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Markers of the oxidative stress and antioxidant system in pulmonary drug susceptible and drug resistant tuberculosis

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Abstract

Background: The oxidative stress biomarkers in tuberculosis were studied, but the differences between the pulmonary drug susceptible and drug resistant forms were not identified.

Material and methods: A prospective, case-control study, which included 51 patients, distributed in 2 groups: the 1st study group (N=24 new cases with drug susceptible tuberculosis) and the 2nd group (N=27 new cases with MDR-TB) similar distributed according to the sex and age were compared with a control group constituted from 36 healthy persons. The intensity of the oxidative stress was appreciated through the serum concentration of the advanced oxidation protein products, lysosomal marker N-acetil-β-D-glucosaminidase, advanced glycation end-products (AGE_s). The antioxidant defense was assessed through the total serum antioxidant activity, the activity of the glutathione enzymes and proteins with antioxidant role.

Results: In MDR-TB carbohydrate peroxidation biomarker versperlysines-like AGE_s was diminshed, as well as the serum antioxidant defense assessed through CUPRAC method. In drug susceptible tuberculosis was established the elevation of the protein peroxidation, high lysosomal membrane damage and increased acute phase protein – ceruloplasmine. Antioxidant enzyme glutathione S-transferase had lower activity in both types of tuberculosis which contributed to the increasing of the γ-glutamyltransferase.

Conclusions: The oxidative stress level was more elevated in drug susceptible tuberculosis and antioxidant defense was more impaired in the MDR-TB group. The antioxidant biomarker – glutathione S-transferase activity, was lower in both types of tuberculosis which increased the γ-glutamyltransferase activity. The polymorphism assessment of the glutathione S-transferase enzyme is important for the individualized therapy and reducing the toxicity of the anti-tuberculosis treatment.

Key words: tuberculosis, oxidative stress, antioxidant system.

Introduction

Tuberculosis evolution and treatment response are determined by the mycobacteria virulence, protective mechanisms and the organism's capacity to fight against the aggression of the oxidative stress through the antioxidant defense [3]. The oxidative stress (OS) is caused by the imbalance between the systemic manifestation of the reactive oxygen species (ROS) and the biological system's ability to detoxify them and to repair the resulting damage [7]. ROS are produced from molecular oxygen following the normal cellular metabolism. As well as, numerous exogenous factors are the sources of the ROS: smoking, ozone exposure, hyperoxia, ionizing radiation, intoxication with heavy metals and some drugs. ROS are divided into 2 groups: free radicals and non-radicals. From the group of the free radicals there are: O_2^- (superoxide radical), OH^-(hydroxyl radical), H_2O_2 (hydroxyl peroxide), ROOH (organic hydroperoxide), RO^- and ROO^- (alkoxy and peroxy radicals), HOCl (hypochlorus acid) and $ONOO^-$ (peroxynitrite) [22]. The biggest physiological damage determines superoxide radical (O_2^-), hydroxyl radical (OH^-) and hydroxyl peroxide (H_2O_2). At the biochemical level severe OS lowers the effectiveness of the enzymatic and non-enzymatic antioxidant defense [7]. The disturbances in the normal redox system cause toxic effects through the peroxidation of the cellular DNA, proteins, lipids, carbohydrates and other biological macromolecules [22]. Mild OS causes cell apoptosis and severe OS– necrosis and functional impairment. Many pathological disturbances and diseases are attributed to the OS: cancer, atherosclerosis, hyperthension, respiratory distress syndrome, chronic obstructive pulmonary disease, asthma, pulmonary fibrosis and neurological disturbances [6, 8, 18, 23]. Advanced oxidation protein products (AOPP) are considered novel markers of the OS [5]. They result from the interaction between the chlorine oxidants (chloramines and hypochlorus acid) with plasmatic proteins [26]. AOPP are carried by oxidized plasma proteins, especially albumin, are excreted by the kidneys and the highest concentrations were identified in patients with severe chronic renal failure, hyperparathyroidism and continuous treatment with calcium and vitamin D [24]. High concentrations of the AOPP are correlated with high level of the advanced glycation end products (AGE_s), resulting from the non-enzymatic glycation of the proteins, lipids and nucleic acid, following the "Maillard" chain of reaction [10]. High AGE_s concentration is identified in patients with diabetes mellitus and represents a marker of the hyperglycemia, non enzymatic glycosilation of the proteins and excessive activation of the polyol way [18]. Close correlation was found between high levels of AOPP and AGE_s in monocyte-mediated inflammatory processes [3].

