

IMPORTANCE OF SERUM BILE ACIDS DETERMINATION IN ADOLESCENTS WITH JUVENILE HYPERBILIRUBINAEMIA

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SUMMARY

The aim of the study was to identify moderate liver impairment in a group of hyperbilirubinaemic adolescents. Using gas chromatography we assessed both total bile acid and primary bile acid levels in 50 adolescents with juvenile hyperbilirubinaemia. At the same time we performed hepatologic examinations and subsequent follow-up assessment of these patients for a period of at least 2 years. As a control group we examined 30 adolescents without any impairment of both the liver and gastrointestinal tract, and 18 patients with low grade (moderately) active chronic hepatitis. In both groups we assessed total and primary bile acids levels as well as conventional liver tests (bilirubin, ALT, AST).

On the basis of the clinical course and laboratory findings we divided our patients with juvenile hyperbilirubinaemia into two groups: a group of individuals with Gilbert's syndrome (30 patients) and a group of individuals with probable moderate liver impairment (20 patients). The latter group consisted of the adolescents who exhibited bilirubinaemia over 90 $\mu\text{mol/l}$ and/or exhibited hepatomegaly or splenomegaly proved by the ultrasound examination and/or exhibited intermittent elevation of the liver aminotransferases serum levels.

In the group of individuals with moderate liver impairment serum total bile acid levels were significantly elevated in 26% of patients, and the serum cholic acid level was significantly elevated in 25% of patients. These two parameters mutually correlated at a high level of significance.

Juvenile hyperbilirubinaemia is one of the common conditions of adolescent age. Its etiology is diverse; it includes both benign conditions like Gilbert's syndrome and post-hepatitic and toxic conditions that require a long-term regimen and follow-up examinations.

The number of people suffering from juvenile hyperbilirubinaemia has been growing in the population. Currently 4-6% of the adolescent population suffers from this disease. This growing number is probably caused by external factors of our environment (infection, toxic effects). The determination of mild liver disease in hyperbilirubinaemic patients and the provision of an adequate regimen of exercise and adequate nutritional measures is of great importance for the health of the adolescent population.

Key words: juvenile hyperbilirubinaemia, adolescence, bile acids, Gilbert's syndrome, hepatotoxic agents, mild liver disease (MLD)

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INTRODUCTION

Juvenile hyperbilirubinaemia is frequently present in the adolescent population, triggering concerns among the physicians or the families of the adolescents about serious diseases of the liver or other organs. The monitoring of disorders of the liver function in these conditions and study of external factors leading thereto might contribute to the prevention and more accurate treatment of juvenile hyperbilirubinaemia. By examining and monitoring the adolescents with juvenile hyperbilirubinaemia, we suggest to introduce more precise terms: the diagnostic, prognostic and assessment aspects of this etiologically heterogeneous group.

When assessing these conditions in adolescents, it is often necessary during the examination or treatment to make decisions about the choice of the professional career, additional preparation for a job, or about the possibility of playing competitive or professional sports. This can all affect the adolescent for the rest of his/her life. Making the right decision during the monitoring of an adolescent can provide a great benefit for the health of that

individual and for the physical fitness of the young generation. We should eliminate useless limitations of these individuals on one hand, and on the other hand we should precisely, if possible, define the period and type of treatment and regimen measures, if any, which should be observed by the adolescent. Examination of serum levels of bile acids, which are one of the few humoral biochemical markers to bring functional aspects into the examination of patients, could be helpful for this decision making.

The objective of this study was to identify mild liver disease in patients with juvenile hyperbilirubinaemia by examining total and primary bile acids in the serum. This marker is probably more precise than determination of conventional liver tests.

According to the scientific literature, the prevalence of juvenile hyperbilirubinaemia in the adolescent population is 5%. Okolicsanyi reported 5% prevalence in the population (1), while Schmidt reported 0.5 - 10% in different groups (2), and Bosma 3 - 10% (3). Chrz, Jirsa and Brodanová described an increasing number of patients in the population of the Czech Republic: it was approximately 6% in the 1970s, and 8.6%

in the 1980s (4). A several-fold increase in the occurrence of hyperbilirubinaemia, as described in Czech blood donors during the 1980s, suggests the significant role of exogenous factors in the development of hyperbilirubinaemia. Only 1.3% of blood donors exhibited hyperbilirubinaemia in 1983, while it was 4.0% of blood donors in the year 1988 (5). The most frequent causes of juvenile hyperbilirubinaemia are Gilbert's syndrome and conditions after a past liver disease (such as infection, toxic effects). Type II Crigler-Najjar syndrome belongs to the group of rarely occurring familial hyperbilirubinaemias. Differential diagnosis of isolated hyperbilirubinaemia during adolescence includes in particular low score chronic hepatitis, Wilson's disease, and α_1 -antitrypsin deficiency. The examination of the external factors affecting the liver functions might be important for the entire adolescent population.

