IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

The Relationship between the Number of Stem Cells and the Concentration of Stromal Cell-Derived Factor-1 with Disease Severity in Patients with Liver Cirrhosis

Ali Mansoursamaei[1], Zahra Forough[2], Amir Mohammad Sadeghi[1], Hani Khalili[1], Hamid Vahedi[3], Amir Atashi[4]

[1]Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[2]Khorasan Razavi, Mashhad, Iran
[3]Imam Hossein Hospital, Shahroud University of Medical Sciences, Shahroud, Iran
[4]Stem Cell and Tissue Engineering Research Center, Shahroud University of Medical Sciences, Shahroud, Iran

Corresponding Author: Hamid Vahedi, Imam Hossein Hospital, Shahroud University of Medical Sciences, Shahroud, Iran
E-mail: vahedi@shmu.ac.ir

Received: 27, Dec, 2023
Accepted: 02, Jul, 2024

ABSTRACT

Background: The chemokine stromal cell-derived factor 1 (SDF-1) is important in tissue repair. In this study, we aimed to investigate the relationship between the number of stem cells in the blood and the blood concentration of stromal cell-derived factor 1 with disease severity in cirrhotic patients.

Materials and Methods: In this study, 15 patients with cirrhosis and 15 healthy individuals aged 18 to 65 were randomly selected between January 2016 and July 2017. The number of circulating stem cells and SDF-1 levels were compared in the patient and healthy control groups. The correlation between circulating stem cells (CSC) and SDF-1 concentration with disease severity was evaluated.

Results: 33% of cirrhotic patients were classified as severity B and 67% as severity C by the Child-Pugh method. The percentage of stem cells and mean SDF-1 concentration in patients with cirrhosis was approximately 2.8 ($p < 0.01$) and 1.81 ($P < 0.01$) times higher than healthy individuals, respectively. Patients with a more severe form of the disease had significantly higher concentrations of SDF-1 in peripheral blood than patients with a milder form ($p=0.04$).

Conclusion: The percentage of stem cells and the concentration of SDF-1 in the serum of cirrhotic patients were significantly higher compared with the control group. In addition, there was no significant relationship between the percentage of circulating stem cells and the severity of the disease, whereas a direct relationship between the severity of the disease and the concentration of SDF-1 was observed.

Keywords: Stem cell; SDF-1; Cirrhosis; CD34+

INTRODUCTION

The liver is one of the body's most important organs because of its functions, such as metabolism and excretion of body toxins [1]. Chronic liver diseases cause cirrhosis and liver failure due to infections, alcohol abuse, and metabolic disorders[2, 3]. 3.5% of deaths in the world are due to cirrhosis[4]. The pathological appearance of cirrhosis is caused by nodule formation, a decrease in cell volume and liver function, fibrosis, and blood flow disorders. Long-term liver disease leads to end-stage liver disease (ESLD), and the only treatment is transplantation[5]. However, transplantation is not always available in most countries and is expensive [6].

The main reason for using stem cell-based therapies is to restore liver function by reactivating its tissue[1]. Hepatocyte-like cells (HLC) are ideal in curing liver diseases[7]. HLC can be induced in vitro and in vivo by manipulating hematopoietic, induced pluripotent, and mesenchymal stem cells (MSCs)[8]. Moreover,

among these cells, MSCs showed the highest similarity and the least immunological effects in the body[9]. They can regenerate, turn into different cells, and be taken from different parts, such as bone marrow [10].

Chemokine stromal cell-derived factor 1 (SDF-1) or CXCL12 is expressed only in healthy liver tissue, although its expression increases in acute or chronic liver injury. Its major secretion sites are liver sinusoidal endothelial cells (LSEC), hepatic stellate cells (HSC), and malignant hepatocytes. This chemokine confirms proinflammatory responses in chronic injury by enhancing the progression of interactions with liver fibrosis, and it regulates the transport of HSCs in BM during fetal and adult hematopoiesis[11,12]. In addition, SDF-1 has an important function in tissue repair. It has been shown that the lack of repair of tissues, especially skin tissues in diabetic patients, is due to the decrease in the ability to produce and secrete this factor [13-15]. In addition, SDF-1 plays an important role in the mobilization, implantation, survival, and proliferation of hematopoietic stem cells[14]. Hematopoietic stem cells are most abundant in the bone marrow and found in the fetal liver and bone marrow, umbilical cord blood, adult bone marrow, and peripheral blood. These cells can be identified by CD34+, CD38-, Lin-, c-Kit+, and Thy-1+ [16-18].