Lysosomal enzymes are mediators of the tissue damage in any type of inflammation [3].

N-Acetyl-β-glucosaminidase (NAG) is a high molecular-weight (~140 kDa) hydrolytic lysosomal enzyme, iden-

tified in many tissues. It breaks chemical bonds of glycosides and amino sugars that form structural components of the tissues and is localized in various parts of the cell, including the cell membrane. There are two main isoenzymes: isoenzyme-A and isoenzyme-B, being different in their heat sensitivity and stability. Isoenzyme-A is contained in the azurophilic granules of polymorphonuclear leukocytes and is excreted during the exocytosis. Isoenzyme-B is localized in the lysosomal membrane and is excreted during lysosomal damage [25]. High activity of NAG is a marker of tissue damage, inflammation and loss of lysosomal integrity [25].

The antioxidant system is composed by the hydrophilic antioxidant compounds identified in the cytoplasm and blood serum, as well as the hydrophobic compounds, which are localized in the biological membranes [7]. Gluthatione (GSH) is the most important antioxidant in animals, plants and bacteria. It prevents the damage of the cellular components produced by ROS and heavy metals. GSH reduces disulfide bonds formed within cytoplasmic proteins and cysteines serving as an electron donor. In the redox process the GSH is converted in its oxidized form, called glutathione disulfide (GSSG.) Once oxidized, the GSH can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio between reduced glutathione (GSH) to oxidized glutathione (GSSG) within cells is used as a measure of the cellular oxidative stress [19]. Glutathione S-transferase (GST) is a cytosolic, mitochondrial and microsomal enzyme, involved in the detoxification of the xenobiotics through the conjugation catalysis of the reduced glutathione in mercapturic compounds [11]. Assessment of the GST activity is a diagnostic tool of the OS and efficiency of the detoxification mechanisms related to GST [1, 14, 16]. The γ-glutamyltransferase (*γ-GPT*) is a key-enzyme of the γ-glutamyl cycle, which catalyses the transfer of the γ- glutamyl group from the glutathione to other amino acids [1, 4]. It is localised in the cell's membrane, and the enzyme's active centre at the exterior cell's border. It has a major role in the detoxification of the inflammatory mediators (cytokines, acute phase proteins), carcinogenic substances and toxine [3]. The synthesis of the *γ-GPT* is induced by drugs, colestasis, alcohol consumption, hepatic tumors and cirrhosis [1]. Actually, the *γ-GPT* is considered advanced biomarkers of the OS [9].

Drug susceptible TB is treated with first line anti-tuberculosis drugs: isoniazid, rifampicin, ethambutol, pirazinamide and streptomycin [27]. Tuberculosis determined by the multidrug resistant strains (MDR-TB) is treated during 18-24 months with 2nd line antituberculosis drugs according to the drug susceptibility test [28]. The standard treatment for MDR-TB consists in injectable antibiotics – aminoglycozides (kanamycin, amikacin or capreomycin) and orally administrated anti-tuberculosis drugs: fluoroquinolones (levofloxacin, moxifloxacin or gatifloxacin), ethionamide, prothionamide, paraaminosalicylic acid and cycloserine) [28]. The rate of adverse drug events, usually correlated with the OS is much higher in patients treated with second line anti-TB drugs, patients with TB-HIV co-infection, than in those treated with first line drugs [12, 26]. The aim of the study was the assessment of the oxidative stress biomarkers and antioxidant system compounds in patients with pulmonary drug susceptible and drug resistant tuberculosis and their comparison with a group of healthy persons.

