

Serum Proinflammatory Cytokines and its Relationship to ¹³C-Methacetin Breath Test Results in Patients with Chronic Hepatitis

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ABSTRACT

Background: Further development and implementation of methods for early noninvasive diagnosis for prognosis and treatment efficiency in patients with chronic hepatitis and development of effective methods of conservative therapy is an important problem of modern hepatology.

Objective: To identify the correlation of proinflammatory cytokines [tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6)] with the results of ¹³C-methacetin breath test (¹³C-MBT) as an indicator of fibrosis in patients with chronic viral hepatitis (CVH), nonalcoholic steatohepatitis (NASH) and alcoholic hepatitis (AH).

Materials and methods: A total of 147 patients with chronic hepatitis and 25 healthy controls were studied. Patients were divided into three groups: I (group CVH): 50 patients with CVH B and C; II (group NASH): 49 patients with NASH; III (group AH): 48 patients with AH. TNF- α and IL-6 was determined by ELISA. Liver biopsy was performed in 46 patients, ¹³C-MBT in 57 patients. A total of 96 patients with F2-F4 Metavir were selected to study the antifibrotic action of losartan: 33 patients with CVH, 33 patients with NASH, 30 patients with AH. Patients were divided into two subgroups. The first subgroup (39 patients) received standard treatment (depending on the etiology of disease). The second subgroup included 57 patients who received standard treatment and antifibrotic therapy (losartan) in a dose of 50 mg daily. Controlled treatment period was 24 weeks.

Results and discussion: Found an inverse correlation between the cumulative dose of ¹³CO₂ and serum levels of TNF- α in patients with CVH ($r = -0.87$, $p = 0.001$), NASH ($r = -0.84$, $p < 0.01$), AH ($r = -0.72$, $p < 0.01$), and IL-6 in patients with CVH ($r = -0.65$, $p = 0.001$), NASH ($r = -0.74$, $p < 0.01$), AH ($r = -0.73$, $p < 0.01$). According to ¹³C-MBT the hepatocytes mass in F3 Metavir patients increased by 34% after the use of losartan, in comparison with the results obtained after the application of basic drugs. For F2 Metavir, this difference was 7%.

Conclusion: Serum TNF- α and IL-6 associated with hepatocyte mass and degree of liver dysfunction. Long-term losartan use (for 6 months) in a daily dose of 50 mg in treatment of patients with CVH, NASH and AH followed by improvements in ¹³C-MBT, indicating an increase functioning hepatocyte mass.

Keywords: Chronic hepatitis, Methacetin breath test, Cytokines, Losartan.

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INTRODUCTION

Recently published data on pathogenesis of hepatitis and liver fibrosis (LF) demonstrate that liver damage almost

always accompanied by immune system dysfunction.^{1,2} It is also known that the proinflammatory cytokines as tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) play important role in the development of liver disease. Clinical studies have demonstrated the role of these cytokines in the pathogenesis of chronic viral hepatitis (CVH),^{1,3,4} nonalcoholic steatohepatitis (NASH)⁵ and alcoholic hepatitis (AH).^{2,6}

At the present stage it is necessary to further improve the determination of the factors influencing the course of chronic hepatitis and the development of LF. It should focus on the impact of cytokine changes for effectiveness of treatment and disease prognosis in patients with CVH, NASH and AH, and to assess the relationship between different methods of noninvasive diagnosis of LF.

OBJECTIVE

To identify the correlation of proinflammatory cytokines (TNF- α and IL-6) with the ¹³C-MBT results in patients with CVH, NASH and AH.

MATERIALS AND METHODS

A total of 147 patients with chronic hepatitis and 25 healthy controls were studied. Patients were divided into two groups. The first group (CVH group) consisted of 50 patients with CVH B and C, the average age (36.96 ± 1.66) years, 28 men and 22 women. The second (NASH group) included 49 patients with NASH, the average age (47.06 ± 1.88), 32 men and 17 women. The third group (AH group) was included 48 patients with AH, the average age (46.25 ± 1.95) years, 31 men and 17 women.

Clinical diagnoses of CVH B and C, NASH, AH are set according to the recommendations of European Association for the Study of the Liver (EASL). Questionnaire GAGE and AUDIT was used when collecting medical history.

Special laboratory tests included the determination of serum levels of TNF- α and IL-6 by enzyme-linked immunosorbent assay.

Percutaneous needle liver biopsy was performed in 46 patients. LF measured by Metavir scoring system.

¹³C-methacetin breath test (¹³C-MBT) was performed in 57 patients. Table 1 shows the normal and pathological total ¹³CO₂ concentration and its relationship to the functioning hepatocyte mass.

