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The Combination of Mesenchymal Stem Cells and Bovine Colostrum in Reducing α-SMA Expression and NLR Levels in Wistar Rats After 50% Fibrotic Liver Resection

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Abstract

BACKGROUND: Liver fibrogenesis will produce α-smooth muscle actin (α-SMA) expression and a continuous inflammatory process, seen through the neutrophil lymphocyte ratio (NLR). The combination of mesenchymal stem cells (MSCs) and bovine colostrum is a novel strategy for repairing hepatic fibrosis tissue.

AIM: The aim of this study is to evaluate the combination of MSCs and bovine colostrum to reduce α-SMA expression and NLR levels in Wistar rats after 50% of fibrotic liver resection.

METHODS: Thirty-six Wistar male rats were randomly divided into five groups (sham, control, colostrum, MSCs, and combination of colostrum-MSCs). Rats were injected with CCl_4 for 8 weeks to induce liver fibrosis then underwent liver resection. NLR levels were determined using hematology analyzer, α-SMA expression of myofibroblast was analyzed by immunofluorescence staining.

RESULTS: A significant reduction in NLR levels on day 3 in the treatment Group I (1.10), Treatment II (0.83), and Treatment III (0.93) compared to the control group. A significant reduction in NLR levels on day 10 in the treatment Group I (0.76), Treatment II (0.64), and Treatment III (0.54) compared to the control group. A significant decrease in α-SMA in treatment Group I (0.134), Treatment II (0.68), and Treatment III (0.42) compared to the control group.

CONCLUSION: In this study, it was found that α-SMA expression and NLR levels on the 3rd and 10th day of administration were reduced in group receiving combination of MSCs and bovine colostrum in the liver of post-resection Wistar rats by 50%.

Introductions

Liver fibrosis occurs due to chronic liver damage and inflammation, from injuries of viral hepatitis, autoimmune, alcohol consumption, and non-alcoholic steatohepatitis (NASH), which characterized by deposition of the extracellular matrix with distortion of the normal liver parenchyma [1], [2], [3], [4]. The process of hepatic fibrogenesis triggers myofibroblasts to become active which then results in the expression of α-smooth muscle actin (α-SMA) as a molecular marker that continues to rise, along with the increase of extracellular matrix in the liver [4], [5]. In addition, there is a systemic increase in immune system cells, such as neutrophils and lymphocytes ratio (NLR), along with fat accumulation in liver is also one of the key contributors of liver fibrosis [6], [7], [8]. In light of the above findings, the fibrosis process will persist, resulting in reduced liver function.

Mesenchymal stem cells (MSCs) have the capacity to self-renew and differentiate into other cell types, thereby facilitating the process of cell regeneration [9], [10], [11], [12], [13]. On the other hand, bovine colostrum has antioxidant properties that can aid in tissue repair [14]. The combination of MSCs and bovine colostrum can aid in the regeneration of cells and tissues in liver fibrosis. At present, the expression of α-SMA is the most widely used marker of myofibroblasts in studies assessing the fibrosis process by taking a role in cell contraction and formation of cytoskeletal tension [15]. Judging from the functional level, myofibroblasts effectively repair defects and rebuild tissue integrity. Collagen scar formation occurs and stiffness leading to decreased tissue function and even organ failure if myofibroblast repair becomes chronic, such as during the development of fibrosis [16].

The neutrophil count was significantly lower in patients with advanced fibrosis than in patients without minimal fibrosis [17]. However, other studies

have shown that NLR is significantly increased in patients with NASH and fibrosis compared with patients without NASH [18], [19]. A previous study reported that MSCs are able to accelerate the liver regeneration process [20]. MSCs will produce several paracrine factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor, insulin growth factor, and interleukin 10 (IL-10) that associated with liver regeneration [11], [12], [21], [22], [23]. In addition, bovine colostrum has a total antioxidant capacity, such as superoxide dismutase, glutathione peroxidase, catalase, Vitamin E, Vitamin C, and β carotene [24]. Therefore, in this study aims to evaluate the effect of combination MSCs and bovine colostrum on the fibrotic liver mouse model.

