Acute phase proteins serum concentrations in children are related to urinary iodine excretion

Lialikau SA[1], Haurylik LL[1], Sobieska M[2]*, Klachko NM[1], Samborski W[2]

[1] Chair of Pediatrics N1, State Medical University, Grodno, Belarus
[2] Department of Physiotherapy, Rheumatology and Rehabilitation, University of Medical Sciences, Poznań, Poland

Abstract

Purpose: The paper presents links between iodine provision and selected acute phase proteins' (APP) serum concentrations as well as their glycosylations profiles (investigated with the use of affinity immunoelectrophoresis with Concanavalin A as ligand) in children.

Material and method: 116 children (58 girls and 58 boys) were enrolled. Iodine level was measured in the morning (7:30-8:30) urine portion, using Cr-As method. According to iodine level children were divided into two groups. The first one consisted of 56 children with decreased iodine level (lower than 100 micrograms/L), second – 60 children with iodine level higher than 100 micrograms/L. In serum the concentration of ferritin, beta2-microglobulin (beta2-MG), thyroxin (T_4), triiodothyronin (T_3), thyrotrophic hormone (TSH) were measured by radioimmunoassay (BELORIS, Belarus). Concentrations of APP: C-reactive protein (CRP), alpha1-acid glycoprotein (AGP), alpha1-antichymotrypsin (ACT), alpha1-antitrypsin (AT), haptoglobin (Hp), alpha2-macroglobulin (A2-M), ceruloplasmin (Cp) and transferrin (Tf) were measured in sera samples by rocket immunoelectrophoresis acc. to Laurell with antibodies and standard from DakoCytomation, Denmark. Microheterogeneity of AGP, ACT and Tf was estimated using affinity immunoelectrophoresis with ConA as a ligand, acc. to Bøg-Hansen.

Results: It was established, that CRP level was lower than upper limit of normal range. Levels of other investigated proteins were reliably dependent on the level of iodine. Especially for AGP lower level was observed for children of the group with low iodine level. In children with low iodine level along with the decrease of serum AGP concentration altered glycosylations profile was observed, namely decrease in the content of variant non-reactive to ConA (W0) and increase in content of weakly reactive (W1) and reactive (W2) variants content, which resulted in increase of the reactivity coefficient (AGP-RC). Similar tendency in alterations of distinctly glycosylated variants in relation to iodine level could be shown for ACT. Serum concentration of any investigated protein was not dependent on the concentration of the hormones of pituitary-thyroid system.

Conclusions: It seems that the influence of the iodine level is direct, not via thyroid hormones. It could be suggested that in euthyroid children with low iodine excretion with urine a hidden iodine deficiency is already registered by the regulatory mechanisms and a kind of acute phase reaction is started, may be in order to increase iodine uptake and storage.

Key words: iodine provision, thyroid hormones, acute phase proteins.

Introduction

A problem of environmental too low iodine level exists in 140 countries all over the world. According to the reports of WHO about 1.5 milliard people live under iodine deficiency conditions [1]. It is known that iodine deficiency may result in several pathological changes, not only in traditionally reported goiter, and ongoing not necessarily along with thyroid enlargement. Iodine plays the most important biological role as a part of thyroidal hormones that take part in regulation of all metabolic processes, physical and mental development, reproductive functions and immunity [2]. But thyroid is not the only organ that makes use of iodine. Also the cells of the immune system use this microelement. As example the bactericidal activity

* CORRESPONDING AUTHOR:
Department of Physiotherapy, Rheumatology and Rehabilitation,
University of Medical Sciences
ul. 28. Czerwca 1956 roku 135/147
61-545 Poznań, Poland
Tel/Fax: +48 61 8310244
e-mail: magdula2811@poczta.onet.pl (Magdalena Sobieska)

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of iodides and peroxiiodides produced by phagocytes may be mentioned [3]. However, no reports were found on relationship between iodine provision and acute phase proteins (APP) level or their microheterogeneity.

