Plasma fibrinogen concentration in pediatric patients treated with an elimination diet based on soy proteins and casein hydrolyzate

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Abstract

Purpose: Fibrinogen is one of the most discussed new risk factors of atherosclerosis. The aim of the study was to assess the relationship between fibrinogen concentration and classic risk markers of atherosclerosis in a group of children aged from 2 to 6 with or without a family history of circulatory system diseases (FHCAD) (American Academy of Pediatrics – AAP criteria). The study also considered the impact of allergies/food intolerance treatment with elimination diets on the concentration of atherosclerosis markers specially fibrinogen.

Material and methods: Inclusion criteria: a) family history of early occurrence of circulatory system diseases (FHCAD+) according to AAP standards; b) the type and duration of elimination diet continued in infancy and early childhood. 134 of 388 children were included in the investigation.

Results: The analysis of data relating to the so-called classic biochemical risk factors of atherosclerosis (total cholesterol – TC, HDL, LDL, triglycerides, glucose) did not reveal any differences between the tested groups. It was found that in the FHCAD+ group the concentration of fibrinogen was statistically higher than in the group with a negative family history. It was discovered that the type of elimination diet had no effect on fibrinogen level in the FHCAD+ group. In the group of children with negative family history the concentration of fibrinogen was statistically lower in the group on casein hydrolysate than in children treated with soy formula.

Conclusions: The initial interview in pediatrics should include information on the patient's family history of atherosclerosis. In case of a positive family history, fibrinogen, as one of atherosclerosis risk factors, should be monitored.

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Introduction

Fibrinogen belongs to a group of new risk factors in atherosclerosis [1-5], being a biochemical marker of inflammatory status and increased procoagulative readiness of the plasma. Fibrinogen is a 340 kDa glycoprotein synthesized mainly in liver. It consists of four different polypeptide chains, coded by genes located on chromosome 4 [6].

Blood plasma contains 80-90% of fibrinogen. Its concentration ranges from 200 to 400 mg/dl (2-4 g/l). Fibrinogen half-time in the circulatory system is 3-6 days, and its synthesis depends on concentration of its degradation products in plasma and concentration of various cytokines produced by stimulated macrophages [7].

The impact of acute or chronic hyperfibrinogenemia on the development of atherosclerosis may be explained by various mechanisms. Fibrinogen infiltrates vascular walls, causing an increased platelet aggregation and the development of mural thrombi. Another consequence is the rheological effect, i.e. the relationship between high concentration of fibrinogen and blood viscosity, as fibrinogen stimulates migration and proliferation of smooth muscle cells [8].

The results of the most significant population studies on the relationship between the concentration of fibrinogen and the development of atherosclerotic changes are presented in *Tab. 1*.

The analysis of these results has shown that fibrinogen is an independent risk factor for atherosclerosis [8,9]. It is worth noting that increased fibrinogen concentration also occurs in asymptomatic patients.

Higher fibrinogen concentration is related to age, high LDL cholesterol and triglycerides concentration, low concentration of HDL cholesterol, obesity, smoking, and lack of physical activity [10].

Studies carried on adult population confirmed that the predictive value of fibrinogen is comparable to the predictive value of high cholesterol concentration [11-14]. So far we do not have

Table 1. **Chosen population research aimed at determining the concentration of fibrinogen**

Type of test	Number of people tested	Age of people tested	Length of test	Conclusions
Northwick Park Heart Study [8]	1 510 men	40-64	10 years	A significant link was confirmed between the concentration of fibrinogen and the mortality caused by circulatory system diseases; this link was independent of other risk factors and stronger than the link with the total cholesterol concentration. About a half of coronary incidents involved patients with the fibrinogen concentration in the top terce.
Caerphilly/Speedwell Study [24]	4 700 men	45-64	3.2-5.1 years	Fibrinogen as an independent risk factor in ischemic heart disease has predictive quality comparable to the total cholesterol concentration, blood pressure and BMI.
Framingham Study [25]	554 men 761 women	47-79	12 years	Fibrinogen as an independent risk factor for the ischemic heart disease and the cerebral stroke.
PROCAM [26]	1 674 men	40-65	2 years	The frequency of myocardial infarction in the tested group was 2.4 times greater for patients with fibrinogen concentration in the top terce than for those whose fibrinogen was in the bottom terce.
GRIPS Study [27]	5 239 men	40-60	5 years	A significant link was found between the fibrinogen concentration and the occurrence of myocardial infarction. The order of the predictive value: LDL, a positive family history, Lp a, HDL, fibrinogen, age, smoking, glucose, blood pressure.

enough data on the relationship between fibrinogen and the development of atherosclerotic process in pediatric population.