Table 1: Evaluation ^{13}C -MBT results

| Total concentration of $^{13}\text{CO}_2$ to 120 min, (%) | Interpretation of results |
|---|--|
| 20-35 | Normal liver function (FGM—100%) |
| 10-20 | Moderate hepatic dysfunction without cirrhotic changes (FGM—50-100%) |
| 2-10 | Severely impaired liver function with cirrhotic changes (FGM—20-50%) |
| >2 | Severely impaired liver function with cirrhotic changes (FGM <20%) |

A total of 96 patients with F2-F4 Metavir were selected to study the antifibrotic action of losartan: 33 patients with CVH, 33 patients with NASH, 30 patients with AH. Among the patients were 59 men and 37 women aged 18 to 77 years, mean age (43.33 ± 1.27) years. Patients were divided into two subgroups. The first subgroup (39 patients) received standard treatment: patients with CVH—antiviral therapy according to conventional protocols, patients with NASH—ursodeoxycholic acid in the average daily dose of 30 mg/kg/d and atorvastatin 20 mg/d, patients with AH—ademetionine at dose of 800 mg intravenously slowly over 14 days, followed by oral administration at dose of 1.600 mg per day (divided into two doses).

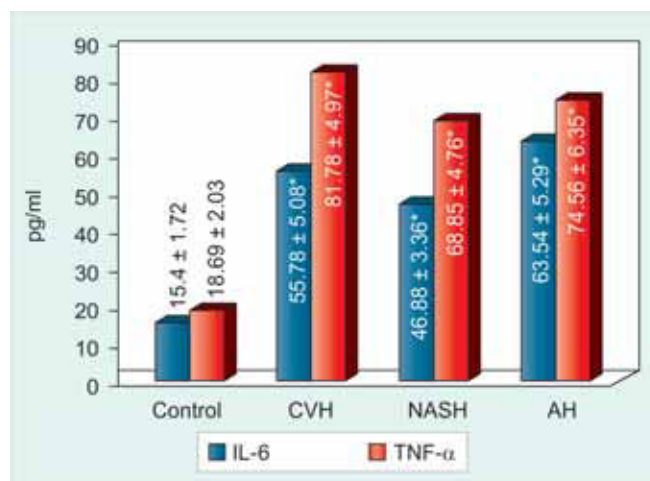
The second subgroup included 57 patients who received the basic therapy (depending on the etiology of the disease) and antifibrotic therapy (losartan) at daily dose of 50 mg. Controlled treatment period was 24 weeks. The effectiveness of the treatment was monitored by the dynamics of the clinical syndromes and laboratory parameters. Laboratory parameters including TNF- α and IL-6 were determined at baseline and after 24 weeks.

All studies were carried out on the certified equipment held metrological control, using certified test kits and consumables.

^{13}C -MBT data were expressed as means with standard deviation. Comparisons between patients with various liver diseases were performed using the Mann-Whitney U test (i.e. nonparametric data). Significance of correlations was determined by the Spearman rank method. Statistical significance was assumed at a p-value of <0.05.

RESULTS AND DISCUSSION

The first stage of this study was to examine the content of TNF- α and IL-6 in serum of patients with CVH, NASH and AH. Also analyzed the relation of proinflammatory cytokines with the results of the ^{13}C -MBT. The average value of TNF- α in group CVH was (81.78 ± 4.97) pg/ml; in group NASH was (68.85 ± 4.76) pg/ml; in group AH was (74.56 ± 6.35) pg/ml. The average level of IL-6 in group CVH was (55.78 ± 5.08) pg/ml, in group NASH was (46.88 ± 3.36) pg/ml; in group AH was (63.54 ± 5.29) pg/ml (Graph 1).



Graph 1: Mean levels of TNF- α and IL-6 in groups CVH, NASH and AH. *The difference between a control group and an experimental group, $p < 0.01$

The data indicate that for CVH B and C, NASH, AH including outcome in LF accompanied by an increased concentration of TNF- α , IL-6 in serum ($p < 0.01$ compared with control group).

During analysis of cytokine levels a direct relationship between TNF- α and IL-6 in patients with CVH ($r = 0.62$, $p < 0.01$), NASH ($r = 0.86$, $p < 0.01$) and AH ($r = 0.78$, $p < 0.01$) was detected.

