The pathogenesis of hepatocellular carcinoma (HCC) is a multifactorial process which has not been fully explained so far. Early diagnosis is still difficult; this neoplasm is frequently diagnosed in the advanced phase. Serum α-fetoprotein (AFP) and ultrasound imaging (US) for many years have been the gold standard of HCC screening. However, sensitivity and specificity of serum AFP in HCC diagnosis are relatively low,[1,2] therefore, it was excluded from the American Association for the Study of Liver Diseases (AASLD) guidelines for HCC screening in 2010[3] and from European Association for the Study of the Liver (EASL) guidelines in 2012,[4] whereas others still recommend testing AFP in addition to US.[5-7] There is a need for new, more sensitive, and reliable diagnostic tests. There are several molecular markers of HCC currently under investigation including cellular markers, cellular cycle regulators, markers of apoptosis, telomerase activity, adhesive molecules, markers of extracellular matrix degradation, markers of angiogenesis, growth factors and their receptors, oncofetal and glycoprotein antigens, enzymes and isoenzymes, and genetic markers.[8]

Pigment epithelium-derived factor (PEDF) is an endogenously produced glycoprotein with a molecular weight of 50 kD, occurring commonly in various organs and exhibiting diverse biological activity. It belongs to the superfamily of serine protease inhibitors. PEDF reaches high...
concentrations in serum, which can be used for diagnostic purposes.\(^9\) Repeatedly confirmed effect of this molecule is inhibition of angiogenesis.\(^10\) It is believed that PEDF has neuroprotective properties,\(^11\) acts as an antioxidant and anti-inflammatory agent,\(^12\) inhibits cellular proliferation and supports cell differentiation, and protects against tumor metastasis.\(^13\) Liver is the major site of PEDF synthesis.\(^14\) PEDF is an important factor in many liver diseases.\(^15\) The mechanism of its action remains largely unclear. Antifibrogenic activity of intrahepatic PEDF has been demonstrated.\(^16\) PEDF is secreted by fat cells as well, playing a role in the development of insulin resistance, metabolic syndrome, and liver steatosis.\(^17-22\)

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent enzyme also called gelatinase B, with a molecular weight of 92 kD. It degrades extracellular matrix proteins, particularly collagen IV, which is a component of basement membrane. This enzyme plays an important role in inflammation, tissue remodeling process, as well as promotes tumor cell migration and metastasis.\(^23,24\) The main sources of matrix metalloproteinases in the damaged liver are stellate cells with endothelium and inflammatory cells.\(^25\) It was found that serum or plasma MMP-9 may be a useful marker of the processes that occur in the tissues.\(^26\) Elevated MMP-9 levels were observed in colorectal cancer and breast cancer,\(^27\) stomach cancer,\(^28\) bladder cancer, acute leukemia, rheumatoid arthritis, melanoma, and HCC.\(^29\) The activity of MMP-9 is checked on several levels: transcription – control of cytokines, activation of the proenzyme – enzyme cascade, including serine proteases and other metalloproteinases, finally – regulation by specific tissue inhibitors of metalloproteinases (TIMP). The data on the serum MMP-9 as a marker of fibrosis are controversial.\(^30\)

In this study, we investigated the usefulness of serum PEDF and MMP-9 in evaluating the progression of liver cirrhosis and development of HCC.

**PATIENTS AND METHODS**

The study group consisted of 212 patients with cirrhosis from the database e-Hepar; 127 men and 85 women, aged from 28 to 86 years, with a mean age of 54.6 years. The study also included a control group of 30 healthy volunteers; 12 men and 18 women, aged from 28 to 52 years, with a mean age 38.3 years. Informed consent was obtained from each patient included in the study. Serum PEDF and MMP-9 were determined by ELISA Kits (PEDF – ChemiKine, Chemicon International; MMP-9 – Platinum ELISA, eBioscience). Standard biochemical serum tests were carried out, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alpha-fetoprotein (AFP). The severity of liver cirrhosis was assessed using the Child-Pugh score. Statistical analysis was performed using Statistica; Spearman’s rank correlation test and the Mann–Whitney U test were used. P value of < 0.05 was considered to be statistically significant.