In one of few published studies conducted on the developmental age population Torbus-Lisiecka and her co-workers found that children's concentration of triglicerides and fibrynolysis activity are strongly determined by parental lipids and haemostasis parameters in children with positive family history of hypertriglyceridemia [15]. Similar findings were described by the same author in a study on children with one of parents suffering from ischemic cerebral stroke [16].

Głowińska and her co-workers evaluated the concentration of fibrinogen in a group of children aged from 4 to 20 years with a positive family history of circulatory system diseases [17]. Children included in the study suffered from arterial hypertension, diabetes, and/or obesity. The results didn't show statistically significant differences among the evaluated groups of children.

The correlation between fibrinogen concentration and atherosclerosis in children still remains to be evaluated. Another open question is the influence of food proteins on fibrinogen concentration.

The available data (Medline, Current Contents) seems to lack research on the impact of food proteins on haemostasis, including fibrinogen, in the pediatric populations.

With this in view, we wanted to assess the concentration of fibrinogen and to evaluate the classic risks of atherosclerosis in a group of children aged from 2 to 6 with or without family history of circulatory system diseases (AAP criteria). We have also studied the influence of treatment of allergies/food intolerance with elimination diets on the concentration of the atherosclerotic process markers, mainly fibrinogen. We wanted to test two hypotheses:

1. Fibrinogen level in the plasma of children with a positive family history pointing to the risk of circulatory system diseases does not differ from the level noted in children with a negative family history.

2. Fibrinogen plasma level in children treated with elimination diet based on soy protein in infancy and early childhood is comparable to the level observed in children treated with casein hydrolysate-based formulas. An alternative hypothesis assumes a statistically significant difference in the concentration of fibrinogen in the above-mentioned groups. The study was designed as a clinical-control investigation.

Material and methods

The research, conducted according to the questionnaire method, involved 2 627 children from the north-east of Poland. We adopted strict criteria of including or excluding children from the study groups.

Inclusion criteria into the study group:

1. Type and duration of elimination diet applied in infancy and early childhood. The dietetic treatment has been recommended to children with diagnosed allergy/food intolerance. The group of children on therapy was limited to those with only one substance replacing milk in the diet (either soya or casein hydrolysate formula), with the diet introduced not later than in sixth month of life and continued for at least 12 months. The children who, at any time, have been treated with both types of substances were not included in the study;

2. Family history of early (before 55 year of life) occurrence of circulatory system diseases. A closer and more detailed look into the family history was then aimed at finding the frequency and duration of various medical conditions among members of the family, back to the second generation. They included arterial hypertension, coronary heart disease, atherosclerosis, diabetes, obesity, and high cholesterol level. The children from families in which these medical conditions occurred before 55 years of age were included in the group with a family history pointing to the risk of circulatory system diseases, according to the standards of the American Academy of Pediatrics [18];

3. Children's age: 2-6 years – according to the conducted research at the age of 2 lipid metabolism profile becomes stabilized;

Table 2. **The characteristics of the tested groups**

Group	Diet followed in infancy and early childhood	Family history of circulatory system diseases	The number of children tested	Age [in years] mean (SD)	BMI [kg/m²] mean (SD)	The month of life in which the diet started mean (SD)	How long the diet was followed (number of months) mean (SD)
1	soya protein	+ positive	24	5.66 (1.10)	15.78 (2.16)	4.08 (1.86)	28.21 (12.12)
2	soya protein	- negative	25	5.09 (1.12)	15.31 (1.69)	4.80 (1.12)	30.76 (15.52)
3	casein hydrolysate	+ positive	25	5.15 (1.22)	16.12 (2.29)	3.28 (1.97)	29.28 (9.13)
4	casein hydrolysate	- negative	18	5.03 (1.20)	15.73 (1.92)	3.61 (1.85)	27.11 (11.43)
5	normal diet	+ positive	42	5.47 (1.26)	15.95 (1.52)		