APP belong to the most ancient part of the unspecific immunity and contribute markedly to the keeping of homeostasis. As much as 30 various proteins are for the moment regarded as APP. Being multifunctional regulators and effectors APP stay in multiple relations to practically all types of cells and molecules. Among APP following functional groups may be described: transport proteins (transferrin, ceruloplasmin and haptoglobin), clotting factors (fibrinogen), antiproteases (α_1-antitrypsin, α_1-antichymotrypsin, α_2-macroglobulin), complement components (C3, C4) and several proteins of hardly known function, like C-reactive protein (CRP), serum amyloid A (SAA), α_1-acid glycoprotein (AGP) and others. Majority of those proteins are produced in hepatocytes, some of them to lesser extent also in lymphocytes, macrophages and endothelial cells, and their synthesis is mediated by interleukin-1 (IL-1), IL-6 and glucocorticosteroids in response to trauma, tissue injury, physical or psychic stress, but also autoimmune, allergic and neoplastic disorders.

Almost all APP (except albumin, CRP and SAA) are glycoproteins. It means that in specific glycosylation sites they bear side oligosaccharide chains, forming bi-, tri- or tetraantennary glycans. The molecules of the same glycoprotein may differ in the structure of side oligosaccharides and this feature is referred to as major microheterogeneity. Molecules with similar sugar chains form so-called variants of the glycoprotein. In physiological conditions the proportion of variants remains stable. There are several reports on microheterogeneity alterations in various pathological conditions, all reporting posttranslational changes in the side sugar structure due to activity of cytokines, such as IL-6, TGFβ, TNFα [4-6].

For the most widely investigated glycoprotein – AGP, the following alterations were described: in acute inflammatory conditions (trauma, acute bacterial infection and exhausting physical stress) there is an increase in biantennary sugar structures along with increase in protein concentration. Chronic inflammation results in increase or decrease of total AGP concentration along with marked increase in tri- and tetraantennary glycans [7,8]. Similar changes were also reported for some other APP.

The aim of the study was to investigate possible links between iodine provision and selected acute phase proteins' serum concentrations as well as their glycosylations profiles (investigated with the use of affinity immunoelectrophoresis with Concanavalin A as ligand) in children.

Material and methods

The study was performed with children whose places of residence are situated on areas affected by the Chernobyl disaster and who were spending their summer vacations in a spa. The whole treatment and investigation performed during this stay were a subject of Ethic Committee Consent and parents of all children expressed in written their approval for the routine laboratory investigation as screening tests for the general health status (also vitamin provision, hormones levels etc.). For the purpose of this study 116 children (58 girls and 58 boys), of them 57 aged from 7 to 12 years and 59 – 12-15 years were enrolled. In all investigated children neither any symptoms of iodine deficiency diseases, acute inflammation nor signs of deterioration of any chronic diseases were found, thus all children were found clinically healthy at the moment of enrollment. Clinical investigation showed also that all children were euthyroid and any substitution of iodine was used prior to investigation.

Iodine level was measured in the morning (7:30–8:30) urine portion, using Cr-As method [9]. According to iodine level children were divided into two groups. The first one consisted of 56 children with decreased iodine level (lower than 100 micrograms/L), second – 60 children with iodine level higher than 100 micrograms/L. In both groups similar proportion of boys and girls was noticed (girls to boys 26:30 in the 1st and 32: 28 in the 2nd group).

Parallel to urine sample and at the same time a sample of peripheral blood was drawn for the routine laboratory investigations. The rests of sera samples were used. In serum the concentration of thyroxin (T4), triiodothyronin (T3), thyrotrophic hormone (TSH) were measured by radioimmunoassay (BELORIS, Belarus). Concentrations of APP: CRP, AGP, alpha1-antichymotrypsin (ACT), alpha1-antitrypsin (AT), haptoglobin (Hp), alpha2-macroglobulin (A2-M), ceruloplasmin (Cp) and transferrin (Tf) were measured in sera samples by rocket immunoelectrophoresis acc. to Laurell [10] with antibodies and standard from DAKOPATTS, Denmark. Microheterogeneity of AGP, ACT and Tf was estimated using affinity immunoelectrophoresis with ConA as a ligand, acc. to Bøg-Hansen [11] with modification of Mackiewicz [12]. For AGP and ACT the so-called reactivity coefficients were calculated acc. to formula: for AGP-RC variants reactive with ConA (W1+W2+W3)/variant non-reactive (W0). For ACT-RC – variants reactive to ConA (A2+A3+A4+A5)/weakly reactive variant A1. Statistical analysis of data was performed using Statistica 6.0 Software. For the differences between the groups the Student t-test was performed. Prior to any other tests the distribution of variables was measured by K-S and Lillefors' tests. To evaluate the influence of a given factor to the alterations of other parameters one way Anova with Fisher's test was performed. Pearson's r was used to expressed significant correlations between the parameters. For all test the level of significance was established for $p<0.05$.