Material and methods

It was realized a prospective research evaluating the biochemical markers of the OS in 87 cases, from which 24 were new cases with drug susceptible pulmonary tuberculosis included in the 1st study group and 27 were MDR-TB patients which were included in the 2nd study group. The groups were compared between them and were compared with a control group (CG) composed from 36 healthy persons assessed according to the clinical and biochemical criteria. The research reported ethics committee approval (nr. 14 of 21/11/2017) and patients' consent was obtained. Patients were diagnosed in the medical specialized institutions of Chisinau during the period 01.01.2016-31.08.2016. Included criteria in the study group were: age more than 18 years old, patient diagnosed with pulmonary tuberculosis, new case type, the diagnosis confirmed through the conventional microbiological methods (microbiological examination and molecular genetic test of the sputum). The study investigation schedule included information about sex, age, radiological aspects, microbiological patient's status, treatment regimen and adverse drug reactions. The included criteria in the control group were: age more than 18 years old, conditioned healthy persons according to the clinical examination, blood test (complete blood count) and biochemical tests (liver transaminases, bilirubin test, hepatitic virus serological tests, HIV serology).

The biochemical investigation of patients was performed during the intensive phase of the treatment. The 36 healthy persons from the control group were investigated in ambulatory conditions. The estimation of the biochemical indices in the serum was performed using the methods with microquantities of the evaluated material. The samples were analysed by spectrophotometry in the maximum standardization conditions. Total proteins were determined using the Lowry modified method [9]. The AOPP were analysed according to the Witko-Sarsat V. modified method [24]. The AGEs were quantified in two types: pentosidine-like AGEs and vesperlysines-like AGEs [17, 21]. The micromethod was based on the fluorescence measure of the intensity of the samples diluted in the phosphate tampon at λexc 335 nm, λem 385 nm (quantification of the pentosidine-like AGEs) and at λexc 370 nm, λem 440 nm (quantification of the vesperlysines-like AGEs) according to the Sero L. modified method [10, 20].

The determination of the total antioxidant activity (tAOA) was performed using two procedures: 1) method based on the degradation of the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical at the interac-

tion with serum compounds with the antioxidant properties and measure of the decreasing absorbance at 734 nm [9]; 2) method CUPRAC (Cupric Ion Reducing Antioxidant Capacity) based on the reducing capacity of the cupric ion through the captation of the hydroxyl radical [20].

The serum antioxidant capacity was assessed by the dosage of the glutathione enzymes (GST and γ-GTP) activity using the analysis kits of the Eliteh (France) producer, according to the attached instructions.

Statistical analysis was carried out by the comparative assessment of the quantitative and qualitative peculiarities of the selected patients using the Microsoft Excel XP programme. Accumulated material was systematized in simple and complex groups. For the testing of significant differences between the studied indices of the compared samples it was performed the statistic non-parametric t test and the significance threshold $p<0.05$.

Results

By distributing patients according to the biological characteristics was established a similar rate of men and women in all three groups, with the predomination of men in the same proportion, which permitted the comparability of the results. The same proportion of young persons, aged less than 44 years old, was established in all groups. All enumerated conditions permitted the comparability of the laboratory data (tab. 1).

Table 1

Segregating patients in sex and age groups

Biological segregation	parameters	1st SG (N=24)	2nd SG(N=27)	CG (N=36)
		N (%)	N (%)	N (%)
Sex stratification	Men	14 (58%)	18 (67%)	24 (67%)
	Women	10 (42%)	9 (33%)	12 (33%)
Stratification in age groups	18-44 years	18 (75%)	21 (77%)	29 (81%)
	≥45 years	6 (25%)	5 (23%)	7 (19%)

Detected by passive way, using standard tools (microbiological examination and chest X-ray) for the investigation of the symptomatic patients were 15 (62%) cases of the 1st SG and 17 (63%) cases of the 2nd SG. The main proportion of both study groups was constituted from the patients with pulmonary infiltrative TB: 22 (91%) in the 1st SG and 24 (89%) in the 2nd SG. Radiological investigations identified lung destruction in all selected TB patients. Microbiological status was positive in all patients and drug susceptibility testing permitted their distribution according to the obtained drug resistance results. Standard treatment for drug susceptible TB was administrated in patients from the 1st SG and standard treatment for MDR-TB in patients from the 2nd SG. There were no major adverse drug reactions identified in the selected patients.