Table 3. **Average values of biochemical parameters for the tested groups of children**

	Group 1		Group 2		Group 3		Group 4		Group 5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total cholesterol [mmol/l]	4.49	0.78	4.16	0.57	4.48	0.79	4.38	0.62	4.54	0.55
LDL Cholesteol [mmol/l]	2.66	0.64	2.62	0.53	2.86	0.67	2.55	0.65	2.69	0.55
HDL Cholesterol [mmol/l]	1.49	0.38	1.20	0.31	1.26	0.22	1.48	0.50	1.44	0.30
Triglicerides	0.75	0.41	0.77	0.34	0.72	0.19	0.77	0.25	0.81	0.33
Fibrinogen [mg/dl]	277.8	50.03	270.7	49.94	284.2	59.83	239.8[a]	46.12	273.9	62.91
Glucose [mmol/l]	4.64	0.41	4.44	0.41	4.49	0.52	4.52	0.57	4.56	0.51

[a] $p<0.05$ vs group 1,2,3 i 5 (Mann-Whitney U-test)

4. The consent of the patients' parents for participation in the study.

Exclusion criteria from the study group:

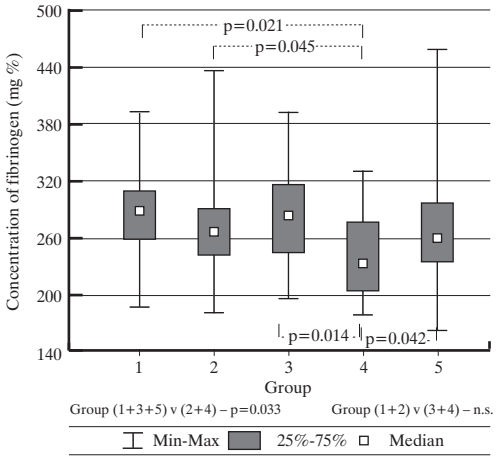
1. Suspected hypertension (diastolic or systolic pressure above 97 centile in two subsequent measurements);
2. Obesity – body weight above 97 centile;
3. History of chronic diseases
   a. glomerulonephritis
   b. chronic bile ducts and liver diseases with and without cholestasis
   c. diabetes
   d. systemic lupus;
4. Infection on the day of inclusion into the study or during the previous 3 weeks;
5. Oral administration of glycocorticosteroids.

The quantity and quality of non-dairy products was similar in all tested groups of children. All children were assign to the study groups and after randomization inside the groups 134 children took part in the tests. *Tab. 2* presents the characteristics of those groups.

Blood samples from all the children were taken from elbow flexure, using minimum stasis. The patients arrived for tests at least 12 hours after the last meal, between 8 and 10 a.m. Blood samples for assessment of lipid metabolism were drawn into glass tubes without any anticoagulant. Routine laboratory methods were used to measure the concentration of total cholesterol, LDL and HDL fractions, as well as of triglycerides and glucose. Blood samples for determination of fibrinogen concentration were taken into test tubes containing 3.8% sodium citrate in the amount allowing to obtain 9:1 blood to anticoagulant ratio. The material was spun at 4°C for 30 minutes. Fibrinogen concentration was measured by Clauss method [19].

The obtained results did not meet the criteria of standard distribution. Therefore they were submitted to statistical analysis using non-parametric Mann-Whitney U-test.

Figure 1. **Mean fibrinogen level in study groups**



The permission to carry out the study was granted by the Bio-Ethical Committee of Białystok Medical University. The parents of the children gave their permission for participation in the test in writing.

