Medical and Biological Sciences, 2013, 27/1, 33-38

ORIGINAL ARTICLE / PRACA ORYGINALNA

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ASSESSMENT OF CONCENTRATION OF TGF-ß IN WOMEN WITH ALCOHOL DEPENDENCE

OCENA STĘŻENIA TRANSFORMUJĄCEGO CZYNNIKA WZROSTU (TGF-ß) W SUROWICY KRWI KOBIET UZALEŻNIONYCH OD ALKOHOLU

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Summary

Alcohol disease is one of the most significant factors for development of fibrosing of the liver Fibrosing process is related to reconstruction of extracellular matrix and precedes the occurrence of cirrhosis. HSC activation is followed by production of ECM elements (2, 3, 5, 7, 8, 9, 10). In the course of fibrosing process, growth factors (mainly TGF, PDGF) and other cytokines responsible for storage, distribution and biological activity of ECM proteins, are involved (3, 11, 12,13). Among growth factors, TGF-β appears to be a key mediator in human fibrogenesis. In HSC TGF-β favors the transition to myofibroblast-like cells, stimulates the synthesis of ECM proteins, and inhibits their degradation. Liver fibrosis is a result of chronic damage to the liver conjunction with the accumulation of ECM proteins, which is a characteristic of most types of chronic liver diseases.

Material and methods. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gammaglutamyltransferase (GTP) transforming growth factor beta (TGF-B) were investigated during this study. Study group consists of 40 inpatients treated in Inpatients Clinic in Toruń. Control group consists of 35 healthy women.

Results. Mean age of females in the study group was 43+/-7 yrs, length of dependence 8+/-6 yrs, AST 32,88+/-32,95, ALT 29,76+/-24,48, GTP 57,21+/-128,4 U/l. The concentration of TGF-β was significantly higher in alcohol dependent female group compared to the healthy subjects (683,45 vs 69,18; p=0,028).

Conclusions. These results imply that prolonged alcohol abuse leads to an increase of concentration of TGF-β as a result of hepatic cirrhosis.

Streszczenie

Choroba alkoholowa jest jednym z czynników o największym znaczeniu dla rozwoju włóknienia wątroby. Patomechanizm włóknienia wątroby jest taki sam niezależnie od etiologii, próbuje się wiec prowadzić próby monitorowania przebiegu patologii wątroby wyrażanej również jako włóknienie tego narządu u osób nadużywających alkoholu poprzez oznaczanie stężenia niektórych składników macierzy pozakomórkowej(extracellular matrix – ECM) lub produktów ich metabolizmu we krwi.

Wielopłaszczyznowość i złożoność czynników biorących udział, leżących u podstaw tak procesu włóknienia jak i regeneracji powoduje poszukiwanie wskaźników najlepiej oddających diagnostycznie stan zaawansowania schorzenia.

Spośród wielu cytokin wpływających na aktywacje HSC wydaje się największe przypisuje się TGF-ß (transforming growth factor ß)

Celem niniejszej pracy była ocena stężenia TGF-ß u pacjentek z zza i próba oceny jego użyteczności diagnostycznej w ocenie stanu wątroby u osób z zza .

Badaniami objęto grupę 40 kobiet z zza leczonych w WOTUiW(Wojewódzki Ośrodek Terapii Uzależnień i Współuzależnień) w Toruniu. Badane kobiety nie były uzależnione od innych prócz alkohol(z wyjątkiem nikotyny) substancji psychoaktywnych; średni wiek 43 lata, średni czas uzależnienia 8 lat. Grupę kontrolną stanowiło 35 zdrowych kobiet. U badanych wykonano oznaczenia aktywności AST , ALT, GTP, stężenia TGF-ß.

Wiek pacjentek wynosił 43+/-7 lat, czas uzależnienia 8+/-6 lat, aktywność enzymów (U/l): AST 32,88+/-32,95, ALT 29,76+/-24,48 , GTP 57,21+/-128,4 U/l. Stężenie TGF-ß było znamiennie wyższe u kobiet z ZZA wobec kobiet zdrowych (683,45 vs 69,18; p=0,028).

Dalszego wyjaśnienia wymaga prognostyczna wartość przydatność TGF-ß jako markera aktywności i chorób przebiegających z włóknieniem.