Materials and Methods

Research design

This study employed a posttest-only control group design to evaluate the efficacy of the combination of MSCs and bovine colostrum in reducing-SMA expression and NLR levels in Wistar rats following a 50% of fibrotic liver resection. The research was conducted for 8 weeks during December 2020–February 2021 at the Stem Cell and Cancer Research Laboratory of Sultan Agung University and the Animal Health Laboratory, Semarang.

Study subjects

The subjects of this study were male rats of Wistar strain, aged 3 months and had body weight around 200–250 g. The number of samples for each study group was six rats. The research subjects were randomly divided into six groups; therefore, the total sample was 36 rats. The research subjects were adapted for 1 week and were given standard feed.

Treatment

Subjects were randomly divided into groups and then received intraperitoneal CCl4 injection 2 μL/g twice a week for 7 weeks, and performed 50% of liver resection. Subjects were divided into control Group I (NaCl 0.9% oral), control Group II (NaCl 0.9% injection), treatment Group I (bovine colostrum 15 μL/g/day for 7 days), treatment Group II (1,000. 000 doses of MSCs), treatment Group III (1,000,000 doses of MSCs and bovine colostrum 15 μL/g/day for 7 days), and treatment Group IV (Sham). Then, the validation by histological examination was performed by making preparations of Hematoxyllin and Eosin (H&E). Blood was collected to assess the levels of hepatic biochemistry on day 3 and day 10 of intervention. A laparotomy was performed after 10 days, liver collection and histological check of the liver samples were also taken.

Variable measurement

Analysis of α-SMA expression was using IHC technique with anti-α-SMA antibody. The result of the examination was the intensity of expression, marked in brown, seen with a 40× magnification microscope in five fields of view with 5–10 expression compartments for each field of view. Neutrophil lymphocyte ratio (NLR) was measured by hematology analyzer and be calculated in quantity/liter units.

Isolation of Umbilical cord (UC)-MSCs

UC derived MSCs (UC-MSCs) were obtained from pregnant single Wistar rats under deep anesthesia and transplanted into an ALF rat model. Briefly, the umbilical cord was cut into pieces after the blood vessels were removed and then transferred to a T25 culture flask containing complete Dulbecco's Modified Eagle's medium (DMEM) (Sigma-Aldrich, Louis St, MO) mixed with 10% of fetal bovine serum (FBS) (GibcoTM Invitrogen, NY, USA), and 100 IU/mL penicillin/streptomycin (Sigma-Aldrich). These cells were incubated in a 5% CO2, 37°C incubator, and the medium was changed every 3 days. After cells reached 80% confluency, the MSC-like cells were passaged with trypsin. Cells from the fouth passage were used for experiments. This study was approved by the Institutional Review Board of the Ethics Committee of the Medical Department, Sultan Agung Islamic University, Semarang, Indonesia.

Induction of post-hepatectomy liver failure (PHLF) with liver fibrosis animals and experimental design

Thirty-six Wistar male rats were randomly divided into Sham group (n = 6) and the model groups (n = 30). The induction LF was performed by injecting intraperitoneally the carbon tetrachloride (CCl_4) (Sigma-Aldrich, USA) with 1 ml/kg twice per week for 8 weeks. After 6 weeks, four rats in the model group were sacrificed at random and liver tissue was obtained to verify LF. All of the PHLF with LF rats undergoing liver resection 50% of the liver in the median and right lateral lobes. The surgical procedure was performed under aseptic condition. Each group consisted of six Wistar rats which administered into the Sham group (negative control was fed with water); NaCl PO group (was fed with NaCl 15μL/g five times a week orally); NaCl injection (was injected 15μL/g five times a week intraparenchymal); Colostrum (Colostrum milk powder (Good Health, NZ) 15 μL/g 5 times a week orally); MSC (doses 1 x 10^6 cells dissolved in 500 μL in NaCl through

intraparenchymal injection), and MTC (MSC doses 5 x 105 cells dissolved in 500 μL NaCl 5 times a week through intraparenchymal injection + Colostrum doses 15μL/g 5 times a week orally).