Analyzing the etiology of liver cirrhosis in the study group, pure viral hepatitis-related cirrhosis was identified in 124 patients, pure alcoholic cirrhosis in 27 patients, cirrhosis of mixed etiology (viral hepatitis-related and alcoholic) in 43 patients, and cirrhosis of other or unknown etiology in 18 patients. From 167 patients infected with hepatotropic viruses, 126 patients were infected with hepatitis C virus (HCV), 33 with hepatitis B virus (HBV), and 8 patients were co-infected with HBV and HCV. HCC was diagnosed in 45 of the 212 patients studied (21%). These were patients with viral hepatitis-related liver cirrhosis (36 patients) and cirrhosis of mixed etiology (8 patients). None of the patients with alcoholic liver disease were diagnosed with HCC. Among patients with HCC, 40 were infected with HCV, 4 patients were diagnosed with HBV, and 1 patient was infected with both viruses.

**RESULTS**

The concentration of both PEDF and MMP-9 was significantly higher in patients with cirrhosis than in the control group (for PEDF, respectively, 11000.7 ± 11367.7 ng/ml vs. 417.3 ± 266.5 ng/ml, P < 0.001; for MMP-9 1863.5 ± 4692.8 ng/ml vs. 94.9 ± 21.6 ng/ml, P < 0.001). There were no significant differences in levels of PEDF or MMP-9 between the groups A, B, and C according to the Child-Pugh classification [Figures 1 and 2]. There were significant differences in the levels of PEDF, depending on the etiology of cirrhosis. In patients with alcoholic or mixed (alcoholic and viral hepatitis-related) cirrhosis, serum PEDF was higher than in other patients (13970.2 ± 13406.9 ng/ml vs. 8563.5 ± 9602.7 ng/ml, P = 0.008) [Figure 3].

In further analysis of the group of patients with viral hepatitis-related cirrhosis, there were significantly higher PEDF levels recorded in patients with HCC (13429.1 ± 12045.8) than that in patients without HCC (6660.1 ± 7927.1; P = 0.04) [Figure 4].

Similarly, there was also a trend for higher serum MMP-9 in patients with HCC (5778.7 ± 12426.6 vs. 1389.8 ± 1944.7 in those without HCC; P = 0.07). The analysis was performed in all patients, regardless of the etiology of cirrhosis [Figure 5].

By examining correlations of various factors associated with the disease to the value of serum PEDF, a negative
correlation between serum PEDF and ALT level \((r = -0.18, P = 0.06)\) was found. This correlation was particularly visible and statistically significant in patients without HCC \((r = -0.27; P = 0.03)\). Significant negative correlation between serum MMP-9 and serum AFP in patients with HCC was observed \((r = -0.54; P = 0.04)\).

**DISCUSSION**

In this study, serum PEDF and MMP-9 were determined in patients with cirrhosis of different etiologies, and their value in the diagnosis of HCC was assessed. PEDF, which has antiangiogenic and antiproliferative activity, is considered to be a protective factor, reducing the risk of HCC development.\(^3\) In contrast, MMP-9 is an enzyme whose activity promotes tumor growth and invasion.\(^3\) In our study, we have shown that both of these markers are elevated in patients with cirrhosis, especially in patients with HCC. There are few reports describing changes in serum levels of PEDF in patients with cirrhosis; decreased level of PEDF in liver tissue was reported in these patients.\(^{16,33}\) Elevated serum PEDF was described in patients with liver steatosis.\(^{18,20}\) As MMP-9 is the enzyme involved in the degradation of PEDF in the liver, it is postulated that there is a relationship of these two markers in certain liver diseases.\(^{33,34}\) There was a decrease of PEDF in liver tissue of humans and animals with alcoholic liver disease with concomitant increase of MMP-9.\(^3\) There is a hypothesis that PEDF inhibits the development of cirrhosis by direct inactivation of the stellate cells and the induction of apoptosis and by stimulating apoptosis in the HCC cells.\(^{35,36}\)