Key words: alcohol dependence, liver disease
Słowa kluczowe: zespół zależności alkoholowej, choroby wątroby

INTRODUCTION

Alcohol disease is one of the most important factors in development of fibrosing of the liver. . Patomechanism of fibrosing remains the same regardless of the etiology. Therefore, there are attempts to monitor the course of liver pathology, also in a form of fibrosing of this organ, in subjects abusing alcohol, by determination of concentration of particular components of extracellular matrix (ECM), or products of their metabolism in the blood. Fibrosing is a consequence of disturbance in balance between synthesis of ECM components and their degeneration. Multifariousity and complexity of factors underlying processes of fibrosing, as well as regeneration, lead to the search of markers that most accurately represent the state of advancement of the disease in terms of diagnosis. The conducted study contributes to these research directions since TGF-ß belongs to the group of factors which stimulate synthesis of ECM elements.

Alcohol disease is one of the most significant factors for development of fibrosing of the liver. Fibrosing process is related to reconstruction of extracellular matrix and precedes the occurrence of cirrhosis (1).

Currently, it is known that regardless of the primary cause of chronic liver damage, the patomechanism of fibrosing is similar and undergoes the general schema of tissue repair process (2,3,4).

Constant effect of damaging factor leads to multiple recurrence of reparation cycle which leads to excessive accumulation of connective tissue's elements. The damaging factor induces a cascade of mechanisms leading to activation of HSC (hepatic stellate cells) and their transformation to myofibroblasts which produce larger amounts of ECM (extracellular matrix) in the damaged liver. Significantly lower amounts of these particles are produced by hepatocytes and sinus endothelial cells (2, 3, 4, 5, 6, 7). Several models of HSC activation and course of fibrosing process have been introduced. In general, at the first stage of activation, an initiation, a pre-inflammatory stage takes place, caused mainly by paracrine stimuli from adjacent liver cells which sensitize HSC to effect of cytokines. Consequently, consolidation and continuation of fibrosing process take place related to paracrine as well as autocrine intercellular transmission.

HSC activation is followed by production of ECM elements (2, 3, 5, 7, 8, 9, 10). In the course of fibrosing process, growth factors (mainly TGF, PDGF) and other cytokines, responsible for storage, distribution and biological activity of ECM proteins are involved (3, 11, 12, 13). Among growth factors, TGF-β appears to be a key mediator in human fibrogenesis. In HSC, TGF-β favors the transition to myofibroblast-like cells, stimulates the synthesis of ECM proteins, and inhibits their degradation. Liver fibrosis is a result of chronic damage to the liver conjunction with the accumulation of ECM proteins, which is a characteristic of most types of chronic liver diseases (14).

Among many cytokines influencing the HSC activation, the biggest significance is ascribed to TGF-ß (transforming growth factor ß). Stimulation of the process by TGF-ß always takes place (1,2), whether TGF-ß is a direct factor stimulating the proliferation of HSC and transformation to myofibroblasts or a factor causing direct intensification of expression of genes responsible for synthesis of particles- ECM elements (5). Since the patomechanism of liver fibrosing remains the same, regardless of etiology, there are attempts to assess fibrosing with non-invasive tests based on examination of several components of extracellular matrix or products of their metabolism in the blood (4).

The aim of this study was to assess the density of TGF-ß and the activity of AST, ALT, GTP which are considered to be markers a liver damage in female patients with alcohol dependence.

SUBJECTS

The study included 40 female subjects with alcohol dependence, treated in WOTUiW in Toruń. Enrolled subjects were not dependent of any psychostimulants, except alcohol (not including nicotine); mean age 43 years old, mean duration of dependence 8 years. The control group consisted of 35 healthy women, which, based on screening, were qualified as healthy and not abusing alcohol.

RESULTS

Studied subjects' clinical and biochemical data: age 43±7, duration of dependence: 8±6, activity of enzymes: GGT 57.21±128.4, AST 32.88±32.95, ALT 29.76±2448. Studied subjects' data was gathered in table 1.

Table 1. *Demographic and biochemical data of patients*
Tabela 1. *Dane demograficzne i biochemiczne pacjentek*

Examined parameter	X	S	Range
Age (years)	43.41	6.88	30-57
Addition period (years)	8.21	5.55	5-20
TGF pg/ml	683.45	1600.991	
AST U/l	32.88	32.95	11-190
ALT U/l	29.76	24.48	7-118
GGT U/l	57.21	128.4	8-756
MCV fl	96.48	5.23	86.1-108.2

Comparison of the achieved results was presented in table 2.