Flow cytometric immunophenotyping of UC-MSCs

The immunophenotypes of MSCs were analyzed at fourth passage. MSCs were stained with antibodies conjugated: fluorescein isothiocyanate-conjugated CD90, Allophycocyanin-conjugated CD73, Peridinin Chlorophyll Protein Complex-conjugated CD105, and phycoerythrin (PE)-conjugated Lin monoclonal antibodies for 30 min at 4°C in the dark. The fluorescence intensity of the cells was evaluated through flow cytometry (BD Bioscience, Franklin Lakes, NJ, USA).

In vitro differentiation

MSCs differentiation potential was determined to characterize the isolated cells. These cells were cultured in DMEM medium supplemented with 10% FBS, 10 mmol/L β-glycerophosphate, mol/L/0.1 μM dexamethasone, 50 μmol/L ascorbate-2-phosphate (all from Sigma-Aldrich, Louis St, MO), at 37°C, and 5% CO2. The fixed cells were stained with 0.2% Alizarin Red solution (Sigma Aldrich) to represent calcium deposition (cells used were from the fourth passage).

Statistical analysis

Test for normality, validity, and reliability was using ANOVA. The degree of significance limit was $p \leq 0.05$ with a 95% confidence interval. Data analysis was performed using a SPSS 21.

Ethical approval

Animal ethics was obtained from submission to the Ethics Committee for Health Research, Faculty of Medicine, Diponegoro University No. 09/EC/H/FK-UNDIP/II/2021. Treatment of animals is adjusted to the standards of animal maintenance and research report was adjusted according to NC3R ARRIVE Guidelines for Animal Research.

Results

NLR Day 3

On day 3, the control group that received injections and oral NaCl 0.9% had mean NLR values of 2.54 and 2.27, respectively. The number of NLRs in the group that received colostrum and MSC alone decreased significantly to 1.10 and 0.83, respectively (Figure 1).

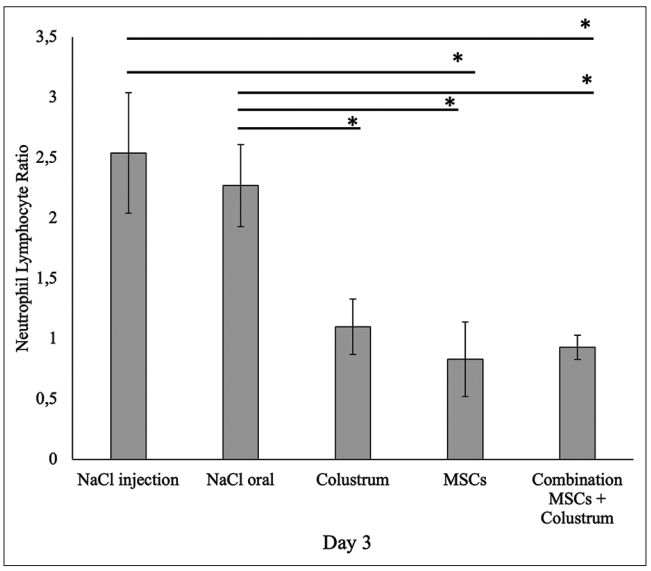

*Figure 1: Neutrophil-lymphocyte ratio on day 3. ANOVA test revealed significant overall differences between colostrum, mesenchymal stem cells (MSCs), and combination MSCs + colostrum groups with control NaCl groups. *p < 0.005*

The control group NLR on day 3 which was given injection 0.9% NaCl had a mean value of 2.54 and that which was given oral 0.9% NaCl had a mean value of 2.27. The NLR treatment group which was given a combination of MSCs and bovine colostrum (MSC+C) on day 3 had a mean value of 0.93. These results were stated to be significant with a decrease in the graphic of treatment group with the combination of MSCs and bovine colostrum compared to the control group of NaCl injection and oral NaCl.

NLR levels on day 10

The NLR profile on day 10 has a similar profile to day 3. The control group that received injections and oral NaCl 0.9% had mean NLR values of 1.49 and 1.05, respectively. The number of NLRs in the group that received colostrum and MSC alone decreased significantly to 0.76 and 0.64, respectively (Figure 2).