There was a decrease of cumulative dose of $^{13}\text{CO}_2$ in patients with CVH, NASH and AH with increase ALT activity, indicating liver dysfunction and reduced percentage of functioning hepatocytes. In addition, cumulative dose $^{13}\text{CO}_2$ reduction accompanied by the increase of TNF- α concentration in patients with CVH ($r = -0.87$, $p = 0.001$), NASH ($r = -0.84$, $p < 0.01$), AH ($r = -0.72$, $p < 0.01$) and IL-6 in patients with CVH ($r = -0.65$, $p = 0.001$), NASH ($r = -0.74$, $p < 0.01$), AH ($r = -0.73$, $p < 0.01$). It concluded that TNF- α and IL-6 levels in patients with chronic hepatitis reflects the degree of liver function impairment.

In patients with CVH with normal liver function (FHM = 100%) of the ^{13}C -MBT results (Table 2), serum concentrations of TNF- α and IL-6 was (46.75 ± 6.28) pg/ml and (27.13 ± 9.17) pg/ml, respectively, exceeded 2.5 (for TNF- α) and 1.8 (for IL-6) times to control. If abnormal liver function (FHM less than 100%) of the ^{13}C -MBT results was a sharp rise in serum concentrations of proinflammatory cytokines. Thus, the level of TNF- α and IL-6 was in these patients (99.04 ± 8.36) pg/ml and (69.44 ± 9.39) pg/ml, respectively, exceeded 5.3 (for TNF- α) and 4.5 (for IL-6) times to control.

TNF- α and IL-6 serum concentrations in NASH patients with normal liver function (FHM = 100%) on the results of ^{13}C -MBT was (28.26 ± 6.27) pg/ml and (33.06 ± 6.98) pg/ml, respectively, exceeded 1.5 (for TNF- α) and 2.1

Table 2: TNF- α and IL-6 concentrations in patients with normal and impaired liver function according to the ^{13}C -MBT, M \pm m

| Groups | ^{13}C -MBT | TNF- α , pg/ml | IL-6, pg/ml |
|--------|--|-----------------------|-------------------|
| CVH | Normal liver function (FHM = 100%) | 46.75 \pm 6.28 | 27.13 \pm 9.17 |
| | Impaired liver function (FHM less than 100%) | 99.04 \pm 8.36 | 69.44 \pm 9.39 |
| NASH | Normal liver function (FHM = 100%) | 28.26 \pm 6.27 | 33.06 \pm 6.98 |
| | Impaired liver function (FHM less than 100%) | 52.95 \pm 6.89 | 82.76 \pm 8.91 |
| AH | Normal liver function (FHM = 100%) | 51.89 \pm 9.67 | 42.28 \pm 7.69 |
| | Impaired liver function (FHM less than 100%) | 158.62 \pm 15.54 | 107.32 \pm 8.85 |

(for IL-6) times to control. In patients with abnormal liver function (FHM less than 100%) on the results of ^{13}C -MBT was a sharp rise in serum concentrations of proinflammatory cytokines. Thus, TNF- α and IL-6 levels in these patients was (52.95 \pm 6.89) pg/ml and (82.76 \pm 8.91) pg/ml, respectively, exceeding the 2.8 (for TNF- α) and 5.4 (for IL-6) times to control.

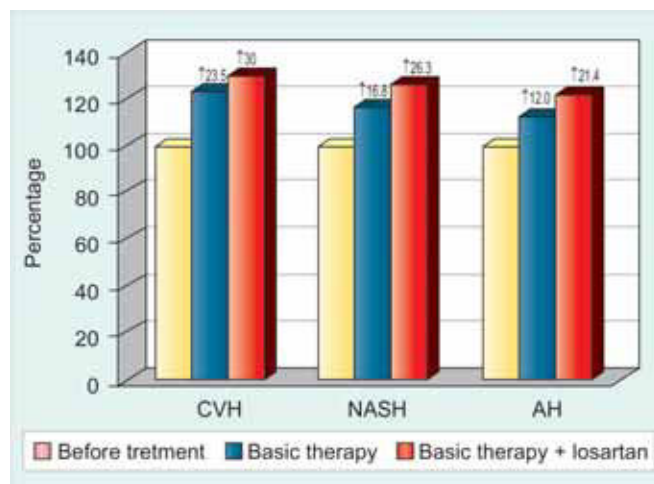
TNF- α and IL-6 serum concentrations in AH patients with normal liver function (FHM = 100%) on the results of ^{13}C -MBT was (51.89 \pm 9.67) pg/ml and (42.28 \pm 7.69) pg/ml, respectively, exceeding by almost three times to control. In patients with abnormal liver function (FHM less than 100%) on the results of ^{13}C -MBT was spike proinflammatory cytokines serum concentration. TNF- α and IL-6 levels in these patients was (158.62 \pm 15.54) pg/ml and (107.32 \pm 8.85) pg/ml, respectively, exceeding the 8.7 (for TNF- α) and 7.1 (for IL-6) times to control.

