 7.5 g/dl with mean 6.3± 0.40 and median 6.3. There is significant difference between the two groups as total protein in group 2 was significantly increased compared to group 1 (p= 0.0001 , Z= 4.67)

Alanine Transaminase:

Alanine transaminase enzyme level in blood was estimated in all experimental groups. In group 1 , the enzyme level ranged between 22 and 90U/l with mean of 54.4± 22.77 and median 59, while group 2 , its level ranged from 3 and 12 with mean 8.1 ± 2.28 and median 8 . There is significant difference between the two groups as alanine transaminase enzyme level in group 2 was significantly decreased compared to group 1 (P= 0.0001 , Z= 4.67)

Aspartate Transaminase:

Aspartate transaminase enzyme level in blood was estimated in all experimental groups. In group 1 , aspartate transaminase enzyme level ranged between23 and 60U/l with mean of 40.7± 11.62 and median 37. On the other hand its level in group 2 ranged from 18 and 5 U/l with mean of 11± 4.11 and median 9. There is significant difference between the two groups as aspartate transaminase enzyme level in group 2 was significantly decreased compared to group 1 (P= 0.0001 , Z= 4.67)

b) Histopathological analysis

The areas showing fibrosis in the liver of all experimental animals were measured .In the untreated rabbits with induced liver fibrosis (group 1) it ranged from 267.65 to 113.03 µm with mean 182.08 ± 43.44 and median 173.94 . While in stem cell treated rabbits with induced liver fibrosis(group 2) the measures of fibrotic areas ranged from 141.38 to 15.05 µm with mean 65.98± 45.94 and median 56.54 which were significantly decreased compared to group 1 (P= 0.0001, Z= 3.824).

Descriptive study

Description of all section taken from all rabbits was done ,the description of two randomly selected images of each group are demonstrated in figures(5-8).

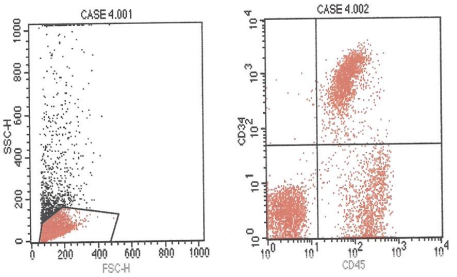

Figure 1: CD34 expression a sample showed 75% pure viableCD34+cells, Clinical Pathology Department, Faculty of Medicine , Alexandria University

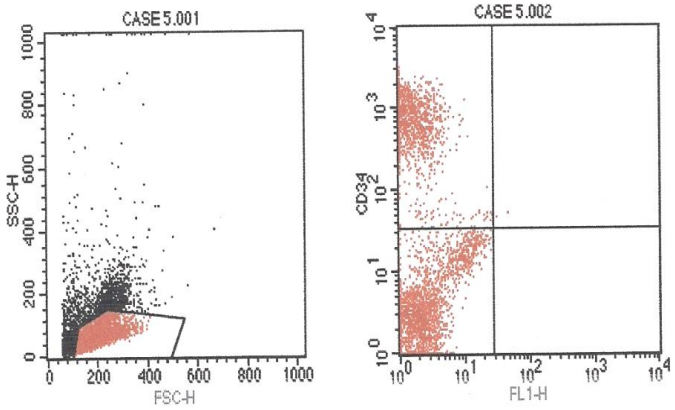

Figure 2: CD34 expression a sample showed 70% pure viableCD34+cells, Clinical Pathology Department, Faculty of Medicine ,Alexandria University