The NLR control group on day 10 that was given injection 0.9% NaCl had a mean value of 1.49 and those given oral 0.9% NaCl had a mean value of 1.05. The NLR treatment group which was given a combination of MSCs and bovine colostrum (MSC+C) on day 10 had a mean value of 0.54 (Figure 3). These results were stated to be significant with a decrease in the graphic of treatment group with the combination of MSCs and bovine colostrum compared to the control group of NaCl injection and oral NaCl.

α-*SMA expression*

The control group which was given 0.9% NaCl orally and by injection had a mean α-SMA expression

value of 0.268 and 0.272, respectively. The combination group experienced a significantly greater decrease in α-SMA expression up to 0.42 compared to the colostrum and MSC single-therapy group (Figure 3a and b). These results were stated to be significant with a decrease in the graphic of treatment group with the combination of MSCs and bovine colostrum compared to the control group of NaCl injection and oral NaCl.

Discussion

Fibroproliferation disorders of the liver are surprisingly common and essentially untreatable disease that results in substantial morbidity and mortality. The previous study reported that neutrophil contributed to the proliferation of fibroblast [25]. However, the role of MSCs and colostrum on the NLR and fibroblast proliferation through α-SMA expression remains unclear. In this study, we found that combination of MSCs and colostrum inhibit liver fibrosis through suppress level of NLR associated with decrease levels of α-SMA.

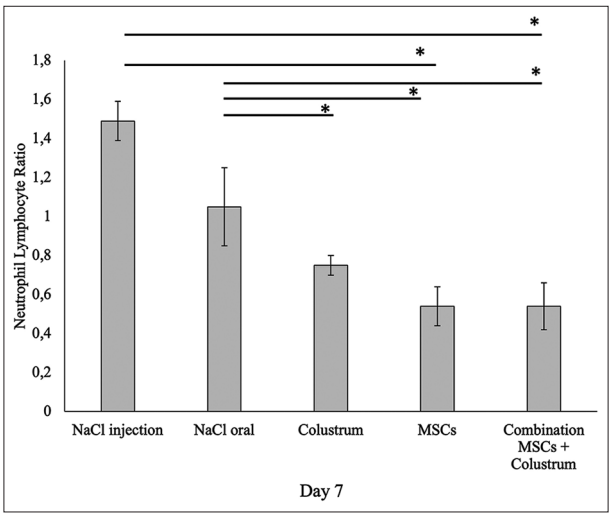

*Figure 2: Neutrophil-lymphocyte ratio on day 10. ANOVA test revealed significant overall differences between colostrum, mesenchymal stem cells (MSCs), and combination MSCs + colostrum groups with control NaCl groups. *p < 0.005*

The research data showed that all NLR levels on day 3 were significantly decrease by MSCs group treatment. This result showed that the MSCs have the role of immunosuppressants during inflammatory process in liver fibrosis. This finding supported the previous study revealed that MSCs-mediated immunosuppression mainly acts through the secretion of soluble molecules that are induces or upregulated indoleamine 2,3-dioxygenase [23], [26]. In addition, MSCs released soluble factor of anti-inflammation such as IL-10 and growth factor including transforming growth factor-ß, fibroblast growth factor, platelet-derived growth factor, and VEGF [27], [28]. This obvious reduction even exceeds the combination of MSCs and bovine colostrum due to insignificant role of bovine colostrum in the early inflammatory phase of liver fibrosis.

NLR levels on day 10 were also reduced. The graphic showed that the combination of MSCs and bovine colostrum (MSC+C) has the lowest value with 0.54. This result showed that the role of immunosuppressants in MSCs is supported by the control of bovine colostrum as an immunoregulator. It could be seen that bovine colostrum needs time to assist MSCs in supporting this role.

In the results of data regarding α-SMA expression, there was a decrease in α-SMA expression,