Thus, TNF- α and IL-6 serum concentration associated with hepatocytes amount and degree of liver dysfunction, as evidenced by cytokines differences in subgroups of patients with normal and impaired liver function on the results of ^{13}C -MBT, which is effective, dynamic and quantitative noninvasive test for the identification of 'fatty liver,' liver cirrhosis with corresponding transport deterioration of liver function and its metabolic capacity.⁷ Negative correlation between the studied cytokines and total $^{13}\text{CO}_2$ concentration is evidence in favor of this statement.

The presented data confirm the high importance of TNF- α and IL-6 to predict the course and outcome of chronic viral hepatitis, NASH and AH.

The second stage of this study was to investigate the losartan efficacy in chronic hepatitis of various etiologies under the control of proinflammatory cytokines levels and ^{13}C -MBT.

It has been found that use as basic treatment so use losartan with basic treatment in CVH, NASH and AH patients leads to a significant reduction in studied proinflammatory cytokines concentration, C-reactive protein and improve ^{13}C -MBT results. It was found that ^{13}C -MBT results have increased by 30% in the CVH group under basic and antifibrotic therapy. For comparison, this result under basic therapy was 23.5% (Graph 2).



Graph 2: ^{13}C -MBT results in patients with CVH, NASH and AH under various therapy regimens (in %)

NASH basic therapy led to an improvement ^{13}C -MBT by 16.8%, whereas, the use of losartan improved the ^{13}C -MBT results at 26.3%.

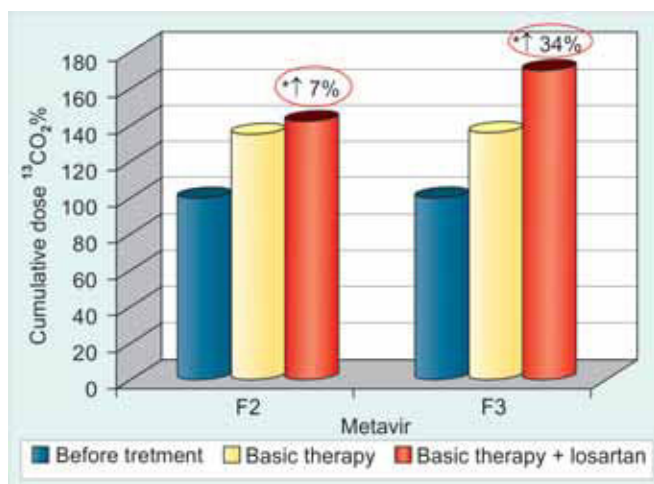
Losartan use in group of patients with AH was associated with improved ^{13}C -MBT results at 21.4%, and basic therapy use increased this test by 12.9%.

Comparative analysis of studied therapy regimens effect in patients with different Metavir fibrosis stages, it has been found that the use of losartan with basic therapy reduces proinflammatory cytokine levels by 4 to 20% more than basic drugs.

At all stages of LF losartan improves basic therapy effectiveness against fibrogenesis. According to ^{13}C -MBT hepatocytes mass after losartan use increased by 34% in F3 Metavir patients, compared with the results obtained after basic drugs application. For F2 Metavir this difference was 7% (Graph 3).

On the basis of presented data it can be concluded that in patients with CVH, NASH and AH there are increased TNF- α and IL-6 levels in response to the progression of LF, which is accompanied by abnormal liver function according to ^{13}C -MBT.

Use basic therapy in combination with losartan in these patients is to reduce studied proinflammatory cytokines concentration and increases functioning hepatocytes mass.



Graph 3: ¹³C-MBT results in patients with chronic hepatitis under the different therapy regimens depending on Metavir liver fibrosis stage (in %). * – The difference compared with the results obtained after basic drugs application

CONCLUSION

1. Serum TNF- α and IL-6 is associated with hepatocytes mass and liver dysfunction degree, as evidenced by the differences in studied cytokines levels in subgroups of patients with normal and decreased liver function according to ¹³C-MBT, as well as an inverse correlation between ¹³CO₂ cumulative dose and serum TNF- α in patients with CVH ($r = -0.87$, $p = 0.001$), NASH ($r = -0.84$; $p < 0.01$), AH ($r = -0.72$, $p < 0.01$); and serum IL-6 in patients with CVH ($r = -0.65$, $p = 0.001$), NASH ($r = -0.74$, $p < 0.01$), AH ($r = -0.73$, $p < 0.01$).
2. Long-term, for 6 months, losartan use in a daily dose of 50 mg in treatment of patients with CVH, NASH and AH followed by a more significant improvements in ¹³C-MBT results, indicating functioning hepatocytes mass increase.

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