Results

Tab. 3 presents the assessed biochemical parameters in the tested groups. The analysis of data linked to the so-called classic risk factors of atherosclerosis, i.e. total concentration of cholesterol and its LDL and HDL fractions, triglycerides and glucose, did not reveal any statistically important differences between the tested groups. A statistically significant difference was found for the concentration of fibrinogen (*Fig. 1*).

It was found that in the group of children with a positive family history of circulatory system diseases the concentration of fibrinogen was statistically higher than in the group with a negative family history (groups 1, 3 and 5, vs 2 and 4; p=0.033).

The analysis of the impact of treatment with an elimination diet on the concentration of fibrinogen in the tested groups revealed that the type of elimination diet did not affect the level of fibrinogen in the group of children with family history of CV diseases. The concentration of fibrinogen was similar in the group of children with a positive family history who have not been treated with an elimination diet.

It is worth noting that among the children with the negative family history the average concentration of fibrinogen was statistically lower in the group on casein hydrolysate diet than the children on soya mixtures (group 4 vs group 2; p=0.045).

Discussion

Our research involved children aged 2 to 6 years. The results show that the concentration of fibrinogen in the group of children with a positive family history of circulatory system diseases of atherosclerotic origin is higher than in children who do not belong to the risk group according to AAP criteria.

It was also found that the concentration of fibrinogen in children from the risk group is not affected by the type of protein used in their nutrition in infancy and early childhood. The research by Pankow et al. [20] was carried on a group of 13 000 men and women aged from 45 to 64, with a positive family history of circulatory system diseases. They evaluated the levels of fibrinogen, VIIc, VIIIc, and von Willbrand factors, protein C, and antithrombin III. The results confirmed that the average concentration of selected parameters, among them classic risk factors of circulatory system diseases, was higher among people with a positive family history [20].

No comparable research has yet been conducted on pediatric population.

The results of published research suggest a positive impact of a soy-based diet on risk factors of atherosclerosis in adults. The research done so far focused on the relationship between the soy-based diet and lipid risk factors of atherosclerosis [21].

According to Anderson and her co-researchers soy protein and soya products reduce the concentration of total cholesterol and its LDL fraction while increasing the concentration of HDL cholesterol [22]. The tests carried among adult patients with hypercholesterolemia revealed that the beneficial effects of soya protein diet are directly correlated to the initial concentration of cholesterol.

Hypercholesterolemia is only one of many risk factors in atherosclerosis. A beneficial, multidirectional effect of soya on the development of atherosclerotic process is well-known [23]. It seems probable that the positive impact of soya also depends on degree of atherosclerotic lesions and the presence of other risk factors in this process.

It is interesting that among the children with negative family history of circulatory system diseases we discovered a significantly lower concentration of fibrinogen in the group whose diet included casein hydrolysates than in the group on soy-based formulas. This result obtained in our study is difficult to interpret, as there is not enough data on the effect of food proteins on haemostasis parameters, including fibrinogen concentration. Solving this problem requires research that could explain the impact of protein amino acid profile on haemostasis parameters. It is possible that the lipid and the amino acid profiles of casein and soya preparations are of some significance. The problem still remains to be solved.

Conclusions

The research conducted on the adults population clearly proves the value of fibrinogen as a risk marker of atherosclerosis. There are still not enough studies on the development of atherosclerotic processes in pediatric population. A frequent occurrence of circulatory system diseases in adult population suggests the necessity of including the patient's family history of atherosclerosis-linked conditions in the pediatric initial interview. In case of a positive family history, fibrinogen could be one of atherosclerosis risk factors to be monitored.

Our study revealed that:

1. In the group of children with a positive family history of circulatory system diseases the concentration of fibrinogen was significantly higher than in the group without such history. The type of diet used did not affect the fibrinogen plasma level in this group of children.
2. A significantly lower concentration of fibrinogen was found in the group of children with a negative family history whose diet included casein hydrolysates in comparison to a similar group treated with soya preparations.

Questions that remain open:

1. Can fibrinogen be considered an independent risk factor of atherosclerotic process in pediatric population?
2. Does the type of diet continued in the infancy and early childhood affect the development of atherosclerotic changes in later life (metabolic imprinting)?

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