ejpmr, 2020,7(12), 100-104

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THE ROLE OF PENTRAXIN-3 IN NON-INVASIVE DIAGNOSIS OF LIVER FIBROSIS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Article Received on 06/10/2020 **Article Revised on 26/10/2020** **Article Accepted on 16/11/2020**

ABSTRACT

The aim of the study: to determine the diagnostic value of Pentraxin-3 for the stages of fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). **Materials and Methods:** 40 patients with NAFLD were examined, the mean age was (38.0 ± 4.1) years. The control group was created by 20 healthy individuals. There were no statistically significant differences in gender and age distribution of groups. Transient electrography of the liver was performed using an ultrasound scanner - SIEMENS - ACUSON S 3000. Determination of the level of pentraxin-3 in blood plasma was performed using the enzyme-linked immunosorbent assay using the ELISA kit. **Results and Discussion:** It was found that the level of Pentraxin-3 depend on the degree of liver fibrosis stage in patients with NAFLD. There was a significant increase of studied biomarker in the case of progression of liver fibrosis stage. Thus, in patients with liver fibrosis stage F0 the level of pentraxin-3 was (254, 35±44, 4) pg/ml, stage F1 – (421, 9±31, 46) pg/ml, and in stage F2 - respectively (430,9±35,86) pg/ml ($p<0.05$). **Conclusion:** In patients with NAFLD, the blood plasma's level of Pentraxin-3 had a significant tendency to increase with the progression of the liver fibrosis stage ($p<0.05$), which confirms the possibility of using this biomarker in the diagnosis of liver fibrosis stages.

KEYWORDS: nonalcoholic fatty liver disease, liver fibrosis, pentraxin-3, transient elastography.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) remains the leading cause of chronic liver disease worldwide, affecting up to 46% of the world's population.[1]

NAFLD includes two different pathomorphological features and prognosis: non-alcoholic hepatic steatosis and non-alcoholic steatohepatitis (NASH), which is considered an active form of NAFLD. [2] According to various data, NASH develops in 10–40% of patients with NAFLD. In turn, 10-30% of patients with NASH develop cirrhosis of the liver within 10 years - a recognized risk factor for hepatocellular carcinoma.[3]

There is evidence that the incidence of hepatocellular carcinoma in patients with cirrhosis on the background of NASH is about 2.6% annually.[4] The progression of NAFLD is accompanied by the development of successive stages of fibrosis with the formation of cirrhosis and liver cancer, which leads to an unfavorable prognosis for life and short survival of this category of patients.[5]

Fibrogenesis in the liver is a universal pathophysiological process in response to its damage and is characterized by excessive deposition of extracellular matrix as a result of increased synthesis of its components and reducing the rate of their destruction. Disruption of metabolic processes between the blood flowing through the portal vein and hepatocytes leads to the development of hypoxia and the involvement of hepatocytes in the process of fibrogenesis.[6] The prognosis and management of patients with NAFLD largely depends on the severity of liver fibrosis.[7]

For the past 50 years, liver biopsy has been the gold standard for determining the stage of liver fibrosis. This method allows not only to obtain information about the degree of fibrosis, but also to detect other liver damage (inflammation, necrosis, accumulation of iron and copper).[8]

However, numerous limitations of morphological examination are the risk of postoperative bleeding, bacteremia, biliary peritonitis, pneumo- or hemothorax, subcutaneous emphysema, inflammation at the puncture site.[9] Also, many patients refuse to perform a biopsy due to patients' fear of performing a biopsy, pain at the site of the biopsy puncture and the risk of side effects of anesthesia, which leads to delayed initiation of active therapy, progression of NAFLD and prolongation of hospitalization.

Thus, non-invasive methods of diagnosing stages of NAFLD are of increasing interest to scientists and

practitioners. Achieving this goal may reduce the need for a liver biopsy (EASL–EASD–EASO Clinical Practice Guidelines, 2016). Recently, the attention of scientists has been drawn to pro-inflammatory cytokine Pentraxin-3, which is produced by the liver in response to inflammatory mediators and is a systemic response to local inflammation.[10] Several research have shown a significantly higher level of pentraxin-3 in patients with histologically confirmed NASH compared with patients with hepatic steatosis.[11]

Thus, **the aim of this study** was to determine the diagnostic value of pentraxin-3 for the stages of fibrosis in patients with NAFLD.

MATERIALS AND METHODS

The study was conducted on the therapeutic department of the Municipal Health Institution "Lozovsky Territorial Medical Association, Ukraine". This study was approved by the ethics commission of Kharkov National Medical University, Ukraine, in accordance with the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as the object of study" in 1964 (revision in 2008). Patients included in the study signed an informed consent to participate in the study. The study was conducted as part of research work of the Department of Internal Medicine №1 Kharkov National Medical University "Clinical significance of markers of inflammation and metabolic disorders in patients with nonalcoholic fatty liver disease» registration № 015U000236.