Biomarkers of the OS constituted the blood concentration of the AOPP, the activity of the NAG, the concentration of the pentosidine-like AGEs and vesperlysines-like AGEs. Assessing the concentration of the AOPP was established a statisticaly higher level in the 1st SG compared with the CG ($p<0,05$) and unsignificantly higher concentration in the 2nd SG comparated with the CG. No differences were established between the study groups. The NAG activity was significantly higher in the 1st SG compared with the 2nd SG, as well as compared with the CG. In the 2nd SG the NAG activity was similar with the results obtained for the CG. The concentration of the pentosidine-like AGEs was significantly lower in the 1st SG compared with the 2nd SG and compared with the CG. The level of vesperlysines-like AGEs was significantly lower in the 2nd SG compared with the 1st SG and compared with the control group ($p<0,001$). Data are shown in the table 2.

The antioxidant defense was evaluated through the glutathione-related enzymes, non-enzymatic proteins and total antioxidant activity (tAOA) of the serum. The serum level of the glutathione-S-transferase (GST) was significantly lower in both study groups compared with the control group at the same statistical level. The activity of γ-glutamyltranspeptidase (γ-GPT) was significantly increased in both study groups in comparison with the control

Table 2

Indices of the oxidative stress

Oxidative system	Parameter	1st SG (N=24)	2nd SG(N=27)	CG (N=36)
		M±SE (%)	M±SE (%)	M±SE (%)
Proteic peroxidation	*AOPP* μMol/l	45.07±22,15(131%) □	38.42±15,77(111%)	34.349±3,58 (100%)
	NAG mMol/s.L	87.79±42.17(133%)■□	66.29±22.27(101%)	65.88±18.63(100%)
Carbohydrate peroxidation	*Pentosidine-like AGEs*	140,79±73,63(66%) ○	216,56±151,64(100,8%)	208,5±16,27(100%)
	Vesperlysines-like AGEs	469,89±166,13(93%)○●	273,82±106,83(62%) ◊	343,2±49,63(100%)

Note: AOPP – advanced oxidation protein products; NAG – N-acetyl-β-D-glucosaminidase; AGEs – Advanced glycated end-products. The percentage was assessed comparing the study groups with the reference value of the control group (100%). Comparison between study groups ● $p<0.001$ ▪ $p<0.05$; comparison between the 1st SG and CG ○ $p<0.001$ □ $p<0.05$; comparison between the 2nd SG and CG ◊< 0.001.

Table 3

Indices of the antioxidant defense

Antioxidant system	Parameter	1st SG (N=24)	2nd SG(N=27)	CG (N=36)
		N (%)	N (%)	N (%)
Glutathione-related enzymes	Glutathione S-transferase nMol/s. L	16.650±5,98 (77%) ○	16.222±8,52 (75%) ◊	21.5±6.75 (100%)
	γ-Glutamyltransferase U/l	62.95±6.56 (147%)Δ	78.24±3.58 (183%)●	427±7.02 (100%)
Total antioxidant activity (tAOA)	Method ABTS mMol/l	0.65±0.03 (91%)	0.67±0.03 (94%)	0.71±0.004 (100%)
	Method CUPRAC mMol/l	1.42±1.49 (276%) Δ●	0.59±0.47 (88%)	0.52±0.04 (100%)
Non-enzymatic antioxidants	Ceruloplasmine mg/ml	911.31±210.70 (125%) □	852.101±256.1 (117%)	724.3±27.8 (100%)
	Total protein	59.21±4.95 (103%)	59.53±2.18 (103%)	57.1±2.3 (100%)

Note: The percentage was assessed comparing the study groups with the reference value of the control group (100%). Comparison between study groups ● p<0,001 ▪ p<0.05; comparison between the 1st SG and CG Δ p<0.001○ p<0.01 □ p<0.05; comparison between the 2nd SG and CG ◊< 0.01.

group. The index was significantly higher in the 2nd SG compared with the 1st SG. The total antioxidant activity (tAOA) of the serum assessed through the ABTS and CUPRAC methods established different results. tAOA evaluated using ABTS method was nonsignificantly lower in both study groups compared with the CG, without differences between groups. Assessing tAOA using CUPRAC method, was identified a significantly higher level in the 1st SG and a mild decreasing tendency in the 2nd SG compared with the CG. Comparing the groups of patients it was identified a statistically higher level of the tAOA using CUPRAC method in the 1st SG compared with the 2nd SG. The concentration level of the ceruloplasmine, known as a protein of the acute phase, with antioxidant role was detected significantly higher in the study groups compared with the CG. The concentration of the total serum proteins (albumin, α1, α2, β and γ globulins), which include and those with an antioxidant role was lower in CG compared with both study groups. No differences were identified between study groups (tab. 3).