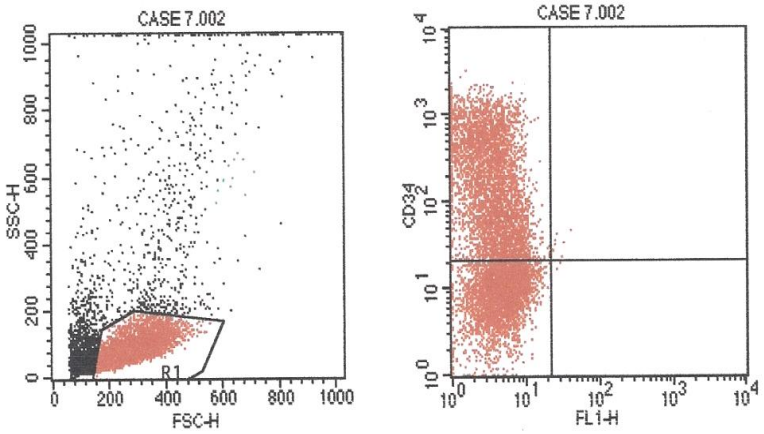

Figure 3: CD34 expression a sample showed 60% pure viableCD34+ cells, Clinical Pathology Department, Faculty of Medicine ,Alexandria University

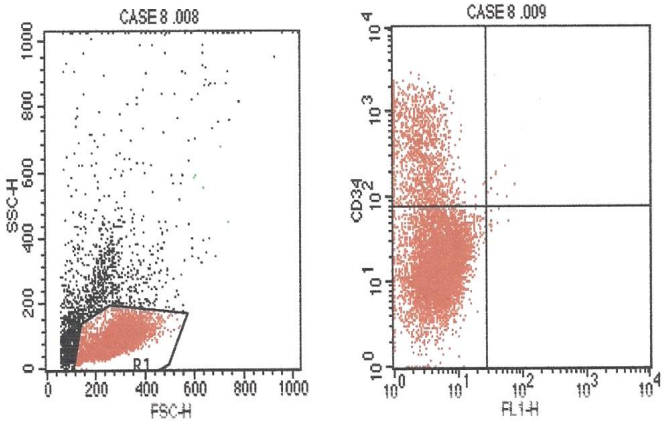

Figure 4: CD34 expression a sample showed 84% pure viable CD34+ cells, , Clinical Pathology Department, Faculty of Medicine ,Alexandria University

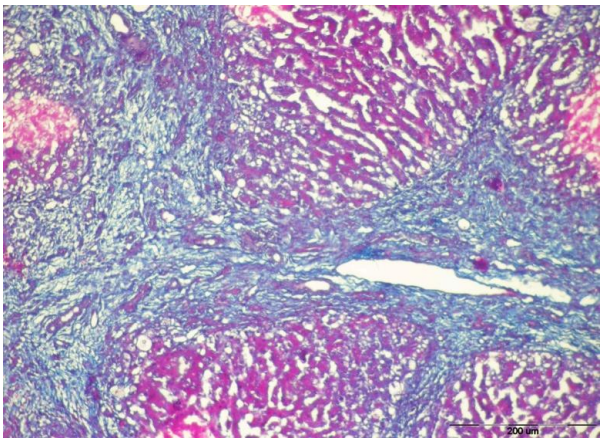

Figure 5: Rabbit liver tissue of group 1. Paraffin section photograph of the Liver tissue of a rabbit from control group (group 1) stained by Masson trichrome stains showing hepatic parenchyma with bridging fibrotic seta(blue) connecting portal tract to another(p-p), also there is dilation of central vein and proliferation of bile ducts. Fibrosis score according to **Ishak scoring system** is 3/6