Hepatic stellate cells (HSCs) are the majority of stem cells in the bone marrow and express CD34+ as a cell surface marker. These cells can self-regenerate and differentiate into progenitor cells. They are known to be liver fibrosis markers [9]. Due to tissue injury, HSCs can be easily obtained from bone marrow and circulate in the blood [20]. In this study, we sought to investigate the relationship between the number of stem cells in the blood and the blood concentration of stromal cell-derived factor-1 with disease severity in cirrhotic patients.

MATERIALS AND METHODS

Patient population and study design

For this study, 15 patients with cirrhosis and 15 healthy individuals aged 18 to 65 years were randomly selected. All patients were selected between January 2016 and July 2017 among patients with liver cirrhosis referred to Imam Hossein Education, Research and Treatment Center in Shahroud. This study was approved by the Ethics Committee of Shahroud University of Medical Sciences with the Code of Ethics "20040" with informed consent. All patients had liver cirrhosis based on clinical, biochemical, and radiological findings, regardless of the cause of cirrhosis. Patients with pregnancy and vital organ problems were excluded from the study. Patients with other diseases unrelated to cirrhosis, such as hepatocellular carcinoma or cancer, including bone marrow malignancies, active infections, history of diabetes, and uncontrolled hypertension, were also excluded. None of the participants took immunosuppressive drugs for at least 4 weeks before participating in the study.

Flow Cytometry and ELISA

Blood samples were collected in tubes containing EDTA anticoagulant. 100 µL of anticoagulated peripheral blood was stained with 5 µL of CD45 conjugated fluorescein isothiocyanate (FITC) (BD Biosciences) and CD34 conjugated phycoerythrin (PE) (BD Biosciences) monoclonal antibodies for 30 minutes at 25 °C in the dark. Then, the blood was lysed with lysis buffer (Biolegend) for 10 minutes. After washing with PBS, cells were fixed with 1% paraformaldehyde until flow cytometric analysis. Then, the cells were analyzed by Attune™ NxT Flow Cytometer flow cytometer (Thermo Fisher Scientific Inc.). At least 50,000 events were recorded and subsequently analyzed using FlowJo software version 7.3 (TreeStar). The frequency of CD34+CD45dim events in peripheral blood was considered circulating stem cells.

The blood concentration of SDF-1 was determined using the SDF-1A Elisa Mini Kit (Peprotech) according to manufacturer instructions.

Statistical analysis

FlowJo X software was used to analyze the results and draw graphs. The number of circulating stem cells and SDF-1 concentration were compared in the patient and healthy control groups. The correlation between circulating stem cells (CSC) and SDF-1 concentration with disease severity was evaluated using the chi-square statistical method. Data were

analyzed using SPSS software (v. 23.0). Severity grading was performed according to the Child-Pugh method for examining disease severity and determining prognosis and mortality [21].

RESULT

The study included 15 healthy subjects and 15 patients with cirrhosis. 47% of the patient and 44% of the healthy groups were male. 46% of the participants were between 51 and 54 years old. The demographic data of the cirrhotic patients are summarized in Table 1. Of these patients, 33% were classified as severity B and 67% as severity C by the Child-Pugh method.

Table 1: Demographic information of 15 cirrhotic patients

	Gender	Age	Cause of Cirrhosis	Esophageal varices	Ascites	Hemoglobin(gr/dl)	Platelet ($\times 10^3$)	Grading based on child-pugh
1	Male	56	HBV	+	+	7.8	82	C
2	Female	62	HBV	+	+	8	71	C
3	Male	57	Cryptogenic	+	-	9.5	96	C
4	Male	59	Cryptogenic	+	+	8.5	68	C
5	Female	52	HBV	+	+	7.5	87	C
6	Female	55	HBV	+	-	9	78	C
7	Male	60	Cryptogenic	-	-	9.8	100	B
8	Female	56	Cryptogenic	-	-	9	111	B
9	Female	53	Cryptogenic	-	-	8.8	90	B
10	Female	51	Cryptogenic	+	+	10	85	C
11	Male	65	HBV	+	+	10.5	75	C
12	Male	58	HBV	+	+	11	81	C
13	Female	60	Cryptogenic	+	-	8	10.2	C
14	Female	55	Cryptogenic	-	-	8.9	91	B
15	Male	58	Cryptogenic	-	-	9	84	B