Table 2. *TGF concentrations in the study and control group*
Tabela 2. *Porównanie stężenia TGF-ß w grupie badanej i kontrolnej*

Examined parameter	Study group	Control group	p
	X ± S	X ± S	
TGF pg/ml	683.45±1600.991	69.18±288.022	0.028

In the next step of the statistical analysis, studied patients were divided based on MCV values and activity of enzymes: AST, ALT, GGT. One of the groups included subjects with correct values of assessed enzymes and MCV, the other one included subjects with values beyond the normative data. Subsequently, concentration of TGFß, values of age and duration of dependence were assessed in both groups. The comparison of TGFß concentration scores in groups with normal and high MCV value and AST, ALT and GGT activity, did not reveal any statistically significant differences in any of the configurations. Achieved results were presented in tables 3, 4, 5, 6, respectively.

Table 3. *TGF concentrations in patients with AST activity within the norm and above the norm*
Tabela 3. *Porównanie stężenia TGF-ß u pacjentek o aktywnościach AST w normie i powyżej normy*

Examined parameter	Patients with AST activity within the norm	Patients with AST activity above the norm	p
	X ± S	X ± S	
TGF pg/ml	1018.37±1946.23		
Age (years)	44.83±6.45	39.13±6.71	0.040
Addition duration (years)	7.84±5.20	9.38±6.78	0.505

Table 4. *TGF concentrations in patients with ALT activity within the norm and above the norm*
Tabela 4. *Porównanie stężenia TGF-ß u pacjentek o aktywnościach ALT w normie i powyżej normy*

Examined parameter	Patients with ALT activity within the norm	Patients with ALT activity above the norm	p
	X ± S	X ± S	
TGF pg/ml	1060.81±1976.24		
Age (years)	44.83±6.45	39.13±6.71	0.040
Addition duration (years)	8.58±5.78	7.22±5.07	0.539

Table 5. *TGF concentrations in patients with GGT activity within the norm and above the norm*
Tabela 5. *Porównanie stężenia TGF-ß u pacjentek o aktywnościach GTP w normie i powyżej normy*

Examined parameter	Patients with GGT activity within the norm	Patients with GGT activity above the norm	p
	X ± S	X ± S	
TGF pg/ml	957.02±1956.13	186.57±439.13	0.189
Age (years)	44.0±7.74	42.91±6.38	0.698
Addition duration (years)	7.95±5.58	8.64±5.95	0.753

Table 6. *TGF concentrations in patients with MCV activity within the norm and above the norm*
Tabela 6. *Porównanie stężenia parametrów u pacjentek o wartościach MCV w normie i powyżej normy*

Examined parameter	Patients with MCV activity within the norm	Patients with MCV activity above the norm	p
	X ± S	X ± S	
TGF pg/ml	580.27±1648.14	995.32±1910.18	0.533
Age (years)	43.62±7.78	42.67±6.04	0.705
Addition duration (years)	7.57±5.41	8.89±5.83	0.519

In the last chapter, values of age, duration of dependence, MCV and AST, ALT iand GGT activity were compared, with regard for the level of TGFß concentration. This was done by excluding subjects with undeterminable level of TGFß concentration and comparing values of age, duration of dependence and AST, ALT and GGT activity with those achieved by the rest of the group. It was observed that subjects with undeterminably low concentration of TGFß were significantly older, but did not differ from the others with regard to the duration of dependence, MCV, and AST, ALT and GGT activity. The scores were presented in table 8.

Table 7. *Values of MCV TGH concentrations, activity of AST, ALT and GGT in patients with addition duration shorter than 10 years and longer than 10 years*

Tabela 7. *Porównanie wartości MCV, stężenia TGF, aktywności AST, ALT, GGT u pacjentek o czasie uzależnienia krótszym niż 10 lat i o czasie uzaleznienia10 lat i dłuższym*

Examined parameter	Addiction duration <10 years	Addition duration >10 years	p
	X ± S	X ± S	
TGF pg/ml	824.31±1965.83	732.45±1306.68	0.894
AST U/l	36.35±38.50	24.9±11.68	0.367
ALT U/l	31.65±27.14	25.40±17.30	0.509
GGT U/l	77.43±169.30	42±042.69	0.544
MCV fl	96.62±6.13	96.17±5.77	0.847

Table 8. *Values of MCV, duration of addition, AST, ALT and GGT activity in patients with undetectable values of TGF and others*

Tabela 8. *Porównanie wartości MCV, wieku czasu uzależnienia, aktywności AST, ALT, GGT u pacjentek o nieoznaczalnych wartościach TGF i pozostałych*

Examined parameter	Patients with TGF=0	Patients with TGF>0	p
	X ± S	X ± S	
Age (years)	42.33±6.48	46.7±6.56	0.091
Addition duration (years)	7.77±5.26	9.50±6.43	0.428
AST U/l	35.91±37.47	20.5±5.46	0.209
ALT U/l	31.23±21.42	17.7±5.23	0.060
GGT U/l	60.96±144.84	50.91±91.58	0.833
MCV fl	95.48±6.41	97.89±5.09	0.409

DISCUSSION

Alcohol abuse, next after viral infections, is one of the most common causes of liver diseases in Poland, (8). A significant role in solving the alcoholism related problems is played by sensitive and specific laboratory diagnosis. Alcohol influences many processes in the organism. Since liver, due to its role in alcohol metabolism and detoxication, is an organ mostly exposed to toxic influence of alcohol, most of the tests applied are aimed at the assessment of liver activity (15).

