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Research Article

Protein oxidation in children with Pancreatic pathology

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Abstract

In this article, the role of protein oxidation and its influence on clinical course of pancreatic diseases in childhood due to research data and our own investigations are described. It is well known that chronic pancreatitis and functional pathology of Oddi's sphincter in children's age are the long-standing processes which result in edema, fibrosis and loss of normal pancreatic cells as well. Clinical diagnosis usually depends on typical morphological changes in the parenchyma of pancreas. This is why initial changes in morphology remain our key points for confirmation of diagnosis and remain one of the major challenges of pediatric pancreatology. We have examined 80 children with pancreatitis. Among them, 50% of patients were 7-9 years old, 50% – 10-18 years old as well. Dysfunction of Oddi's sphincter (pancreatic type) was confirmed in children of early school age (70,0%). Chronic pancreatitis prevailed in adolescents (52,5%). In a group of adolescents, significant pain in abdomen was present (80%). Among dyspeptic symptoms - nausea (65%), bitter belching (45%) as well as symptoms of intoxication (100%) were verified also. For these children of course, chronic pancreatitis was more characteristic. The data of investigation shows increased indexes of protein oxidation in patients with dysfunction of Oddi's sphincter. The results of studies proved that in chronic pancreatitis there are more expressed violations of protein synthesis and detoxicative function of the liver than in functional pathology. These changes were combined with clinical peculiarities of diseases. So, in functional pathology of pancreas we could find more significant violations of protein oxidation like initial stage of the pathological processes. These changes were related to age of children under our supervision, with peculiarities of autonomic regulation. Among early markers of these violations are protein oxidation subproducts which damage of tissues and organs. Increased amount of sub products at the length of wave 350 nm was seen. It could be the significant indicator of hypoxic changes in pancreatic parenchyma.

Key words: children, chronic pancreatitis, dysfunction of Oddi's sphincter, protein oxidation.

INTRODUCTION

It is well known that chronic pancreatitis in children's age is a long-standing process which results in fibrosis and loss of normal pancreatic cells as well. Clinical diagnosis usually depends on typical morphological changes in the parenchyma of pancreas [1].

As a result, researchers all over the world try to find out methods of early verification of diagnosis and prevention of chronic pancreatitis in childhood.

In recent years new studies about role of protein oxidation were found [2]. Protein oxidation is defined as the covalent modification of a protein induced directly by reactive oxygen species (ROS) and/or indirectly by reactions with secondary byproducts of oxidative stress [3].

The following general types of protein modification are listed below:

• Sulfur oxidation (Cys disulfides, S-thiolation, Metsulfoxide)
• Protein carbonyls (side chain aldehydes, ketones)
• Tyrosine cross-links, chlorination, hydroxylation
• Tryptophanyl modifications
• Hydro(pero)xy derivatives of aliphatic amino acids
• Chloramines, deamination
• Aminoacid interconversions (e.g., His to Asn; Pro to OH-Pro)
• Lipid peroxidation adducts (MDA, HNE, acrolein)
• Aminoacid oxidation adducts (e.g., p-hydroxyphenylacetaldehyde)
• Glixocidation adducts (e.g., carboxymetillysine)

• Cross-links, aggregation, pepdide bound cleavage [4, 5].

Different forms of oxidative modification have biochemical consequences such as: loss of protein functions, loss of protease inhibitor activity, diminished sensitivity to proteolysis, modified transcription of certain genes et cetra [6].

Among diseases related to increased levels of protein carbonyls, acute pancreatitis was found, but the role of protein oxidation in development of chronic pancreatitis in children's age hasn't been studied [6].

OBJECTIVES OF INVESTIGATION

To estimate the role of protein oxidation in school-aged children with pancreatic diseases.

MATERIAL AND METHODS OF INVESTIGATION

Protein oxidation we verified on spectrophotometer by evaluation of its intensive absorption in blood serum [7]. Biochemical investigations were done on the basis of certificated biochemical laboratory (certificate №001479 from 03.02.2010) of Ivano-Frankivsk National medical university (Chief of the biochemical department, Erstenjuk A.M, Ph.D).

Monitoring of ECG with analysis of variability of heart rhythm (Apparatus CardioLAb VHR, Kharkiv-Ukraine) was done in the department of functional diagnostics of regional children's hospital (Chief of the department ,Tsaruk O.Ya ,MD). All investigations in children we have done 1-1.5 hours after meal. We determined index of summary regulation of autonomic nervous system. It includes five categories: summary index of regulation due to frequency of heartbeat, summary power of spectrum, index of strain, activity of vasomotor and cardio-vascular centers.

Also we have determined liver functions (cholinesterase, hipuric acid, AlT, AsT) by routine methods of investigation.