The percentage of stem cells in the peripheral blood circulation of cirrhotic patients and healthy people is shown in Figure 1. In healthy people, the percentage of stem cells was 0.046%, but in patients with cirrhosis, it was 0.13%, about 2.8 times more than in healthy people ($p < 0.001$). An example of the flow cytometry graphs related to the measurement of CD34+ stem cells and WBC in the peripheral blood circulation in patients with cirrhosis and healthy people is shown in Figure 2.

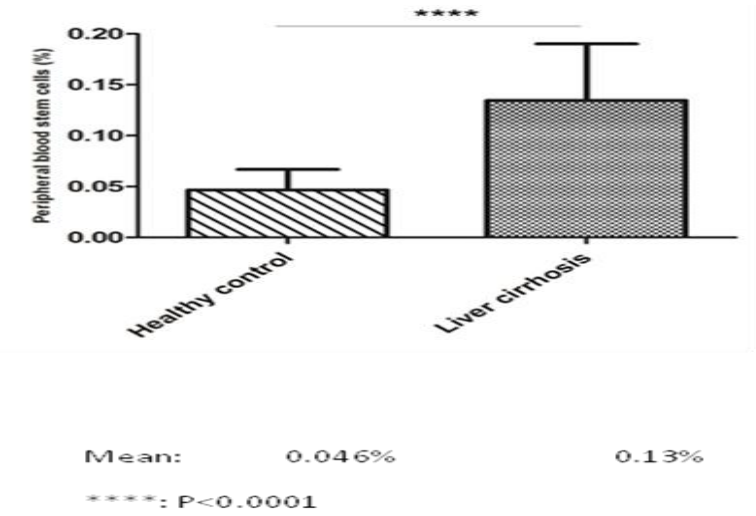

Figure 1. percentage of circulating stem cells in cirrhosis patients and healthy control

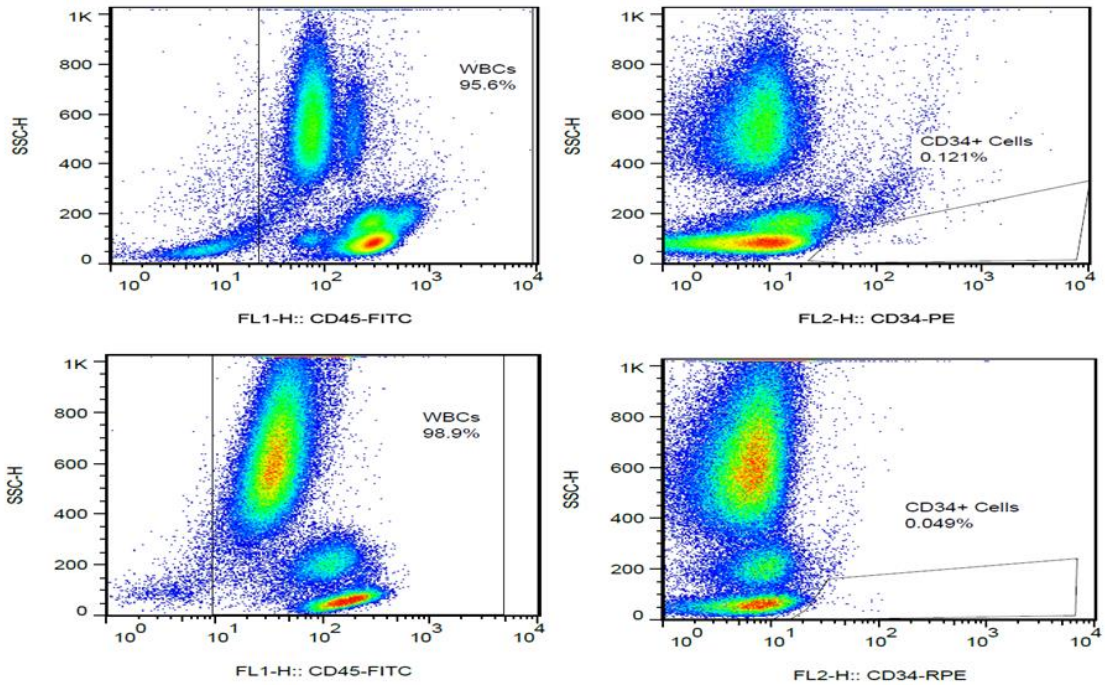

Figure 2. Flowcytometry charts of CD34+ stem cells in patients with cirrhosis and healthy control blood circulation