Epidemiological data concerning the course of liver diseases caused by abuse of alcohol is similar; however, the character and intensity of functional changes may be different. In all alcohol dependent subjects the metabolic function of liver is altered (16).

It is known that the first step of alcohol-induced liver disease is fatty degeneration of the liver, which may progress to inflammation of the liver. Cirrhosis is he last step. According to different authors, it is estimated that fatty degeneration of the liver develops in about 21% (17), or almost all subjects abusing alcohol (18); inflammation of liver in about 10% (15) or 17% (19). The cirrhosis develops, according to different data, in 15-20% of patients (16, 18), in 7.4 of patients (17).

Some diversity of epidemiological data is probably caused by the fact that the specific feature of alcohol-induced liver damage is the occurrence of symptoms depending, to a large degree, on individual variance, which is dependent on many not definitively explained factors (17). In some individuals, despite permanent alcohol use, only functional disturbances occur with no damage of liver accompanied by morphological changes (16).

The most characteristic consequence of alcohol abuse is fatty degeneration of the liver. It is reversible, although often co-occurs with fibrous alterations which may lead to cirrhosis (17). There is no reason to state that fatty degeneration of the liver leads to chronic alterations in form of fibrosis and cirrhosis, however, alcohol, an etiological factor for fatty degeneration, is simultaneously a substance inducing fibrosing and various kinds of damage to liver cells (16). Alcohol induced damage of the liver does not occur in all subjects abusing alcohol. Even those regularly consuming large amounts of alcohol develop damage of the liver only in 77% (16). Fibrosing of the liver is a dynamic process depending on intensity and duration of damage to the organ. It is accepted that the crucial examination of diagnosis of fibrosing of the liver is its biopsy. Due to the invasiveness of this examination, there are attempts to assess condition of the liver with less invasive methods – determination of biochemical markers of developing fibrosing process is implemented (5, 11).

The contribution of cytokines in the course of liver diseases has not been definitively explained. However, it is known that they act profibrogenous, as well as antifibrogenous (3). Influence of many cytokines on activation of lipocytes and their transformation to myofibroblasts producing largest amounts of ECM is considered (3, 5). The transforming growth factors induce fibrosing (7, 10, 13, 14). In normal conditions, the levels of pro-inflammatory cytokines and the inflammatory response decrease once the infection is under control. If the levels of inflammatory cytokines remain elevated, the chronic inflammation ensues. This is the case in alcohol induced chronic inflammation of the liver which can be a precursor to fibrosis and cirrhosis (20, 21).

The biggest significance is ascribed to TGF-ß. One of the most important functions that TGF-ß play in organism is the influence on composition of ECM extracellular substance, related to fibrosing processes. Within the liver, TGF-ß inhibits proliferation of hepatocytes, stimulates synthesis of extreacellular matrix proteins and plays a significant role in the process of apoptosis (1, 3, 12, 13, 14, 15).

It has been shown that the concentration of this cytokine in the liver increases significantly in course of liver diseases, proportionally to the degree of advancement of fibrosing (3, 22). Similarly, Mazur et al. (1) confirmed the increased concentration of TGF-ß in patients with chronic inflammation of the liver. In the own study, according to data from the literature, the concentration of TGF- ß was significantly higher in the alcohol dependent women compared to the healthy subjects, what may indicates the intensification of changes within the liver.

However, advancement of the liver disease seems to be undeterminable using concentration of TGF-ß as marker or diagnosing parameter. Prognostic value of TGF-ß as a marker of activity and diseases with fibrosing in course need further explanation.

CONCLUSIONS

1. Significantly higher concentration of TGF-ß in alcohol dependent patients compared to healthy subjects has been observed.
2. Determination of TGF-ß concentration in blood serum of alcohol dependent patients is a test of small usefulness in assessment of advancement of liver disease.
3. Prognostic value of TGF-ß as a marker of activity and diseases with fibrosing in course need further explanation.

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Received: 11.05.2011
Accepted for publication: 31.08.2012