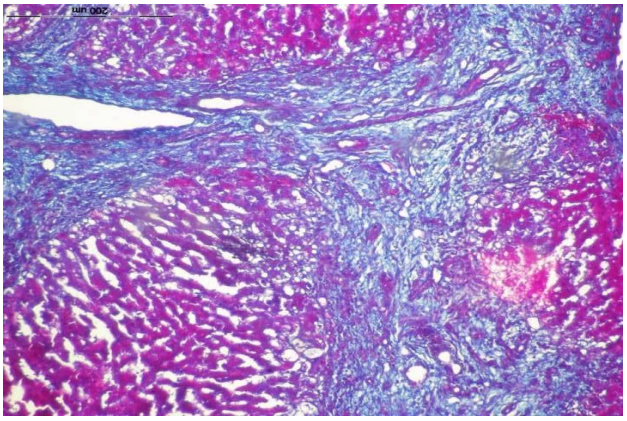

Figure 6: Rabbit liver tissue of group 1. Paraffin section photograph of the Liver tissue of a rabbit from control group (group 1) stained by Masson trichrome stains showing hepatic parenchyma with bridging fibrotic seta connecting portal tract to another(p-p) and to the central vein (p-c) with dilation of blood sinusoids and proliferation of bile ducts. Fibrosis score according to **Ishak scoring system** is 4/6

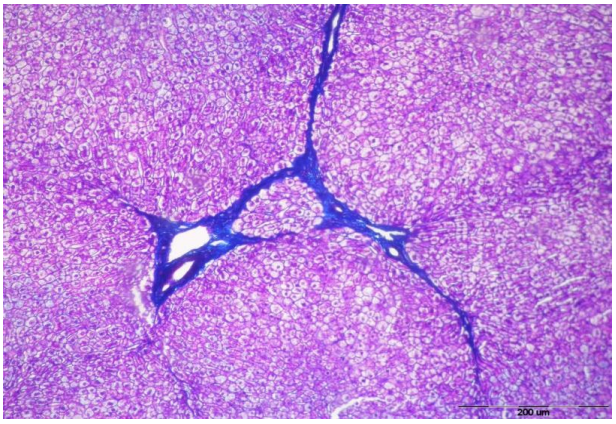

Figure 7: Rabbit liver tissue of group 2. Paraffin section photograph of the Liver tissue of a rabbit from stem cell treated group (group 2) stained by Masson trichrome stains showing hepatic lobules surrounded with connective tissue stroma (blue). Also there is moderate microvascular steotosis and regeneration of the hepatic parenchyma surrounding the periportal and centrilobular areas which appears like the normal morphological structure of hepatocytes of the normal liver tissue of the rabbit .Fibrosis score according to **Ishak Fibrosis score system** 1/6

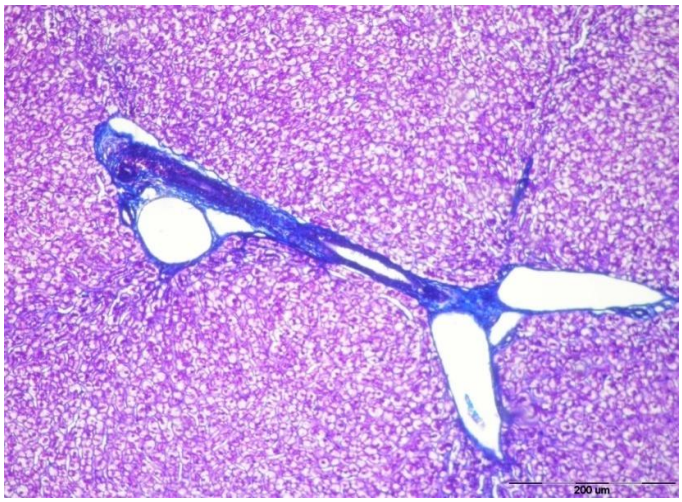

Figure 8: Rabbit liver tissue of group 2. Paraffin section photograph of the Liver tissue of a rabbit from stem cell treated group (group 2) stained by Masson trichrome stains showing three hepatic lobules trios of neighboring hepatic lobules denoting blood vessels surrounded by connective tissue stroma (blue) , observe the restarting of hepatic parenchyma to apparently normal morphological structure of hepatocytes compared to the normal liver tissue. Fibrosis score according to **Ishak Fibrosis score system** 1/6

4. Discussion

Stem cells offer many new opportunities for novel therapeutic approaches in cell based therapy .Cell therapy can be defined as the use of living cells to restore, maintain or enhance the function of tissues and organs. The use of isolated, viable cells has emerged as an experimental therapeutic tool in the past decade, due to progress in cell biology and particularly in techniques for the isolation and culture of cells derived from several organs and tissues [41].