It was also found that the concentration of SDF-1 was higher in cirrhotic patients than in healthy individuals (Figure 3). It is shown that the average SDF-1 concentration in cirrhotic patients was 703.9 pg/ml, while in the healthy group, it was 387.1 pg/ml, 1.81 times higher ($P < 0.001$).

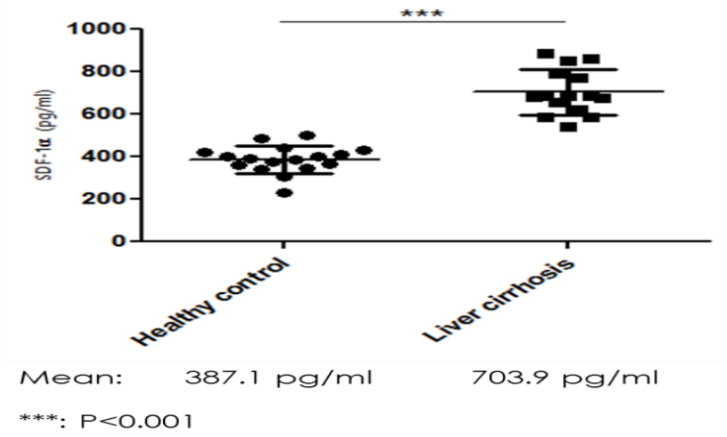

Figure 3: Average SDF-1 concentration in groups of healthy individuals and patients

Correlation analysis between the percentage of circulating stem cells and disease severity showed no significant correlation between disease severity and the percentage of circulating stem cells in cirrhotic patients (P=0.46). In other words, the number of circulating stem cells is probably not a determining factor for the severity of cirrhosis. However, statistical analysis showed that more severe disease increases SDF-1 levels (Table 3). It was found that patients with a more severe form of the disease had significantly higher concentrations of SDF-1 in peripheral blood than patients with a milder form (p=0.04).

Table 3: The result of the Chi-Square test to determine the association between the concentration of SDF-1 and CD34+ stem cells with the severity of cirrhosis

	Child B (N = 5) Mean ± SD	**Child C (N = 10) Mean ± SD**	**P value**
SDF-1	650.2 ± 45.5	721.4 ± 109.1	0.04
CD34+	0.142 ± 0.061	0.131 ± 0.055	0.46

DISCUSSION

Cirrhosis can result from various causes, such as hepatitis B and long-term alcohol consumption. Stem cells are very important in repairing human tissue, especially the liver. Korbling et al. have shown that hepatocytes and epithelial cells related to the transplant donor have appeared in liver tissue, skin, and digestive tract in patients receiving peripheral blood stem cell transplantation from the recipient. This shows that circulating stem cells can transform into various adult cells, including liver tissue cells (22). Therefore, it was hypothesized that due to liver tissue damage and lack of replacement of new hepatocyte cells, the number of circulating stem cells in cirrhotic patients might be lower than in healthy people. However, our result showed that the number of stem cells was higher in cirrhotic patients than in healthy people. This contradiction might be because the liver itself cannot repair and reconstruct itself using stem cells due to chronic inflammation. In other words, more stem cells are mobilized from the bone marrow in response to liver tissue damage, but probably the stem cells are unable to be implanted into the liver because of chronic inflammation, or they become apoptotic after implantation because of the unsuitable microenvironment of the liver (23). Consistent with our results, Kaur et al. and Dalakas et al. have shown that a subset of stem cells characterized by CD34+, CD133+, CD31+ is increased in patients with alcoholic cirrhosis (24). The statistical analysis results showed no correlation

between the severity of the disease and the number of circulating stem cells. And since the number of stem cells increased in the peripheral circulation and there was no correlation between this number of stem cells and the severity of cirrhosis, the mobilization of stem cells does not seem to contribute to the pathogenesis of cirrhosis.