STUDY GROUP AND METHODS

During the years 1995 to 1999 we examined serum levels of bile acids in 98 patients.

These patients were divided into 4 study groups:

Based on the results of clinical examinations during at least a two-year follow-up period, we divided the juvenile hyperbilirubinaemia group into group 1 (compatible with the diagnosis of Gilbert's syndrome and comprising 20 patients) and group 2 (incompatible with the diagnosis of Gilbert's syndrome and comprising 30 patients).

The inclusion criteria used for the enrollment of patients into group 2 were as follows:

At least one of the following three criteria was found during the two-year follow-up period

1. the level of bilirubin exceeded 90 $\mu\text{mol/l}$ at least once;
2. hepato- and/or splenomegaly;
3. transient elevation of the originally absolutely normal aminotransferase levels up to twice the upper level of the reference range.

Group 3 was a control group and comprised 30 patients, while group 4 comprised 18 patients with low grade chronic hepatitis.

Characteristics of the Individual Groups

The group of patients with juvenile hyperbilirubinaemia (group 1 and 2): The average age of patients with juvenile hyperbilirubinaemia was 17.6 years, ranging from 13 to 23 years of age. The group comprised 44 boys and 6 girls. The patients were monitored in our outpatient ward for 2 to 4 years. Upon admission in our counseling center or for hospitalization, the hyperbilirubinaemia was known in the patients for the period of one month up to 6 years. Hyperbilirubinaemia was most frequently detected during examinations for visible jaundice of the sclerae or skin, followed by symptoms of increased, non-specific gastrointestinal disorders and by coincidence. The maximum hyperbilirubinaemia found during the follow-up period was 130 $\mu\text{mol/l}$, the maximum proportion of the conjugated fraction of bilirubin was 20%. Two patients had a history of infectious mononucleosis in childhood, none of the studied patients had infectious hepatitis A, B, or C or other liver disease. Abuse of alcohol was found neither in the juvenile hyperbilirubinaemia group nor in the control group.

None of the patients had a history of other hepatotoxic effects. Isolated hyperbilirubinaemia in family history was found in 10 patients (20% of the group) and in 2 of them the isolated hyperbilirubinaemia was found in several family members (namely: mother, father and brother in both these patients); in 4 patients from the study group we found a family history of other liver disease in first-level relatives. These were liver cysts in the patient's mother; liver cirrhosis in the father of the patient's mother, two cases of unclear hepatopathy very likely to be caused by EBV infection (one in the patient's father and one in the patient's mother).

Liver examination (Table 1) was performed during the first examination (in 30 patients during the hospitalization), or in 20 patients during comprehensive outpatient examination. Liver-type hyperbilirubinaemia was identified and confirmed by triple examination of reticulocytes and by normal levels of plasmatic haptoglobin. Routine liver tests were performed, including bilirubin, conjugated fraction of bilirubin, aminotransferases ALT, AST and ALP and GMT. Basic immunological examination was carried out, including immunoglobulins, C 3, CIK, ANAb, with normal findings in all cases; as well as serological examination for hepatotropic infections: such as hepatitis A, B, C (which was negative in all patients); EBV (Fig. 1); in 58 % of the study group, we found positive IgG against viral capsid antigen (VCA) only; in 24% we found one positive result for IgM and IgG against VCA, which means active infection, while the anti-EBNA antibodies were always positive, which suggests the activation of the infection. In 6% of cases we found a repeated activation of EBV infection

Table 1. Evaluation methods in patients with juvenile hyperbilirubinaemia

Evaluation	Tests
Hematological	blood picture, reticulocytes (3x) (haptoglobin)
Biochemical	bilirubin direct (indirect) and total ALT, AST, ALP, GMT Quick test, INR cholinesterasis, prealbumin, ceruloplasmin α_1 -antitrypsin
Immunological	Ig, CIK, ANAb
Serological	hepatitis A, B, C EBV, CMV, HSV toxoplasmosis
Parasitological	stool sample (3x)
Imaging methods	ultrasonography of the liver, spleen and biliary tract cholescintigraphy

ANAb – antinuclear antibody

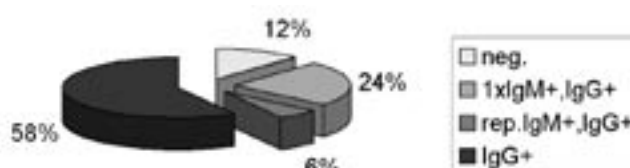


Fig 1. EBV - VCA antibody in hyperbilirubinaemic patients (group 1,2).