Results and discussion

The investigation of thyroid hormones showed normal values for all children. No differences were found if children were divided according to age, sex or iodine urine excretion. For the group with low iodine level slightly higher level of TSH was observed than in the group with normal iodine level, but the difference was not significant (3.7 ± 0.7 versus 3.1 ± 0.3 microunits/mL, respectively).

It was established, that CRP level was lower than upper limit and no differences were found between the groups. This could

Table 1. **Serum concentration of APP and percentage of their variants (mean ± SD) in relation to iodine excretion in urine, in children with low iodine level and with normal iodine level. Statistical significance of the difference between the groups is given in the last column and "NS" means p>0.1**

Parameters	1st group – low iodine	2nd group – normal iodine	p
	Mean ± SD	Mean ± SD	
AGP (mg/L)	603.79 ± 211.25	829.71 ± 397.26	0.0004
W0 (%)	41.05 ± 7.84	44.10 ± 5.29	0.02
W1 (%)	45.97 ± 6.06	43.59 ± 5.23	0.03
W2 (%)	11.28 ± 3.22	9.68 ± 3.37	0.01
W3 (%)	1.72 ± 2.04	1.93 ± 1.81	NS
AGP-RC	1.46 ±0.41	1.28 ± 0.26	0.009
ACT (mg/l)	279.32 ± 108.50	362.67 ± 142.98	0.001
A1 (%)	19.34 ± 4.09	21.53 ± 4.48	0.03
A2 (%)	29.02 ± 2.94	30.44 ± 5.59	NS
A3 (%)	32.27 ± 4.79	21.59 ± 7.01	0.000001
A4 (%)	16.38 ± 5.36	23.54 ± 11.44	0.001
A5 (%)	2.75 ± 2.41	5.13 ± 2.63	0.00008
ACT-RC	4.41 ± 1.34	3.93 ± 1.10	0.08
TF (mg/l)	2325.68 ± 478.54	2627.87 ± 935.70	0.06
T1 (%)	5.65 ± 3.80	6.29 ± 3.26	NS
T2 (%)	16.88 ± 5.27	17.99 ± 7.39	NS
T3 (%)	65.66 ± 8.53	63.91 ± 9.53	NS
T4 (%)	12.78 ± 4.54	14.07 ± 7.68	NS
A2-M (g/l)	4.84 ± 1.36	5.20 ± 1.62	NS
Cp (mg/l)	412.64 ± 112.39	525.41 ± 162.99	0.0001
Hp (mg/l)	0.58 ± 0.43	0.79 ± 0.48	0.05
AT (g/l)	1.72 ± 0.38	2.09 ± 0.59	0.0007

Table 2. **ANOVA analysis of the influence of selected factors (age, sex and iodine in urine) on investigated APP. Shown is only those parameters for which the influence of iodine level was proved. "NS" means p>0.1**

Parameters	Sex		Age		Iodine in urine	
	F	p	F	p	F	p
AGP	2.77	NS	3.40	0.07	14.99	0.0002
W1 (%)	1.33	NS	3.40	0.07	4.17	0.04
AGP-RC	0.96	NS	0.56	NS	4.78	0.03
ACT	2.12	NS	3.82	0.06	12.80	0.0005
A3 (%)	0.44	NS	0.01	NS	44.35	0.000001
A4 (%)	0.13	NS	0.01	NS	8.66	0.004
A5 (%)	0.92	NS	0.06	NS	16.43	0.0001
Tf	0.51	NS	0.13	NS	3.85	0.06
Cp	2.62	NS	8.28	0.005	19.07	0.00003
Hp	3.98	0.05	0.58	NS	6.62	0.01
AT	0.84	NS	0.04	NS	12.56	0.0006

be regarded as exclusion of any inflammatory processes in all investigated children.

Levels of other investigated proteins were reliably dependent on the level of iodine. Especially for AGP lower level was observed for children of the first group (low iodine level), (*Tab. 1*).