RESULTS OF INVESTIGATION AND CONCLUSION

We have examined 80 children with pancreatitis. Among them 50% of patients were 7-9 years old, 50% – 10-18 years old as well. Dysfunction of Oddi's sphincter (pancreatic type), was confirmed in children of early school age (70,0%). In adolescents chronic pancreatitis prevailed (52,5%).

Duration of both diseases makes 1-5 years but frequent exacerbations were noticed throughout last half a year. These exacerbations were closely related to violations of diet (prevalence of fatty food, fresh juices and stewed cabbage (this is a meal often prepared in Ukraine during the winter). Majority of patients were suffering from acute abdominal pain which was relieved by the administration of spasmolitics (no-spa) and nonsteroid anti-inflammatory medicines (ibuprofen).

Symptoms of functional pathology of pancreas were similar to clinic of main somatic diseases of upper abdomen (f.e. chronic gastritis or cholecystitis).

Biochemical examinations done by us estimated intensification of peroxidation in both groups of patients (table1).

The data of investigation shows increased indexes of protein oxidation in patients with dysfunction of Oddi's sphincter.

At the same time nobody estimated direct relation between level of protein oxidation and expression of clinical symptoms of pancreatic diseases such as: abdominal pain (80,0%), recurrent vomiting (68,2%), sour belching (63,6%), tendency to diarrhea (50,0%), subfebrile temperature (27,5%).

These results may indicate activation of biochemical processes in children with functional disease of pancreas. Damage effect of free reactive molecules is released in synthesis of byproducts of protein oxidation which inactivate kation pumps, channels of ion conductivity, proteins and enzymes of membrane. These changes are also connected with more severe course of the disease: frequent exacerbations of abdominal spastic pain (98,0%), vomiting (75,0%), subfebrile temperature (65,0%). During attack of pain children take specific posture to relief the pain (60,0%). Signs of intoxication were seen like: dizziness, increased perspiration, loss of consciousness.

Thus results of studies revealed increasing of aldegid and ketonic acids of the main character as the result of protein oxidation in children with pathology of pancreas ($p<0,05$).

We have found that more expressed changes were noticed in children with dysfunction of Oddi's sphincter, pancreatic type. That could be significant indicator of early pathological changes in parenchyma of pancreas as well as sign of damage of protein molecules. The length of waves in this case makes 430 and 530 nm. In norm, the amount of proteins at such length of waves should be lower. At the same time, amount of proteins in such length of waves wasn't defined from normal values.

In group of adolescents significant pain in abdomen took place (80%). Among dyspeptic symptoms nausea (65%), bitter belching (45%) as well as symptoms of intoxication (100%) were verified also. For these children of course, chronic pancreatitis was more characteristic. Disease was accompanied with anxiety (78,0%), depression (75,0%), violations of quality of life (60,0%). Patients of this age group didn't believe in efficiency of therapy, especially boys (45,0%).

Levels of protein oxidation in these patients are described below (table 4).

As it is well seen in table 2, content of carbonile groups of proteins closely related to type of pathology and was significantly higher than in dysfunction of Oddi's sphincter, pancreatic type . At the result of protein oxidation there is denaturation and increased proteolytic degradation because endogenous proteases occurred.

Violations of secretion or activity of these enzymes resulted in accumulation of protein oxidation sub products in blood serum [8,9].

Due to modern outlook and new approaches in medical science, carbonile sub products are the early indicators of injury of organs and tissues.

They are active metabolites which could effectively estimate level of damage, improve results of clinical and laboratory findings in pancreas and liver.

Estimation of liver functions due to reactivation of protein oxidation is described in picture below (table 3).

The results of studies proved that in chronic pancreatitis there are more expressed violations of protein synthesis and detoxicative function of the liver than in dysfunction of Oddi's sphincter, pancreatic type. These changes were combined with clinical peculiarities of diseases.

We also have noticed moderate violations of index of summary regulation of autonomic nervous system (table 4).

Table 1
Protein oxidation in children with pancreatic diseases, n=40

Length of wave, nm	Dysfunction of Oddi's sphincter, pancreatic type, n=20	Normal (predicted) values	Chronic pancreatitis, n=20	Normal (predicted) values
356	0,65±0,2* p<0,05	1,33±0,01	0,77±0,2* p<0,05	1,33±0,01
370	0, 55±0,1 p<0,05	1,31±0,01	0,79±0,1 p<0,05	1,31±0,01
430	2, 35±0,7 p<0,05	0,71±0,05	2,01±0,6 p<0,05	0,71±0,05
530	2, 38±0,2 p<0,05	0,03±0,002	1,17±0,7 p<0,05	0,03±0,002