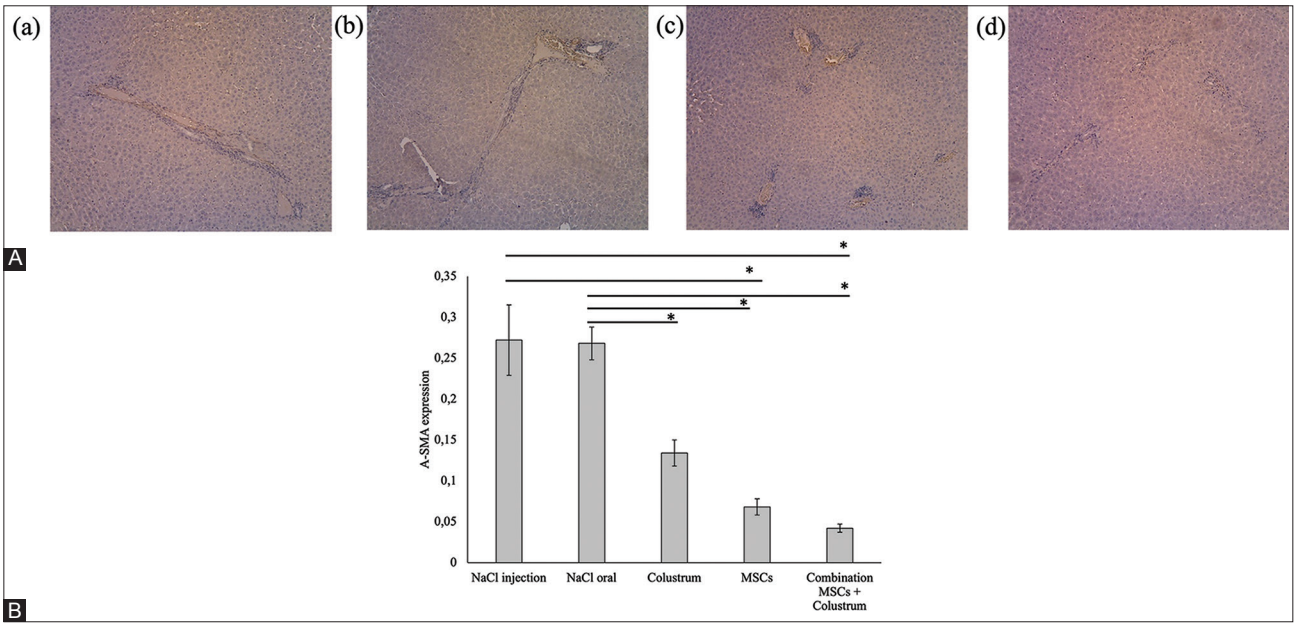

*Figure 3: α-SMA expression in the five groups. (A) Representative immunohistochemical staining of α-smooth muscle actin (α-SMA) expression in liver tissues (magnification, ×40) (a) NaCl oral, (b) NaCl injection, (c) colostrum, and (d) mesenchymal stem cells treatment. (B) Relative quantitation of α-SMA expression. Data were obtained from three independent experiments in each condition. * p < 0.05*

and it was seen that the combination of mesenchymal stem cells and bovine colostrum (MSC+C) had the lowest value with 0.042. This result also supported the facts that the role of immunosuppressants in mesenchymal stem cells is assisted by bovine colostrum function as an immunoregulator.

Conclusion

In this study, α-SMA expression was reduced in the group receiving combination of mesenchymal stem cells and bovine colostrum in the liver of Wistar rats after 50% of liver resection. NLR levels were also reduced on day 3 and day 10 of intervention in the group receiving combination of mesenchymal stem cells and bovine colostrum in the liver of Wistar rats after 50% of liver resection.

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Author Contributions

MMH, YWP, and AP were responsible for experimental design and developed methodology. MMH and NDA carried out the experiments. MMH, AP, and YP interpreted the results, performed data analysis, and prepared the figures and tables. AP and NDA wrote, reviewed, and revised the manuscript. AP provided administrative, technical, and material support. AP and YP supervised the study.