In children with low iodine level along with the decrease of serum AGP concentration altered glycosylation profile was observed, namely decrease in the content of W0 variant (non-reactive with ConA) and increase in W1 and W2 content, which resulted in increase of AGP-RC. Similar tendency in alterations of distinctly glycosylated variants in relation to iodine level could be shown for ACT. In children with iodine deficiency total serum ACT concentration was decreased, along with decrease in percentage of A1 variant and significant increase in A3 variant. Percentages of A4 and A5 variants decreased in comparison to group 2. All these alterations together resulted in increase of ACT-RC value. Thus, similarly as for AGP, decreased total ACT concentration was accompanied by higher reactivity with ConA, i.e. relative domination of biantennary glycans. For both proteins the decrease of ConA non-reactive variants (W0 of AGP and A1 of ACT) was the common feature. This tendency of decreasing ConA non-reactive variants seemed to be the most probable candidate for a link between iodine and APP.

Mean serum transferrin concentration was slightly (not significantly) lower for children with iodine deficiency than in group 2. The percentages of its variants did not differ significantly, T1, T2 and T4 being slightly lower and T3 slightly higher than for group 2. Also serum A2-M concentration did not differ between the groups. On the contrary, serum concentrations of Cp, Hp and AT seemed tightly related to iodine level, with normal concentrations in group 2 and decreased below the normal values in group 1.

It is known that during development in children several processes take place that influence basic biochemical parameters. Especially during sex maturation these changes are caused not only by age, but mainly by sex. To determine the influence of all factors mentioned (age, sex and iodine deficiency) on the investigated parameters, the multi-factorial analysis of dispersion (ANOVA) was conducted. The influence of each factor was calculated according to Fisher's test (F). The results of this analysis showed that the influence of iodine deficiency on AGP, ACT, Tf, Cp, and Hp and AT was independent from age and sex (*Tab. 2*), but when the influence of sex and age was excluded, the link between iodine deficiency and the concentration of variants W0, W2 of AGP and A1 of ACT lost its statistical significance.

The level of iodine in the organism is tightly bound to the production of thyrotrophic hormone and the function of thyroid gland. This could explain the results obtained in the correlation analysis, showing that majority of the investigated proteins' serum concentrations correlated significantly (and with the same sign) with both urinary excretion of iodine and the concentrations of pituitary-thyroid hormones (*Tab. 3*).

As conclusion one question appeared: which of the investigated factors – iodine level or hormones influenced the concentration and glycosylation of the investigated APP. And again the dispersion analysis was performed for each pair of factors: TSH and iodine, T_3 and iodine, T_4 and iodine. It was shown that the level of iodine with high significance and directly influenced the serum concentration of AGP, the percentage of its ConA strongly reactive variants and the AGP-RC value, as well as the serum concentration of ACT and the percentage of its variants A3, A4 and A5 (also strongly reactive with ConA), and concentration of Cp, Hp and AT (*Tab. 4*). Serum concentration of any

Table 3. **The correlation (Pearson's r) between TSH, T_3, and T_4 in the peripheral blood, iodine in urine and serum APP concentrations. Shown are only those parameters for which the correlation was proved to be statistically significant. "NS" means p>0.1**

Parameters	Iodine in urine		T_3		T_4		TSH	
	r	p	r	p	r	p	r	p
AGP	0.32	0.001	0.31	0.001	0.28	0.002	-0.06	NS
W1 (%)	-0.12	NS	-0.18	0.05	-0.04	NS	0.09	NS
W2 (%)	-0.19	0.05	-0.03	NS	0.06	NS	-0.16	0.08
ACT	0.36	0.001	0.30	0.001	0.32	0.001	0.04	NS
A1 (%)	0.12	NS	0.23	0.02	0.22	0.03	-0.08	NS
A3 (%)	-0.47	0.0001	-0.59	0.0001	-0.42	0.0001	-0.11	NS
A4 (%)	0.20	0.05	0.11	NS	0.20	0.05	0.05	NS
A5 (%)	0.43	0.0001	0.48	0.0001	0.33	0.001	0.17	0.09
ACT-RC	-0.11	NS	-0.25	0.01	-0.20	0.05	0.07	NS
Tf	0.30	0.003	0.16	0.08	0.05	NS	0.15	NS
T2 (%)	0.12	NS	0.14	NS	0.04	NS	-0.19	0.05
T4 (%)	0.14	NS	0.18	0.06	-0.03	NS	0.24	0.01
A2-M	0.24	0.01	0.06	NS	-0.13	NS	-0.06	NS
Cp	0.35	0.001	0.37	0.001	0.14	NS	-0.06	NS
AT	0.34	0.001	0.33	0.001	0.001	NS	0.20	0.05