40 patients with NAFLD were examined. 40 patients with NAFLD were examined. The mean age was (38.0 ± 4.1) years. They were divided into 3 groups: group A included 13 patients with hepatic fibrosis stage F0, group B included 12 patients with hepatic fibrosis stage F1, and group C included 15 patients with hepatic fibrosis stage F2. Control group (group D) was formed of 20 apparently healthy people.

Inclusion criteria were the following: persistently (at least 6 months) elevated aminotransferases, presence of ultrasonography brightness in liver without any other liver or biliary tract disease.

Exclusion criteria were the following: consume more than 20 g of ethanol daily, serologically confirmed liver infectious diseases (including viral hepatitis A, B and chronic hepatitis C), primary biliary cirrhosis, sclerosing cholangitis, chronic inflammatory diseases, chronic cardiac insufficiency, autoimmune rheumatologic diseases (that may also increase pentraxin-3 level in blood), thyroid disorders, oncology diseases, renal insufficiency. Pregnant women, as well as aged over 55 years were also excluded from the research.

The Ethics Committee of the Kharkov National Medical University approved the study and all participants gave their consent to the study, which was conducted according to the Helsinki Declaration Biological measurements.

NAFLD was diagnosed in accordance with the criteria of the European Association for the Study of the Liver (EASL), the European Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO).

The level of Pentraxin-3 was determined according to the enzyme multiplied immunoassay method using Human Pentraxin-3 ELISA KIT produced by Multisciences (Lianke) Biotech Co. (China) with Immunochem-2100 immunoenzymometric analyzer (USA).

Transient electrography of the liver was performed using an ultrasound scanner - SIEMENS - ACUSON S 3000. Assessment of the severity of liver fibrosis according to transient electrography corresponded to the values of the international scale METAVIR, where F0 - no fibrosis, F1 - portal fibrosis (stellar expansion of the portal tract) without septa, F2 - portal fibrosis and single septa, F3 - portal fibrosis and multiple septa without F4 - cirrhosis

The results statistical processing was performed with Microsoft Office Excel 2013 and Statistica 13.1 computer programs on a personal computer with the use of parametric (Student's t-test) and non-parametric (Mann–Whitney U-test) statistical methods. The relationship between variables was analyzed using Spearman's correlations. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Analysis of metabolic parameters in patients with NAFLD depending on the existing stage of liver fibrosis (Table 1) showed an increase in the studied parameters in accordance with the increase in the stage of hepatic fibrosis. When comparing the indicators of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutaminetranspeptidase (GGT) significantly higher values were demonstrated in groups of patients with hepatic fibrosis stage F2 and F3 compared with hepatic fibrosis stage F0 ($p < 0,05$). The obtained results of the level of alkaline phosphatase (AF) were nonspecific and no significant differences were found ($p > 0.05$).

Table 1: Analysis of metabolic parameters in patients with NAFLD depending on the hepatic fibrosis stage.

Parameters	Group A (n=13)	Group B (n=12)	Group C (n=15)	Group D (n=20)
AST, U/L	43,1 ± 10,2 $p^1 < 0.05$	51 ± 11,4 $p^1 < 0.05$ $p^2 < 0.01$	53 ± 12,8 $p^1 < 0.05$ $p^3 < 0.05$ $p^4 < 0.05$	24,5± 10,4
ALT, U/L	65 ± 21,3 $p^1 < 0.01$	87,2 ± 12,3 $p^1 < 0.05$ $p^2 < 0.05$	103 ± 10,5 $p^1 < 0.05$ $p^3 < 0.05$ $p^4 < 0.05$	22,5± 13,6
GGT, U/L	60,7 ± 21,6 $p^1 < 0.01$	67 ± 10,6 $p^1 < 0.05$ $p^2 < 0.05$	75 ± 16,2 $p^1 < 0.05$ $p^3 < 0.05$ $p^4 < 0.05$	22,5± 11,3
AF, U/L	158,8 ± 34,3 $p^1 < 0.05$	169,5 ± 23,5 $p^1 > 0.01$ $p^2 > 0.05$	173,2 ± 20,8 $p^1 < 0.05$ $p^3 > 0.05$ $p^4 > 0.05$	98± 21,3
Total cholesterol, mg/dL	5,0 ± 0,8 $p^1 < 0.01$	5,8 ± 0,9 $p^1 < 0.05$ $p^2 < 0.05$	6,2 ± 0,5 $p^1 < 0.05$ $p^3 < 0.05$ $p^4 < 0.05$	4,3± 0,5
LDL-C, mg/dL	5,9 ± 1,9 $p^1 < 0.01$	6,3 ± 1,5 $p^1 < 0.01$ $p^2 < 0.05$	6,6 ± 1,3 $p^1 < 0.05$ $p^3 < 0.05$ $p^4 < 0.05$	2,3± 1,7
HDL-C, mg/dL	1,5 ± 2,5 $p^1 > 0.05$	1,2 ± 1,3 $p^1 > 0.01$ $p^2 > 0.05$	1,2 ± 1,5 $p^1 < 0.05$ $p^3 > 0.05$ $p^4 > 0.05$	1,38± 1,4
CRP, mg/l	3.8 ± 3.1 $p^1 < 0.01$	4.1 ± 2.9 $p^1 < 0.01$ $p^2 < 0.05$	4.3 ± 2.3 $p^1 < 0.05$ $p^3 < 0.05$ $p^4 < 0.05$	0.4 ± 0.6