Discussion

Distributing the patients, according to the sex and age, it was determined the predomination of the men at reproductive age (18-44 years) in both study groups, as well as in the control group, which allowed the comparability of the results.

Two thirds of both study groups were detected by using standard microbiological examination and chest X-ray investigation. Similar data were obtained in the national studies. The majority of both study groups was diagnosed with pulmonary infiltrative TB with lung destruction. Microbiological status was positive in all patients and drug susceptibility testing permitted their distribution according to the obtained drug resistance results. Standard treatment for drug susceptible TB was administrated in patients from the drug susceptible group and standard treatment for MDR-TB in patients from the 2nd SG. The regimens were used according to the WHO recommendations [27, 28].

The biomarker of the OS – the concentration of the AOPP was higher in the drug susceptible TB group, compared with the MDR-TB group and healthy group demonstrating a higher level of the oxidative damage and protein catabolism in the 1st SG. In the speciality literature, comparable studies about AOPP concentration in patients with drug susceptible and MDR-TB have not been identified. The serum concentration of the pentosidine-like advanced glycation end-products determined a significantly lower concentration in patients with drug susceptible tuberculosis compared with the patients with MDR-TB and healthy group. The results confirmed the state of organism's starvation and exacerbation of catabolic processes assessed through the AOPP dosage. The serum level of vesperlysines-like advanced glycation end-products was much lower in the group of MDR-TB patients, compared with the drug-susceptible group. Both biomarkers of the carbohydrate peroxidation demonstrated the disturbances in the glycemic metabolism, which cannot be compared with the data issued from the international studies where enrolled diabetic patients with tuberculosis were. The high activity of the NAG is considered a biomarker of the xenobiotic-induced lysosomal membrane damage. The activity level was significantly higher in the drug susceptible tuberculosis group compared with the patients with MDR-TB and healthy group. It demonstrated the sustained oxidative damage of the leukocytes by the acute inflammatory process. No similar studies were found in the international review. The biomarker of the antioxidant defense – total antioxidant activity assessed using ABTS method was mildly decreased in the groups of tuberculosis-patients; on the other hand, CUPRAC method established contradicto-

ry results. So, in the drug susceptible group AOA was three times higher and in MDR-TB group it was slightly decreased compared with the healthy group. It could be explained by different components of the serum involved in the antioxidant defense identified through the ABTS and CUPRAC methods. The concentration of the ceruloplasmine, acute phase proteine with antioxidant role, was higher in the drug susceptible group compared with the MDR-TB and control group, and confirmed a higher toxic damage due to acute inflammatory process, as did the lysosomal marker (NAG), ceruloplasmine and advanced oxidation protein products.

The activity of the glutathione S-transferase enzyme (GST) was significantly diminished in the group of patients with tuberculosis. The literature data correlated the epigenetic disturbances with the low level of the GST gene expression, especially of the isoformes associated with the hepatotoxicity of the anti-tuberculosis drugs [15]. In consequence, the level of γ- glutamyltransferase at patients with both types of tuberculosis was significantly increased.

Conclusions

1. In patients with drug susceptible tuberculosis was established the increased level of the protein peroxidation, lysosomal membrane damage and acute phase protein.
2. In patients with MDR-TB were identified severe disturbances of the carbohydrate peroxidation evaluated through the verspelysines-like AGEs and reduced antixodant defense assessed through CUPRAC method.
3. Enzymatic antioxidant defense – glutathione S-transferase activity, was lower in both types of tuberculosis which contributed to the increasing of the γ- glutamyltransferase during the antituberculosis treatment.
4. The polymorphism assessment of the glutathione S-transferase enzyme is important for the individualized therapy and reducing the toxicity of the anti-tuberculosis treatment.

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