SDF-1 can be produced and secreted by all tissues of the body, especially damaged cells, and it causes the migration of stem cells from the bone marrow to the peripheral blood and eventually to tissues in need of cell repair (25). In a study of diabetic wounds, Fiorina et al. showed that healing of diabetic wounds requires the presence of factor SDF-1 and stem cells in the circulation (26). Dalakas et al. showed that chemokine levels increase as a result of liver damage caused by long-term alcohol consumption. However, the transplantation of a woman's liver to an alcoholic man in the same study also showed that chemokines and stem cells do not affect liver repair (23). Considering the high degree of liver cell destruction and apoptosis in cirrhosis and SDF-1 secretion from the damaged cells, higher concentrations of SDF-1 in the blood of cirrhotic patients are expected, which was confirmed by the results of this study and has also been shown in other studies (27-30). Chalin et al. showed that SDF-1 can be a diagnostic biomarker (31). It can be concluded that liver tissue responds well to destruction and fibrosis due to the higher concentration of this important factor in cirrhotic patients. Moreover, SDF-1 increased directly with the increase in disease severity in cirrhotic patients, which was confirmed by Bihari et al. (27). Therefore, the concentration of SDF-1 can be used as a biomarker to determine the severity of the disease.

This study has some limitations, including the small sample size and the restriction to patient selection from a single hospital in a short period. In addition, it was not possible to sample cirrhotic patients with a single etiology or to compare patients with different etiologies. Therefore, future studies may investigate and explore the biomarker ability of SDF-1 to determine disease severity and prognosis. It is also suggested that other factors that stimulate stem cell migration, such as CXCL11, will be measured.

CONCLUSION

The percentage of stem cells and the concentration of SDF-1 in the serum of cirrhotic patients were significantly higher compared with the control group. In addition, there was no significant relationship between the percentage of circulating stem cells and the severity of the disease, whereas a direct relationship between the severity of the disease and the concentration of SDF-1 was observed.

CONFLICT OF INTERESRT

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

Not applicable.

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Shahroud University of Medical Sciences with the Code of Ethics "20040" with informed consent.

Consent for Publication

Not applicable

Availability of Data and Materials

Not applicable. All data can be found in the article.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Siapati EK, Roubelakis MG, Vassilopoulos G. Liver Regeneration by Hematopoietic Stem Cells: Have We Reached the End of the Road? Cells. 2022;11(15):2312.

2. Kim G, Kim MY, Baik SK. Transient elastography versus hepatic venous pressure gradient for diagnosing portal hypertension: a systematic review and meta-analysis. Clin Mol Hepatol. 2017;23(1):34-41.

3. Shim KY, Eom YW, Kim MY, et al. Role of the renin-angiotensin system in hepatic fibrosis and portal hypertension. Korean J Intern Med. 2018;33(3):453-461.