References

1. Kitano M, Bloomston PM. Hepatic stellate cells and microRNAs in pathogenesis of liver fibrosis. J Clin Med. 2016;5(3):38. https://doi.org/10.3390/jcm5030038
   PMid:26999230
2. Böttcher K, Pinzani M. Pathophysiology of liver fibrosis and the methodological barriers to the development of anti-fibrogenic agents. Adv Drug Deliv Rev. 2017;121:3-8. https://doi.org/10.1016/j.addr.2017.05.016
   PMid:28600202
3. Chen L, Zhang C, Chen L, Wang X, Xiang B, Wu X, *et al*. Human Menstrual blood-derived stem cells ameliorate liver fibrosis in mice by targeting hepatic stellate cells via paracrine mediators. Stem Cells Transl Med. 2017;6(1):272-84. https://doi.org/10.5966/sctm.2015-0265
   PMid:28170193
4. Hinz B. The myofibroblast: Paradigm for a mechanically active cell. J Biomech. 2010;43(1):146-55. https://doi.org/10.1016/j.jbiomech.2009.09.020
   PMid:19800625
5. Rockey DC, Du Q, Shi Z. Smooth muscle α-actin deficiency leads to decreased liver fibrosis via impaired cytoskeletal signaling in hepatic stellate cells. Am J Pathol. 2019;189(11):2209-20. https://doi.org/10.1016/j.ajpath.2019.07.019
   PMid:31476284
6. Kekilli M, Tanoglu A, Sakin YS, Kurt M, Ocal S, Bagci S. Is the neutrophil to lymphocyte ratio associated with liver fibrosis in patients with chronic hepatitis B? World J Gastroenterol. 2015;21(18):5575-81. https://doi.org/10.3748/wjg.v21.i18.5575
   PMid:25987782
7. Ramadan OI, Nasr M, El-Hay OM, Hasan A, Abd-Allah EE, Mahmoud ME, *et al*. Potential protective effect of *Zingiber officinale* in comparison to rosuvastatin on high-fat diet-induced non-alcoholic fatty liver disease in rats. Open Access Maced J Med Sci. 2022;10(A):916-23.
8. Jia F, Hu X, Kimura T, Tanaka N. Impact of dietary fat on the progression of liver fibrosis: Lessons from animal and cell studies. Int J Mol Sci. 2021;22(1):10303. https://doi.org/10.3390/ijms221910303
   PMid:34638640
9. Kim D, Cho GS, Han C, Park DH, Park HK, Woo DH, *et al*. Current understanding of stem cell and secretome therapies in liver diseases. Tissue Eng Regen Med. 2017;14(6):653-65. https://doi.org/10.1007/s13770-017-0093-7
   PMid:30603518
10. Restimulia L, Ilyas S, Munir D, Putra A, Madiadipoera T, Farhat F, *et al*. Rats' umbilical-cord mesenchymal stem cells ameliorate mast cells and Hsp70 on ovalbumin-induced allergic rhinitis rats. Med Glas (Zenica). 2022;19(1):52-9. https://doi.org/10.17392/1421-21
    PMid:35048629
11. Drawina P, Putra A, Nasihun T, Prajoko YW, Dirja BT, Amalina ND. Increased serial levels of platelet-derived growth factor using hypoxic mesenchymal stem cell-conditioned medium to promote closure acceler-ation in a full-thickness wound. Indones J Biotechnol. 2022;27(1):36.
12. Hamra NF, Putra A, Tjipta A, Amalina ND, Nasihun T. Hypoxia mesenchymal stem cells accelerate wound closure improvement by controlling α-smooth muscle actin expression in the full-thickness animal model. Open Access Maced J Med Sci. 2021;9:35-41.
13. Darlan DM, Munir D, Putra A, Alif I, Amalina ND, Jusuf NK, *et al*. Revealing the decrease of indoleamine 2,3-dioxygenase as a major constituent for B cells survival post-mesenchymal stem cells co-cultured with peripheral blood mononuclear cell (PBMC) of systemic lupus erythematosus (SLE) patients. Med Glas. 2022;19(1):12-8. https://doi.org/10.17392/1414-21
    PMid:35048623
14. Restimulia L, Ilyas S, Munir D, Putra A, Madiadipoera T, Farhat F, *et al*. The CD4+ CD25+ FoxP3+ Regulatory T Cells Regulated by MSCs Suppress Plasma Cells in a Mouse Model of Allergic Rhinitis. Medical Archives. 202;75(4):256.
15. Talele NP, Fradette J, Davies JE, Kapus A, Hinz B. Expression of α-smooth muscle actin determines the fate of mesenchymal stromal cells. Stem Cell Reports. 2015;4(6):1016-30. https://doi.org/10.1016/j.stemcr.2015.05.004

