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TUMOR-NECROTIZING FACTOR α AND ITS ROLE IN PATHOGENESIS OF HEART AND GALLBLADDER DISORDERS

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Abstract. Tumour-necrotizing factor α (TNFα) is a key proinflammatory cytokine, which may increase in patients with heart failure, Alzheimer's disease, psoriasis, or inflammatory bowel diseases. TNFα decreases the absorptive function of the gallbladder (GB) wall and takes part in GB changes from hyperplasia of mucous membrane to carcinoma. The aim of this study was to estimate the TNFα concentration in serum and its correlation with GB status and cardiovascular disorders.
Materials and methods. We have estimated TNFα level in 20 patients, who were divided into group 1 (intact GB) and group 2 (GB changes). The results were analysed with the help of Statistica 5.0 software (USA).
Results and discussion. In the case of GB changes the serum TNFα concentration of the included patients was 81.7% higher than in case of intact GB ($p<0.05$), which confirms the activation of TNFα production by biliary system cells. We also uncovered a strong correlation ($r=0.96$, $p<0.05$) of serum TNFα level with GB wall thickness in the group 2. ECGs of patients with GB disorders and elevated TNFα were characterized by the signs of increase of left atrium electric activity and pre-excitation.
Conclusions. 1) Blood concentration of TNFα depends on GB condition. 2) GB changes and elevated levels of serum TNFα are accompanied by the significant elongation of electrical systole of ventricles and the domination of electrical activity of left heart chambers.

Key words: tumour-necrotizing factor α, gallbladder, cardiovascular disorders.

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Introduction

Tumour-necrotizing factor α (TNFα) is a key proinflammatory cytokine of humans. It was first discovered as a death mediator of neoplastic cells, but now it is known as a regulator of progress, proliferation, angiogenesis, invasion and metastasis development of tumours [1–4]. Apart from that, this cytokine of monocyte and macrophage origin mediates such systemic processes as shock and tissue damage [5], cachexia, anaemia and chronic inflammation [6, 7]. TNFα is often called a conductor of pro-inflammatory cytokines cascade [8,9]. Clinical value of TNFα has not been completely established yet. Serum TNFα concentration was shown to increase in patients with heart failure, tumours, depression and Alzheimer's disease, psoriasis, inflammatory bowel diseases etc., so TNFα as a biochemical marker lacks nosological specificity. Scarce and heterogeneous clinical data underline the need of future studies on TNFα clinical value.

The role of TNFα in gallbladder (GB) disorders has not been thoroughly investigated . It is known that the biliary epithelium of mice synthetizes and secretes TNFα at bacterial lipopolysaccharide exposure [10]. Apart from that, TNFα together with interleukin-1α can directly decrease the absorptive function of the GB wall, consequently promoting gallstones formation [11]. The proportion of GB cells with expression of TNFα mRNA increases in parallel with the evolution of GB changes from hyperplasia of mucous membrane to its dysplasia and GB carcinoma. Hence, it can be speculated that TNFα is involved in evolution of cholelithiasis with chronic inflammation to GB carcinoma [12]. Some polymorphisms of TNFα gene (–308(G/A)) can influence predisposition to GB cancer even in the absence of cholelithiasis. It is interesting that this tendency was revealed especially in women [13]. Apart from that, it was reported that TNFα is able to stimulate lymphangiogenesis of GB carcinoma by the means of increasing the expression of vascular endothelial growth factor [14].

During the last decade the interest of scientists in TNFα has significantly increased. It can be explained by the fact that TNFα became a target of treatment by its antibodies (infliximab, adalimumab etc). The aim of this study was to estimate the TNFα serum concentration in patients with GB disorders and its correlations with heart conditions.

Materials and methods

TNFα serum concentration was estimated by the immunoenzyme method using Stat Fax 303 Plus apparatus (USA) in 20 patients with chronic obstructive pulmonary diseases without clinical signs and/or established diagnosis of cardiovascular disease, which were further divided into 2 groups: with intact GB (n=8, group 1) and with GB changes, revealed with the help of ultrasonography (n=12, group 2). These changes included biliary sludge; signs of prior cholecystitis, thickened GB wall, GB neck or body deformations; GB stones or prior cholecystectomy. The groups were compared according to age, sex, diagnosis, anthropometric parameters, clinical parameters of haemodynamics, as well as the sonographic sizes of the liver, spleen and pancreas (Table 1).