4. Asrani SK, Devarbhavi H, Eaton J, et al. Burden of liver diseases in the world. J Hepatol. 2019;70(1):151-71.
5. Kang SH, Kim MY, Baik SK. Novelties in the pathophysiology and management of portal hypertension: new treatments on the horizon. Hepatol Int. 2018;12(Suppl 1):112-21.
6. Kang SH, Kim MY, Eom YW, et al. Mesenchymal stem cells for the treatment of liver disease: present and perspectives. Gut Liver. 2020;14(3):306-315.
7. Corbett JL, Duncan SA. iPSC-derived hepatocytes as a platform for disease modeling and drug discovery. Front Med (Lausanne). 2019;6:265.
8. Si-Tayeb K, Noto FK, Nagaoka M, et al. Highly efficient generation of human hepatocyte–like cells from induced pluripotent stem cells. Hepatology. 2010;51(1):297-305.
9. Kim G, Eom YW, Baik SK, et al. Therapeutic effects of mesenchymal stem cells for patients with chronic liver diseases: systematic review and meta-analysis. J Korean Med Sci. 2015;30(10):1405-15.
10. Secunda R, Vennila R, Mohanashankar A, et al. Isolation, expansion and characterisation of mesenchymal stem cells from human bone marrow, adipose tissue, umbilical cord blood and matrix: a comparative study. Cytotechnology. 2015;67(5):793-807.
11. Gilbert W, Bragg R, Elmansi AM, McGee-Lawrence ME, Isales CM, Hamrick MW, et al. Stromal cell-derived factor-1 (CXCL12) and its role in bone and muscle biology. Cytokine. 2019;123:154783.
12. Kinoshita M. CELLULAR, MOLECULAR, GENOMICS, AND BIOMEDICAL APPROACHES | Germ Cell Migration and Trans Sex. In: Farrell AP, editor. Encyclopedia of Fish Physiology. San Diego: Academic Press; 2011. pp: 2046-54.
13. Lataillade JJ, Clay D, Dupuy C, et al. Chemokine SDF-1 enhances circulating CD34+ cell proliferation in synergy with cytokines: possible role in progenitor survival. Blood. 2000;95(3):756-68.
14. Motabi IH, DiPersio JF. Advances in stem cell mobilization. Blood Rev. 2012;26(6):267-78.
15. Nagasawa T. Cxcl12/sdf-1 and cxcr4. Front Immunol. 2015;6:301.
16. Donnelly DS, Krause DS. Hematopoietic stem cells can be CD34+ or CD34. Leuk Lymphoma. 2001;40(3-4):221-34.
17. Le Guern AC, Giovino MA, Abe M, et al. Stem cell activity of porcine c-kit+ hematopoietic cells. Exp Hematol. 2003;31(9):833-40.
18. Matsuoka Y, Sasaki Y, Nakatsuka R, et al. Low level of c-kit expression marks deeply quiescent murine hematopoietic stem cells. Stem Cells. 2011;29(11):1783-91.
19. Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev. 2017;121:27-42.
20. Kwak KA, Cho HJ, Yang JY, et al. Current perspectives regarding stem cell-based therapy for liver cirrhosis. Can J Gastroenterol Hepatol. 2018;2018:4197857.
21. Thüring J, Rippel O, Haarburger C, et al. Multiphase CT-based prediction of Child-Pugh classification: a machine learning approach. Eur Radiol Exp. 2020;4(1):20.
22. Körbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med. 2002;346(10):738-46.
23. Dalakas E, Newsome PN, Boyle S, et al. Bone marrow stem cells contribute to alcohol liver fibrosis in humans. Stem Cells Dev. 2010;19(9):1417-25.
24. Kaur S, Sehgal R, Shastry SM, et al. Circulating endothelial progenitor cells present an inflammatory phenotype and function in patients with alcoholic liver cirrhosis. Front Physiol. 2018;9:556.
25. Lataillade JJ, Domenech J, Le Bousse-Kerdilès MC. Stromal cell-derived factor-1 (SDF-1)\CXCR4 couple plays multiple roles on haematopoietic progenitors at the border between the old cytokine and new chemokine worlds: survival, cell cycling and trafficking. Eur Cytokine Netw. 2004;15(3):177-88.
26. Fiorina P, Pietramaggiori G, Scherer SS, et al. The mobilization and effect of endogenous bone marrow progenitor cells in diabetic wound healing. Cell Transplant. 2010;19(11):1369-81.
27. Bihari C, Anand L, Rooge S, et al. Bone marrow stem cells and their niche components are adversely affected in advanced cirrhosis of the liver. Hepatology. 2016;64(4):1273-88.
28. Kedarisetty CK, Anand L, Bhardwaj A, et al. Combination of granulocyte colony-stimulating factor and erythropoietin improves outcomes of patients with decompensated cirrhosis. Gastroenterology. 2015;148(7):1362-70. e7.
29. Spahr L, Chalandon Y, Terraz S, et al. Autologous bone marrow mononuclear cell transplantation in patients with decompensated alcoholic liver disease: a randomized controlled trial. PLoS One. 2013;8(1):e53719.
30. Chalin A, Lefevre B, Devisme C, et al. Serum CXCL10, CXCL11, CXCL12, and CXCL14 chemokine patterns in patients with acute liver injury. Cytokine. 2018;111:500-504.
31. Chalin A, Lefevre B, Devisme C, et al. Circulating levels of CXCL11 and CXCL12 are biomarkers of cirrhosis in patients with chronic hepatitis C infection. Cytokine. 2019;117:72-78.