*Note – improved difference between indexes of sick and healthy children

Table 2
Protein oxidation in adolescents with pancreatic pathology, n=40

Length of wave, nm	Dysfunction of Oddi's sphincter, pancreatic type n=20	Normal (predicted) values	Chronic pancreatitis, n=20	Normal (predicted) values
356	0,66±0,2* p<0,05	1,33±0,01	0,75±0,2* p<0,05	1,33±0,01
370	0, 68±0,1 p<0,05	1,31±0,01	0,85±0,1 p<0,05	1,31±0,01
430	2, 11±0,7 p<0,05	0,71±0,05	1,25±0,6 p<0,05	0,71±0,05
530	2, 08±0,2 p<0,05	0,03±0,002	1,07±0,7 p<0,05	0,03±0,002

Table 3
Violations of protein synthesis and detoxication in children with pancreatic diseases, n=80

Index	Chronic pancreatitis, n=40	Dysfunction of Oddi's sphincter, pancreatic type, n=40	Improved difference
Common protein, g/l	55,2±1,4	61,5±1,3	p<0,05
Albumins, g/l	36,8±0,5	42,2±0,5	p<0,05
Globulins, g/l	22,8±0,3	25,1±0,2	p<0,05
α_1-globulin,%	4,78±1,2	3,55±1,2	p<0,05
β- globulin,%	6,11±1,4	12,1±1,4	p<0,05
γ- globulin,%	13,8±1,3	11,2±1,3	p<0,05
Cholinesterase of the liver in blood serum, мкkag*l	65,1±0,5	76,2±0,5	p=0,05
Hipuric acid in urine, g/l	2,17±0,11	1,67±0,07	p<0,05

Table 4
Violations of autonomic nervous system, n=80

Disease	Number of patients, percentage	Index of summary regulation of autonomic nervous system
Dysfunction of Oddi's sphincter, pancreatic type	21 (52,5%)	2-4 marks
	12 (30%)	5-6 marks
	7 (17,5%)	7-8 marks
Chronic pancreatitis	8(20%)	2-4 marks
	25 (62,5%)	5-6 marks
	10 (25%)	7-8 marks

Table 5
Protein oxidation in school-aged children with pancreatic diseases, n=80

Length of wave, nm	Dysfunction of Oddi's sphincter, pancreatic type, n=38		Chronic pancreatitis, n=42	
	Before treatment	After treatment	Before treatment	After treatment
356	0,66±0,2	0,33±0,2 p<0,05	0,75±0,2*	0, 53±0,2 p<0,05
370	0, 68±0,1	0,55±0,1 p<0,05	0,85±0,1	0,65±0,1 p<0,05
430	2, 11±0,7	1,04±0,8 p<0,05	1,25±0,6	1,03±0,7 p<0,05
530	2, 08±0,2	2,01±0,8 p<0,05	0,75±0,2*	0,52±0,2 p<0,05

We have found moderate strain of adaptive mechanisms in children with pancreatitis which could be background of future pathological process. Index of summary regulation of autonomic nervous system at this case makes 2-4 marks. Thus it is possible to indicate violations of autonomic nervous system only after influence of stress or physical strain.

In patients with chronic pancreatitis, we have recognized a wide range of pathological conditions. Expressed functional stress of regulative systems was measured by index of summary regulation of autonomic nervous system (5-6 marks) and it was seen in 62,5% patients. Significantly expressed strain of psychoemotional and autonomic deregulation was noticed in 25% patients with chronic pancreatitis (index was equal 7–8 marks). Mild form of strain was recognized in 20,0% of children with chronic pancreatitis.

Due to violations of protein oxidation we have offered a certain antioxidant, Quercetin applied in granules and tablets (depending on age).

Dynamics of protein oxidation indexes after application of antioxidant medicine Quercetin is shown below (table 5).

Significant decreasing of main indexes was proved after therapy especially at length of waves 430 nm and 530 nm.

These changes were more informative in group of patients with dysfunction of Oddi's sphincter, pancreatic type. In group of comparison increased indexes were found only in half of cases that could be the indicator of organic changes in pancreas.

Functional condition of liver was changed in children with chronic pancreatitis only after 7-10 days of therapy: level of hipuric acid became 1.5 times lower than in norm. Cholinesterase remained constant only in group of comparison. Changes of main indexes in this group took place only at 14-20 days of therapy.

Thus, in children with dysfunction of Oddi's sphincter, pancreatic type, decreased levels of aldegid and ketonic subproducts at the length of waves 430 and 530 nm was seen in 75 % of cases, which show the efficiency of treatment with application of antioxidants. The other indexes were not different from predicted values before and after treatment.

We also noticed positive dynamics of indexes responsible for condition of autonomic regulation. Main index of autonomic regulation turned back to norm only in patients who received quercetin throughout 12-14 days of therapy.

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