Note: p^1 - the probability of changes compared to the control group;
p^2 - the probability of changes in the group NAFLD with hepatic fibrosis stage F0 compared to the group NAFLD with hepatic fibrosis stage F1;
p^3 - the probability of changes in the group NAFLD with hepatic fibrosis stage F0 compared to the group NAFLD with hepatic fibrosis stage F2
p^4 - the probability of changes in the group NAFLD with hepatic fibrosis stage F1 compared to the group NAFLD with hepatic fibrosis stage F2.ALT - alanine aminotransferase;
AST - aspartate aminotransferase;
ALT - alanine aminotransferase;
GGT - γ-glutaminetranspeptidase;
AF - alkaline phosphatase;
LDL-C - Low-density lipoprotein cholesterol;
HDL-C - High-density lipoprotein cholesterol;
CRP — C-reactive protein;

Also, similar results were obtained when assessing the lipid profile, where the indicators of total cholesterol and Low-density lipoprotein cholesterol (LDL-C) increased in accordance with the progression of the hepatic fibrosis stage when compared between groups with different stages of fibrosis ($p < 0,05$) and control group ($p < 0.01$).

C-reactive protein (CRP) level analysis evidenced it's increase in the group of patients with hepatic fibrosis stage F0, F1 and F2 ($p < 0.05$) compared to the control group ($p < 0.01$). Also there was the significant difference of this parameter between the groups of patients with hepatic fibrosis ($p < 0.05$).

Analysis of the dynamics of Pentraxin-3 level depending on the hepatic fibrosis stage in patients with NAFLD (Fig. 2) showed a significant increase the studied biomarker in the case of progression of fibrosis.

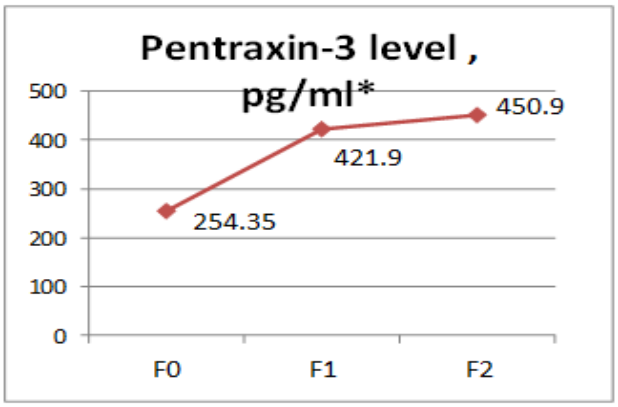

Fig. 2 Dynamics of pentraxin-3 levels depending on the degree of liver fibrosis in patients with NAFLD.

* The difference in the indicators of the main group is statistically significant (p <0,05).

Thus, in patients with hepatic fibrosis stage F0 the level of Pentraxin-3 was (254.35 ± 44.4) pg/ml, in stage F1 – (421.9 ± 31.46) pg/ml, and in stage F-2 - respectively (450.9 ± 35.86) pg/ml.

Pentraxin-3 is a novel biological marker of fibrogenic processes in liver. In a study (2016), it was investigate that Pentraxin 3 can be a new noninvasive marker for prediction of liver fibrosis in patients with NAFLD. It was shown that elevated plasma Pentraxin-3 levels are associated with the presence of fibrosis in patients with NAFLD.[12]

Narciso-Schiavon. et al. (2017) demonstrated that the median Pentraxin-3 level was significantly higher in stable cirrhotic patients compared to controls. The results of that study indicate the potential for use of Pentraxin-3 as an inflammatory biomarker for the prognosis of patients with hepatic fibrosis and cirrhosis.[13]

Previous studies demonstrate that Pentraxin-3 has been shown to affect tissue remodelling and fibrosis by stabilizing all types of amyloid fibrils and by regulating monocyte to fibrocyte differentiation.[14]

These data are also consistent with our study findings. According to our results, there is a close association between elevated Pentraxin-3 levels and the formation of liver fibrosis in patients with NAFLD. Furthermore, our data are also consistent with the literature findings which have shown the potential importance of serum Pentraxin-3 levels as compared to some other markers in the determination of clinical diagnosis and prognosis of hepatic fibrosis and cirrhosis.

CONCLUSIONS

In patients with NAFLD, the blood plasma's level of Pentraxin-3 had a significant tendency to increase with the progression of the liver fibrosis stage ($p<0.05$), which confirms the possibility of using this biomarker in the diagnosis of liver fibrosis stages.

ACKNOWLEDGEMENT

We thank Oksana Chervona and Timur Alexandtov for their technical assistance. This work was supported by a research work of the Department of Internal Medicine №1 Kharkov National Medical University "Clinical significance of markers of inflammation and metabolic disorders in patients with nonalcoholic fatty liver disease».

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