Some studies have confirmed elevated levels of MMP-9 in patients with chronic liver disease.\(^{29,37}\) Studies have also shown a correlation of serum MMP-9 with the progression of liver fibrosis,\(^7\) as well as transaminase levels,\(^{37,38}\) which
was not confirmed in our study. Other authors, in contrast to us, found reduced in serum MMP-9 in patients with chronic hepatitis C and cirrhosis. Many studies have demonstrated higher MMP-9 levels in plasma and in liver tissue in patients with HCC. In tumor tissue, higher expression of this enzyme was detected than in adjacent liver tissue. Hayasaka et al. determined the sensitivity (53%) and specificity (89%) of plasma MMP-9 for the detection of HCC. It was also found that MMP-9 correlated with the progression of cancer: vascular invasion, low tumor cell differentiation, capsule infiltration, the incidence of metastasis, overall stage of HCC, and with poor prognosis. The value of simultaneous determination of MMPs and tissue inhibitors of MMP (TIMPs) is emphasized. The ratios of MMP:TIMP and MMP-9:TIMP-2 are important in the diagnosis and prognosis of HCC.

In our study, we found that serum MMP-9 correlates negatively with serum AFP. Other studies do not confirm these results. Our results can be partially explained by a possible diversity of histopathological forms of HCC in the study group. Carcinoma with low cell differentiation may not produce AFP. In this case, the determination of MMP-9 would increase the likelihood of the diagnosis of HCC.

In the study group, we found significantly higher level of serum PEDF in patients who abuse alcohol (alcoholic or mixed cirrhosis) than that in patients with viral hepatitis-related cirrhosis. Sogawa et al. also confirmed that PEDF levels may be an independent exponent of excessive alcohol consumption. A negative correlation of PEDF and ALT level in our study suggests that PEDF is not a marker of inflammation in the liver.

These are only preliminary studies regarding the potential role of serum PEDF and MMP-9 as adjunctive diagnostic biochemical makers in HCC. The limitations of these tests is that their cut-offs are not defined. Further studies are needed to better characterize the suitability of these tests in clinical practice.

In conclusion, serum PEDF and MMP-9 may aid in the diagnosis of HCC, especially in patients with low AFP, where the diagnosis is questionable. Alcohol consumption can affect the level of serum PEDF. Serum PEDF and MMP-9 are higher in cirrhosis, however, their level does not differentiate the stages of cirrhosis.

Acknowledgement

Grant number 12710 from The National Centre for Research and Development, Ministry of Science and Higher Education, Poland.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Chen JG, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, et al. Screening for liver cancer: Results of a randomised controlled trial in Qidong, China. J Med Screen 2003;10:204-9.
2. Paul SB, Gulati MS, Sreenivas V, Madan K, Gupta AK, Mukhopadhyay S, Acharya SK. Evaluating patients with cirrhosis for hepatocellular carcinoma: Value of clinical symptomatology, imaging and alpha-fetoprotein. Oncology 2007;72(Suppl 1):117-23.
3. Bruix J, Sherman M. Management of hepatocellular carcinoma: An update. Hepatology 2011;53:1020-2.
4. European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: Management of hepatocellular carcinoma. J Hepatol 2012;56:908-43.
5. World Health Organization. Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. March 2015. Available from: URL: http://www.who.int/hiv/pub/hepatitis/hepatitis-b-guidelines/en/. [Last accessed on August 08, 2016].
6. World Health Organization. Guidelines for the Screening, Care, and Treatment of Persons with Hepatitis C Infection. April 2014. Available from: URL: http://www.who.int/hiv/pub/hepatitis/hepatitis-c-guidelines/en/. [Last accessed on August 08, 2016].
7. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int 2010;4:439-74.
8. Singhal A, Jayaraman M, Dhanasekaran DN, Kohli V. Molecular and serum markers in hepatocellular carcinoma: Predictive tools for prognosis and recurrence. Crit Rev Oncol Hematol 2012;82:116-40.
9. Petersen SV, Valnickova Z, Enghild JJ. Pigment-epithelium-derived factor (PEDF) protects motor neurons from chronic glutamate-mediated neurodegeneration. J Neuropathol Exp Neurol 1999;58:719-28.
10. Zhang SX, Wang JJ, Gao G, Shao C, Mott R, Ma JX. Pigment epithelium-derived factor (PEDF) is an endogenous anti-inflammatory factor. FASEB J 2006;20:233-5.
11. Hoshina D, Abe R, Yamagishi SI, Shimizu H. The role of PEDF in tumor growth and metastasis. Curr Mol Med. 2010;10:292-3.
12. Tombran-Tink J, Mazuruk K, Rodriguez IR, Chung D, Linker T, Englander E, et al. Organization, evolutionary conservation, expression and unusual Alu density of the human gene for pigment epithelium-derived factor, a unique neurotropic serpin. Mol Vis 1996;2:11.
13. Acharya SK. Evaluating patients with cirrhosis for hepatocellular carcinoma: Results of a randomised controlled trial in Qidong, China. J Med Screen 2003;10:204-9.
epithelium-derived factor is an intrinsic antifibrosis factor targeting hepatic stellate cells. Am J Pathol 2010;177:1798-811.