Experimental cell therapy has a longer tradition in hepatology, since it has been known for more than 30 years that isolated hepatocytes infused into the portal vein engraft into the liver cords and express normal cell function [42]. At present, there is growing interest in the therapeutic use of stem cells as they are representing the future of cell transplantation for treatment of advanced liver disease.

Cell based therapy is unlike liver transplantation as the diseased liver is not removed but the cells are transplanted in it . So how these cells could reverse the fibrosis resulted from any disease is a point of concern to many investigators , as hepatic fibrosis and/ or cirrhosis is traditionally thought to be irreversible. However, evidence from animal studies and human clinical observations indicate that even advanced fibrosis is still reversible [43].

The most effective intervention in the treatment of liver fibrosis is to remove the causative agents, such as the utilization of anti-viral therapy and the abstinence of alcohol intake. It may take years for significant recovery to be achieved; the time varies depending on the underlying cause of the liver injury and its severity. It is unlikely to reach a complete return to normal histology. So, the term of "regression" is more relevant to the real situation rather than "reversal" [44]

The challenge in this field is to identify a reliable source of stem cells for transplantation that can be derived by reproducible methods. These cell lines would ideally be highly viable preparations with robust function and engraftment capacity and well-characterized so that results of transplantation can be more readily studied and followed.

In the present study, the umbilical cord blood as a source of stem cells was employed to explore the ability of its derived CD34+ hematopoietic stem cells to survive, engraft and regress drug induced liver fibrosis in rabbits.

We had chosen the umbilical cord blood in our study, for many reasons that make it a more promising source of stem cells in the future than bone marrow and peripheral blood.

Starting with the availability of cord blood[45], the less invasive technique of collection which carries no harm to the donor unlike that of the BM which requires 100–200 punctures in iliac crests under general anaesthesia in the operating room and always associated with acute side effects[46], the same as collection of stem cells from PB which may be less aggressive but still carries many risks to the patient owing to the serious side effects that may result not only form the procedure itself but also from the received G-CSF[47] . Cord blood is collected immediately after the baby is born, which means that HUCB stem cells are among the youngest types of cell that can be isolated from a human being. This is important, as cells from adult donors may have an

impaired DNA quality caused by environmental and endogenous factors during their lifetime. Moreover, the HUCB cells carry a lower risk of viral contamination due to the minimal exposure of donor babies to viruses during prenatal life [48].One of the main advantages of HUCB stem cells is hidden in their core, they have very long telomeres as they undergo a limited number of cell divisions prior to birth, this is in addition to telomerase enzyme that they contain [49].

This is in addition to the most important advantage of cord blood derived stem cells which is their immunological immaturity [50,51] which permits a higher degree of HLA disparity while demonstrating a reduction in the incidence and severity of GVHD compared to other transplantation modalities(bone marrow and peripheral blood)[52]

For the best of our knowledge this is the first study to be held on rabbits, that are considered a larger animal model than mice and rats which are commonly used in other studies investigating the same point. Working on a large animal model, is a step forward for the clinical application of cord blood stem cells in liver fibrosis.

In addition , it was observed by our team that in other studies the stem cells xenogenic transplantation take place in immune deficient animal models as the non obese diabetic-severe combined immunodeficient (NOD/SCID). In our study the rabbits did not receive any immunosuppressant or undergo irradiation to attenuate their immune system through the whole study period.

The present study was held in two phases; the first was the isolation of CD34+ cells from human umbilical cord blood , the second was investigating the effect of these cells transplantation in a drug induced liver fibrosis animal model.

The first phase , the mononuclear cell layer was separated from the human umbilical cord blood using the density gradient centrifugation technique "Ficoll Paque", then CD34+ hematopoietic stem cells were purified by their positive selection using the magnetic cell sorting technique " autoMACS" . Confirming and testing the success of the purification , and assessing the purified cells viability were done by the flowcytometric immunophenotyping technique .Using this technique , an absolute CD34+ viable cells could be determined in order to calculate the needed number of viable CD34+ cells for each transplantation.