Table 1: Clinical parameters of investigated patients

Parameter, units of measurement	Group 1 (intact GB), n=8	Group 2 (GB changes), n=12	p
Sex (coded as 1 – male, 2 – female)	1.75±0.25	1.33±0.14	>0.05
Age, years	52.26±5.75	61.17±4.41	>0.05
Forced vital lung capacity, %	71.72±7.31	70.75±6.31	>0.05
Vital lung capacity, %	78.35±6.60	73.59±7.01	>0.05
Forced expired volume in 1 second, %	67.72±12.50	65.61±7.50	>0.05
Liver, right lobe, mm	149.75±15.12	154.00±6.04	>0.05
Liver, left lobe, mm	75.00±4.34	72.27±4.42	>0.05
Pancreas, head, mm	26.00±1.87	22.78±1.78	>0.05
Pancreas, body, mm	17.50±0.96	15.43±1.15	>0.05
Pancreas, tail, mm	23.00±0.91	19.97±1.36	>0.05
Spleen, length, mm	95.50±7.58	108.09±3.44	>0.05
Spleen, width, mm	37.00±3.58	45.23±3.08	>0.05
Systolic blood pressure, mm Hg	137.50±6.29	143.00±7.33	>0.05
Diastolic blood pressure, mm Hg	82.50±2.50	87.58±3.83	>0.05
Heart rate, bpm	76.00±3.81	77.50±2.44	>0.05

Electrocardiogram records and analysis of the participants of both groups were conducted with the help of "Yukard-200" apparatus ("Yutas", Ukraine). Patients with clinical signs of arrhythmias and ischemia were excluded from the study. The results were analysed with the help of Statistica 5.0 software ("Statsoft", USA). After a normality check we used parametric statistical methods. The results were considered significant if $p < 0.05$.

Results

The results of ECG analysis of both groups are represented in the Table 2. We noticed that QT interval and its corrected values were significantly higher in patients with GB changes. This can certify that these patients are more prone to various arrhythmias.

It was also revealed that group 2 patients were characterized by thicker GB wall and longer GB length (Table 3).

Correlation analysis showed that TNFα directly correlated with GB wall thickness (Table 4), whereas other correlations of TNFα revealed to be insignificant (Table 4).

Table 2: ECG parameters of investigated patients

Parameter, units of measurement	Group 1 (intact GB), n=4	Group 2 (GB changes), n=12	p
QRS, ms	97.25±6.00	105.50±0.50	>0.05
PR, ms	151.50±8.77	123.50±17.29	>0.05
QT, ms	365.25±9.78	482.25±41.74	<0.05
QTc, ms	412.25±5.14	553.25±49.08	<0.05
P, °	64.10±7.30	43.67±5.24	<0.05
QRS, °	15.97±23.11	17.37±25.95	>0.05
T, °	67.00±21.58	38.22±10.13	>0.05
PR, ms	151.50±8.77	123.50±17.29	<0.05

Table 3: GB parameters of investigated patients

Parameter, units of measurement	Group 1 (intact GB), n=4	Group 2 (GB changes), n=12	p
GB wall thickness, mm	2.07±0.13	3.55±0.03	**<0.05**
Cystic duct width, mm	4.63±0.58	5.74±0.69	>0.05
GB length, mm	53.33±2.02	66.27±3.29	**<0.05**
GB width, mm	27.33±1.20	25.70±2.09	>0.05

Table 4: Correlations of TNFα

Parameters	r	P
TNFα – cystic duct	0.99	>0.05
TNFα – pancreas body	0.94	>0.05
TNFα – GB wall thickness	0.96	<0.05

Discussion

According to the literature, TNFα plays a significant role not only in pathogenesis of tumours, psychic and nervous system diseases, psoriasis, inflammatory bowel diseases but also in cardiovascular disorders development. In particular, mice with the TNFα gene knockout were characterized by less prominent apoptosis and unfavourable heart remodelling in the experimental model of myocardial infarction [15]. Moreover, an increase of concentration of soluble receptors to TNFα is associated with the increase of mortality and cardiovascular events in general population after standardization according to the levels of other inflammatory markers [16–23]. A.C. Carlsson et al. (2018) established that the association between TNFα serum concentration and cardiac diseases does not depend on renal function, classic factors of cardiovascular risk nor pharmacotherapy [14]. It was also shown that TNFα serum concentration directly correlates with heart failure incidences [24–27]. TNFα was shown to mediate negative inotropic effects both in vitro and in vivo [28–30], acting like a cardiodepressant. There are some reports on associations of sympathetic overdrive (chronic activation of β-adrenoreceptors) with hyperproduction of TNFα [31–34]. Particularly, it was established that the usage of β-blockers significantly decreases expression of TNFα in myocardium [35]. This fact draws the attention of scientists to this class of medications.