17. Famulla S, Lamers D, Hartwig S, Passlack W, Horrigs A, Cramer A, et al. Pigment epithelium-derived factor (PEDF) is one of the most abundant proteins secreted by human adipocytes and induces insulin resistance and inflammatory signaling in muscle and fat cells. Int J Obes 2011;35:762-72.

18. Yilmaz Y, Eren F, Ayyildiz T, Colak Y, Kurt R, Senates E, et al. Serum pigment epithelium-derived factor levels are increased in patients with biopsy-proven nonalcoholic fatty liver disease and independently associated with liver steatosis. Clin Chim Acta 2011;412:2296-9.

19. Stejskal D, Karpiszek M, Svesták M, Hejdák P, Sporová L, Kotolová H. Pigment epithelium-derived factor as a new marker of metabolic syndrome in Caucasian population. J Clin Lab Anal 2010;24:17-9.

20. Yamagishi S, Adachi H, Abe A, Yashiro T, Enomoto M, Furuki K, et al. Elevated serum levels of pigment epithelium-derived factor in the metabolic syndrome. J Endocrinol Metab 2006;91:2447-50.

21. Borg ML, Andrews ZB, Duh EJ, Zechner R, Meikle PJ, Watt MJ. Pigment epithelium-derived factor regulates lipid metabolism via adipose triglyceride lipase. Diabetes 2011;60:1458-66.

22. Chung C, Doll JA, Gattu AK, Shugrue C, Cornwell M, Fitchev P, et al. Anti-angiogenic pigment epithelium-derived factor regulates hepatocyte triglyceride content through adipose triglyceride lipase (ATGL). J Hepatol 2008;48:471-8.

23. Stamenkovic I. Matrix metalloproteinases in tumor invasion and metastasis. Semin Cancer Biol 2000;10:415-33.

24. Hurst NG, Stocken DD, Wilson S, Keh C, Wakelam MJ, Ismail T. Elevated serum level of matrix metalloproteinase-9 (92-kd type IV collagenase/gelatinase B) in hepatocellular carcinoma. Hepatology 2007;97:971-7.

25. Takahara T, Furui K, Yata Y, Jin B, Zhang LP, Nambu S, et al. Dual expression of matrix metalloproteinase-2 and membrane-type 1-matrix metalloproteinase in fibrotic human livers. Hepatology 1997;26:1521-9.

26. Zucker S, Hymowitz M, Conner C, Zarrabi HM, Hurewitz AN, Matrisian L, et al. Measurement of matrix metalloproteinases and tissue inhibitors of metalloproteinases in blood and tissues. Clinical and experimental applications. Ann N Y Acad Sci 1999;878:212-27.

27. Zucker S, Lysik RM, Zarrabi MH, Moll U, M (r) 92,000 type IV collagenase is increased in plasma of patients with colon cancer and breast cancer. Cancer Res 1993;53:140-6.

28. Wu CY, Wu MS, Chiang EP, Chen YJ, Chen CJ, Chi NH, et al. Plasma matrix metalloproteinase-9 level is better than serum matrix metalloproteinase-9 level to predict gastric cancer evolution. Clin Cancer Res 2007;13:2054-60.

29. Hayasaka A, Suzuki N, Fujimoto N, Iwama S, Fukuyama E, Kanda Y, et al. Elevated plasma levels of matrix metalloproteinase-9 (92-kd type IV collagenase/gelatinase B) in hepatocellular carcinoma. Hepatology 1996;24:1058-62.