PMid:26028530

16. Darby IA, Laverdet B, Bonté F, Desmoulière A. Fibroblasts and myofibroblasts in wound healing. Clin Cosmet Investig Dermatol. 2014;7:301-11. https://doi.org/10.2147/CCID.S50046
PMid:25395868
17. Ruta VM, Man AM, Alexescu TG, Motoc NS, Tarmure S, Ungur RA, *et al*. Neutrophil-to-lymphocyte ratio and systemic immune-inflammation index-biomarkers in interstitial lung disease. Medicina (Lithuania). 2020;56(8):381. https://doi.org/10.3390/medicina56080381
PMid:32751302
18. Yilmaz H, Yalcin KS, Namuslu M, Celik HT, Sozen M, Inan O, *et al*. Neutrophil-lymphocyte ratio (NLR) could be better predictor than C-reactive protein (CRP) for liver fibrosis in non-alcoholic steatohepatitis(NASH). 2015;45(3):278-86.
PMid:26116591
19. Lesmana CR, Kencana Y, Rinaldi I, Kurniawan J, Hasan I, Sulaiman AS, *et al*. Diagnostic value of neutrophil to lymphocyte ratio in non-alcoholic fatty liver disease evaluated using transient elastography (TE) with controlled attenuated parameter (CAP). Diabetes Metab Syndr Obes. 2022;15:15-22. https://doi.org/10.2147/DMSO.S330526
PMid:35023936
20. Darlan DM, Munir D, Jusuf NK, Putra A, Ikhsan R, Alif I. In vitro regulation of IL-6 and TGF-ß by mesenchymal stem cells in systemic lupus erythematosus patients. Med Glas (Zenica). 2020;17(2):408-13.
21. Prajoko YW, Putra A, Dirja BT, Muhar AM, Amalina ND. The ameliorating effects of MSCs in controlling treg-mediated B-Cell depletion by Indoleamine 2, 3-dioxygenase Induction in PBMC of SLE patients. Open Access Maced J Med Sci. 2022;10(A):6-11.
22. Sungkar T, Putra A, Lindarto D, Sembiring RJ. Intravenous umbilical cord-derived mesenchymal stem cells transplantation regulates hyaluronic acid and interleukin-10 secretion producing low-grade liver fibrosis in experimental rat. Med Arch. 2020;74(3):177-82. https://doi.org/10.5455/medarh.2020.74.177-182
PMid:32801431
23. Prajoko YW, Putra A, Dirja BT, Muhar AM, Amalina ND. The ameliorating effects of mscs in controlling treg-mediated b-cell depletion by indoleamine 2, 3-dioxygenase induction in PBMC of SLE patients. Open Access Maced J Med Sci. 2022;10:6-11.
24. Gregory AD, Kliment CR, Metz HE, Kim KH, Kargl J, Agostini BA, *et al*. Neutrophil elastase promotes myofibroblast differentiation in lung fibrosis. J Leukoc Biol. 2015;98(2):143-52. https://doi.org/10.1189/jlb.3HI1014-493R
25. Lim JY, Kim BS, Bin RD, Kim TW, Park G, Min CK. The therapeutic efficacy of mesenchymal stromal cells on experimental colitis was improved by the IFN-γ and poly(I: C) priming through promoting the expression of indoleamine 2,3-dioxygenase. Stem Cell Res Ther. 2021;12(1):1-13.
26. El Agha E, Kramann R, Schneider RK, Li X, Seeger W, Humphreys BD, *et al*. Mesenchymal stem cells in fibrotic disease. Cell Stem Cell. 2017;21(2):166-77. https://doi.org/10.1016/j.stem.2017.07.011
27. Putra A, Antari AD, Kustiyah AR, Intan YS, Sadyah NA, Wirawan N, *et al*. Mesenchymal stem cells accelerate liver regeneration in acute liver failure animal model. Biomed Res Ther. 2018;5(11):2802-10.
28. Hermansyah D, Putra A, Munir D, Lelo A, Amalina ND, Alif I. Synergistic effect of Curcuma longa extract in combination with Phyllanthus niruri extract in regulating Annexin A2, epidermal growth factor receptor, matrix metalloproteinases, and pyruvate kinase M1/2 signaling pathway on breast cancer stem cell. Open Access Maced J Med Sci. 2021;9(A):271-85.