TNFα concentration of the patients was characterized by significant variations (from 0.8 to 8.2 pg/ml), which can explain differences of TNFα levels in analysed referen-

ces [36,37]. It seems important that in case of GB changes serum TNFα concentration was by 81.7% higher than in case of intact GB (2.18±0.37 and 1.20±0.24 pg/ml consequently, $p<0.05$), which confirms activation of TNFα production by pathological biliary system cells, which was described above, and can play a pathogenetic role in cardiovascular disorders. The above hypothesis is confirmed by the finding of a strong correlation of TNFα with GB wall thickness in the group 2 ($r=0.96$, $p<0.05$) (Table 4) Hence, GB condition influences serum TNFα level, and GB disorders may provoke TNFα hyperproduction, consequently, setting off pathological mechanisms, which were described above.

To check the hypothesis of a relation of GB condition with serum TNFα concentration and cardiac diseases, we analysed screening parameters of cardiovascular system in patients with GB disorders and high level of TNFα. It was revealed that the groups with normal and elevated TNFα did not differ in systolic and diastolic pressure (Table 1). However, in case of elevated serum TNFα concentration (above 6 pg/ml) and GB disorders we noticed a trend of mean systolic pressure increase above the normal values. Mean systolic pressure was in the range of the first grade of arterial hypertension, whereas other parameters exceeded the norm by 2-6% only.

We also found a significant difference in the results of automatized ECG interpretation. ECGs of patients with GB disorders and elevated TNFα were characterized by significantly smaller angle of P wave axis ($43.67 \pm 5.24^\circ$ vs. $64.10\pm7.30^\circ$, $p<0.05$) (Table 2) and lower velocity of supraventricular conduction (123.50 ± 17.29 ms vs. 151.50 ± 8.77 ms, $p>0.05$) (Table 2), which can be a sign of increase of left atrium electric activity and pre-excitation, which was diagnosed in 50% of participants of this group. Such changes of electrical activity of atria were accompanied by the change of electrical systole of ventricles. GB disorders and elevated serum TNFα were associated with significant elongation of electric systole (by one–third: 32–34%). This tendency was noted for both absolute (482.25 ± 41.74 ms vs. 365.25 ± 9.78 ms, $p<0.05$) and corrected (553.25 ± 49.08 ms. vs. 412.25 ± 5.14 ms, $p<0.05$) values (Table 2), which exceeded the normal level (440 ms). Elongation of electric systole is often associated with myocardial ischemia and is concerned to be a predictor of life–threatening arrhythmias [38]. The length of ventricular complex did not differ between the groups, but in the second group it exceeded the normal value in all patients (105.50±0.50 ms vs. 97.25±6.00 ms, $p>0.05$) (Table 2). The mean angle of T wave axis in group 2 was lower by 43% ($38.22 \pm 10.13^\circ$ vs. $67.00 \pm 21.5^\circ$, $p>0.05$) (Table 2), which can be a sign of repolarization retardation in the left ventricle as a result of asymptomatic ischemia. Consequently, according to automatized analysis of ECG parameters, GB changes and high levels TNFα are accompanied by the significant elongation of the electric systole of ventricles (QT and its corrected value) and domination of electrical activity of left heart chambers. These changes can be considered indirect signs of asymptomatic ischemia and a marker of high risk of arrhythmias.

Taking into account the above mentioned facts and tendencies, it can be stated that there is a need for future studies of TNFα action mechanisms, estimation of influence of traditional medications on TNFα production and optimization of indications for TNFα antagonists' usage in clinical practice. As for cardiology, we think that the scientific community has to review the usage of ursodeoxycholic acid, which regulates cell signalling pathways in the heart and protects it from hypoperfusion damage [39] together with inhibition of pathological signalling pathway TNFα/caspase8/caspase3 in GB [40]. It can also be helpful to review the role of β–blockers, which were shown to decrease the concentration of TNFα and its soluble receptors in patients with dilatational cardiomyopathy [41].

Conclusions

1. Serum concentration of TNFα is characterized by significant fluctuations and depends on GB condition. Serum concentration of TNFα significantly increases in case of pathological changes of GB, which can set off various mechanisms of heart disorders.
2. According to automatized analysis of ECG, GB changes and elevated serum concentrations of TNFα are accompanied by the significant elongation of electrical systole of ventricles and domination of electrical activity of left heart chambers, which can be considered signs of asymptomatic ischemia and markers of high risk of arrhythmias.

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