30. Bruno CM, Valenti M, Bertino G, Arditi A, Consolo M, Mazzarino CM, et al. Altered pattern of circulating matrix metalloproteinases-2,-9 and tissue inhibitor of metalloproteinases-2 in patients with HCV-related chronic hepatitis. Relationship to histological features. Panminerva Med 2009;51:191-6.

31. Ek ET, Dass CR, Choong PF. PEDF: A potential molecular therapeutic target with multiple anti-cancer activities. Trends Mol Med 2006;12:497-502.

32. Korn WM. Moving toward an understanding of the metastatic process in hepatocellular carcinoma. World J Gastroenterol 2001;7:777-8.

33. Chung C, Shugrue C, Nagar A, Doll JA, Cornwell M, Gattu A, et al. Ethanol exposure depletes hepatic pigment epithelium-derived factor, a novel lipid regulator. Gastroenterology 2009;136:331-40.

34. Pollina EA, Legesse-Miller A, Haley EM, Goodpaster T, Randolph-Habecker J, Coller HA. Regulating the angiogenic balance in tissues. Cell Cycle 2008;7:2056-70.

35. Lai IJ, Ho TC. Pigment epithelial-derived factor inhibits c-FLIP expression and assists cigitazone-induced apoptosis in hepatocellular carcinoma. Anticancer Res 2011;31:1173-80.

36. Broadhead ML, Dass CR, Choong PF. Cancer cell apoptotic pathways mediated by PEDF: Prospects for therapy. Trends Mol Med 2009;15:461-7.

37. Capone F, Guerriero E, Sorice A, Maio P, Colonna G, Castello G, et al. Characterisation of metalloproteinas, oxidative status and inflammation levels in the different stages of fibrosis in HCV patients. Clin Biochem 2012:45:525-9.

38. Helaly GF. Differences in circulating MMP-9 levels with regard to viral load and AST: ALT ratio between chronic hepatitis B and C patients. Br J Biomed Sci 2011;68:38-42.

39. Badra G, Lotfy M, El-Refaie A, Obada M, Abdelmonem E, Kandeel S, et al. Significance of serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in chronic hepatitis C patients. Acta Microbiol Immunol Hung 2010;57:29-42.

40. Ariti S, Mise M, Harada T, Furutani M, Ishigami S, Niwano M, et al. Overexpression of matrix metalloproteinase 9 gene in hepatocellular carcinoma with invasive potential. Hepatology 1996;24:316-22.

41. Kim KR, Bae JS, Choi HN, Park HS, Jang KY, Chung MJ, et al. The role of serum response factor in hepatocellular carcinoma: An association with matrix metalloproteinase. Oncol Rep 2011;26:1567-72.

42. Nart D, Yaman B, Yilmaz F, Zeytunlu M, Karasu Z, Kiliç M. Expression of matrix metalloproteinase-9 in predicting prognosis of hepatocellular carcinoma after liver transplantation. Liver Transpl 2010;16:621-30.

43. El Tayebi HM, Salah W, El Sayed IH, Salam EM, Zekri AR, Zayed N, et al. Expression of insulin-like growth factor-II, matrix metalloproteinases, and their tissue inhibitors as predictive markers in the peripheral blood of HCC patients. Biomarkers 2011;16:346-54.

44. Abdel-Moneim SS, Abdou Mostafa EF, Osama A. Circulating matrix metalloproteinase-1 (MMP-1) and tissue inhibitor metalloproteinase-1 (TIMP-1) as serum markers of liver fibrosis in patients with chronic viral hepatitis. Arab J Gastroenterol 2008;9:39-43.

45. Yeh HC, Lin SM, Chen MF, Pan TL, Wang PW, Yeh CT. Evaluation of serum matrix metalloproteinase (MMP)-9 to MMP-2 ratio as a biomarker in hepatocellular carcinoma. Hepatogastroenterology 2010;57:98-102.

46. Ghada F, Helaly GF. Impact of hepatitis B viral load on serum IL-6, MMP-9 and alpha-fetoprotein levels and their correlation with liver injury in chronic hepatitis B infection. Cytokine 2011;53:119-23.