The purification of cells for transplantation was done in several studies as Tanabe et al [53] ; Nonome et al [54] Wang et al [55] and Kawamoto et al[56]

The transplantation of purified cells means the transplantation of a known cell phenotype , unlike the mononuclear cells transplantation which include known and unknown cell types that may influence the engraftment of cells , as graft characteristics are the most important parameters in determining engraftment. Earlier, nucleated cell dose was the parameter most widely assessed,[57] for engraftment .Recently, CD34 count seems to be a better quantitative indicator than nucleated cell count [58] . Moreover , mononuclear cells transplantation may result in unwanted effects caused by cell populations other than stem cells.

The second phase of this study was the *in vivo* study ,where 4 x 10^5 HCB derived CD 34+ cells in one ml media were infused over 5 minutes into the portal vein of rabbits fibrotic liver that was induced by allyl alcohol injections for two months. The portal vein was exposed by an open surgery performed carefully in a sterile environment. Eight weeks after CD34+ cells transplantation the rabbits were sacrificed to assess the goal of the study. The assessment was done through biochemical and histopathological analysis.

Our results showed significant increase in serum albumin and total protein level in stem cell treated group. On the other hand, serum alanine transaminase and aspartate transaminase level were significantly decreased in them.

The histopathological analysis using image analysis system (Olympus light microscope equipped with the spot digital camera and soft imaging system 2005- analysis life science serious) showed that the CD34+ HUCB derived cells have a significant antifibrotic effect as evidenced by the decrease in liver collagen deposition stained with Masson's trichrome in stem cell treated group.

Stem cell therapy for the regression of liver fibrosis was investigated by a number of researchers that used different types and sources of stem cells in other animal models. The usage of **bone marrow mesenchymal stem cells (MSC)** for autologus transplantation was demonstrated by Aziz *et al.*[59] Sakaida *et al.* [60] Luk *et al.* [61] *et al.* [62] Zhao *et al.* [63] and Terai *et al.*[64] all showed that MSC have antifibrotic effects in injured liver of animal models of liver fibrosis

Other researchers investigated the effect of **hematopoietic stem cells derived from bone marrow** in mice or rat fibrotic liver and came out with the same results(Jang *et al.* [65] ;Terai *et al.* [66] Khurana *et al.* [67].

Human umbilical cord blood derived stem cells were also investigated A number of previously published studies[68-73] have reported the capacity of HUCB cells to give rise to hepatocytes in mouse liver, they used different cell populations in different liver injury models .

The exact mechanism by which stem cells can ameliorate liver fibrosis is not clear. But, via parallel developments in liver cell biology and stem cell biology, it seems likely that a cell based approach to liver fibrosis may become a reality within the next coming years.

Previous studies[74,75] proposed if a new protocol based on stem cell infusion following liver damage in the absence of irradiation and impairment of the immune system will represent a step forward for the clinical application of stem cell transplantation .Our study adopted this proposal, as the experimental animals in this study survived the whole period after human umbilical cord derived stem cells transplantation in a completely healthy life without even minor manifestation of any abnormal condition This proves the ability of the xenogenic transplantation of human umbilical cord derived stem cells and not only the allogenic transplantation with more HLA mismatches.

Conclusion

- CD34+ hematopoietic stem cells can be successfully isolated from human umbilical cord blood using the magnetic cell sorting technique with high degree of purity and viability .
- The purified viable human umbilical cord blood CD34+ stem cells are able to survive and engraft in fibrotic liver and thus produce a regression of liver fibrosis and improvement in the impaired liver functions .
- The ability of the xenogenic transplantation of human umbilical cord derived stem cells without impairing the immune system of the experimental animals .This proves the immunological immaturity of cord blood cells and gives the availability of stem cell transplantation to any patient with better toleration across the HLA barrier ,which overcomes the problem of compatible donor limitations.

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