Table 4. **Investigation of the link between thyroid hormones and APP. The F value and significance (p) of the influence of TSH, T_3, T_4 and iodine on the investigated parameters. Shown are only those parameters for which the influence was proved. "NS" means p>0.1**

Parameter	TSH		Iodine		T_3		Iodine		T_4		Iodine	
	F	p	F	p	F	p	F	p	F	p	F	p
AGP	0.39	NS	9.07	0.001	1.32	NS	9.63	0.001	1.31	NS	5.42	0.005
W1 (%)	0.76	NS	3.84	0.02	0.74	NS	2.41	0.09	1.51	NS	1.80	NS
W2 (%)	0.37	NS	2.58	0.08	0.13	NS	2.59	0.08	2.52	0.08	4.41	0.01
W3 (%)	1.95	NS	3.33	0.04	0.47	NS	4.65	0.01	0.02	NS	2.92	0.05
AGP-RC	1.38	NS	3.29	0.04	0.31	NS	3.01	0.05	1.26	NS	6.22	0.003
ACT	0.42	NS	10.5	0.001	0.61	NS	11.1	0.001	1.44	NS	6.87	0.001
A3 (%)	0.67	NS	28.9	0.001	5.12	0.01	17.3	0.001	3.32	0.04	13.5	0.001
A4 (%)	2.30	NS	5.48	0.005	0.21	NS	4.76	0.01	0.01	NS	2.36	NS
A5 (%)	1.49	NS	10.5	0.001	2.60	0.08	4.60	0.01	0.08	NS	5.01	0.01
T2 (%)	3.29	0.04	0.26	NS	1.69	NS	0.28	NS	1.65	NS	0.56	NS
Cp	0.31	NS	10.4	0.001	1.00	NS	9.27	0.001	0.06	NS	15.3	0.001
Hp	1.83	NS	5.42	0.005	0.04	NS	4.71	0.01	0.42	NS	1.36	NS
AT	2.40	NS	5.82	0.004	0.21	NS	6.82	0.001	0.21	NS	6.74	0.001

investigated protein was not dependent on the concentration of the hormones of pituitary-thyroid system. However, the percentage of variant A3 of ACT was dependent on triiodothyronin and the percentage of T2 variant of Tf was dependent on TSH.

Thus, low iodine in urine was associated with decreased AGP, ACT, along with altered glycosylation of those glycoproteins, with decreased Cp, Hp and AT. The investigated proteins were not reliably bound to the hormones of pituitary-thyroid system but to the level of iodine in the organism, which could be shown by the high correlation values. Also the glycosylation profiles of the investigated proteins correlated with iodine provision, not with the hormones. This was mainly true for the AGP and ACT variants strongly reactive with ConA, thus possessing mainly biantennary glycans. All the described links were not dependent on age or sex of the enrolled children and were stable for the whole investigated group. The iodine provision seemed not to influence the serum concentration of Tf and the percentage of its variants, the serum concentration of A2-M nor the AGP or ACT variants non-reactive with ConA (with mainly triantennary glycans).

The explanation of these associations observed between iodine and investigated APP is difficult. It could be suggested that in euthyroid children with low iodine excretion with urine a hidden iodine deficiency is already registered by the regulatory mechanisms and a disturbance of liver protein synthesis appears, first for the most important acute phase reactants. It may be postulated that these alterations appear in order to increase iodine uptake and storage or they play a regulatory role. It cannot be stated that such a small iodine deficit is already causing protein deficiency as general health status of the investigated children was satisfying. Besides the observed alterations of the glycosylations profiles were rather similar to what was previously described as acute inflammatory changes rather than a sign of deficit (according to our previous observations liver cirrhosis caused dramatic decrease of ConA reactivity along with decreased APP concentrations; unpublished data). According

to our own experience any inflammatory process would cause elevation in at least AGP level (if not other glycoproteins, even in absence of elevated CRP), along with elevated AGP-RC in acute inflammation or decreased in chronic inflammatory processes. Finally, decreased levels were also found in pregnancy and in allergy, mainly in allergy to food, but in this cases reactivity to ConA was also diminished. Thus, this is the first report on decreased AGP level with increased AGP-RC. Though we are not able to show the exact mechanism of iodine influence on the synthesis and glycosylation of acute phase proteins, the proven links seem interesting and we would like to describe them as preliminary study.

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