during the follow-up, which means repeated positive findings of IgM and IgG against VCA and positive anti-EBNA antibodies. During the CMV examinations, positive IgG antibodies were found in 6 cases, while no active infection including positive finding of IgM antibodies was found; a test for toxoplasmosis was negative in all patients. Triple examination of stools for the presence of parasites was always negative. We examined ceruloplasmin and α_1 -antitrypsin in all patients with normal findings. We performed sonography of the liver, spleen, gall-bladder and bile ducts. Alterations to these organs were found in 10 patients (20% of the group), of which 5 patients were diagnosed with splenomegaly, 3 patients were diagnosed with hepatomegaly and 2 with hepatosplenomegaly. In two patients, liver biopsy was performed after the examination and follow-up, revealing the finding of S/P past liver damage with microdroplet steatosis and minor periportal fibrosis, without any signs of chronic inflammation.

The control group (group 3 - controls) comprised 30 patients aged between 14 and 22 years, who were examined for other reasons and had no history of liver disease, gastrointestinal tract disorders or serious renal diseases with impairment of renal functions. The average age in this group was 17.9 years.

In addition to bile acids, we performed a conventional liver test, and examined bilirubin, ALT and AST in these patients.

The group of patients with low grade chronic hepatitis (group 4) comprised 18 patients, of which 9 were recruited from the patients attending the Gastroenterological Advisory Center of the Clinic of Pediatric and Adolescent Medicine, Medical Faculty I, Charles University (age range from 9 to 22 years) and other 9 patients from the Gastroenterological Advisory Center of Ass- Prof. Dr. Stránský of the Department of Internal Medicine, Medical Faculty III, Vinohrady, Charles University (age range 18 to 56 years). Conventional liver tests, ALT and AST were examined in these patients in addition to bile acids.

Collection of Blood Samples for Bile Acids and Laboratory Examinations

The bile acids samples were taken from the patients in the morning under standard conditions after overnight fasting. The collected blood samples were centrifuged and the serum was frozen to -20°C and stored.

In all patients evaluated, we examined levels of bile acids in the serum using gas chromatography, as described in detail in relevant literature (6, 7). The examinations included total bile acids levels, primary bile acids levels (cholic acid and chenodeoxycholic acid), and secondary bile acids (deoxycholic, lithocholic, and ursodeoxycholic acid). At the same time, conventional liver tests, including bilirubin, ALT and AST, were performed in all 4 patient groups.

Gas Chromatography Used

To evaluate the serum levels of bile acids, we used gas chromatography, performed in the Research Biochemical Laboratory of the Medical Faculty I, Charles University, using the Dorcus analyzer, capillary glass columns and anion ion-exchanger AMBERLYST A - 26. The values obtained were compared with the internal standard, Bisnor, graphically plotted over the curve and processed into tables on the computer (8).

Statistical Methods

We determined basic statistical characteristics, such as means, standard deviations, minimal and maximal (peak) values and medians for values of total and individual bile acids and for indices thereof, and for the values of conventional liver tests.

Additional statistical evaluation was performed using analysis of variance ANOVA, including the determination of statistically significant differences between all groups studied and t-tests between 2 groups with the determination of Bonferroni's level of significance.

We determined correlation coefficients with a designated level of significance between the total bile acids and cholic acid and conventional liver tests (bilirubin, ALT, and AST).

RESULTS

Serum levels of total bile acids (TBA), primary bile acids: cholic acid (CA) and chenodeoxycholic acid (CDCA) (Tables 2, 3, 4) and secondary bile acids: lithocholic acid (LA), deoxycholic acid (DCA) and tertiary ursodeoxycholic acid (UDCA) were evaluated in 98 individuals in 4 groups. Using the analysis of variance, ANOVA, the values obtained were compared between the groups and the statistically significant differences were determined according to Bonferroni's level of significance when using a t-test between the 2 groups (Table 5).

Bile Acids in the Individual Groups

The values of total, primary and secondary bile acids were compared in groups 1 and 2, in the controls and in the group with chronic mild active hepatitis (chronic group).

Total bile acids (TBA) showed a statistical difference at the 5% level of significance between the control group and group 2, patients incompatible with Gilbert's syndrome. Significant difference in TBA was also found between groups 1 and 2, with mean concentrations of 3.31 and 5.36 $\mu\text{mol/l}$ respectively ($p=0.05$). In contrast, almost identical mean values were found between the control group and group 1 (patients compatible with Gilbert's syndrome). As expected, a difference in the TBA values was found

Table 2. Total bile acids (TBA)

Group	No of samples	Results up normal		Mean	SE	TBA levels		Median
		n=	%			min	max	
Group 1	20	0	0.00%	3.3135	1.1884	1.59	5.43	3.1150
Group 2	30	8	26.67%	5.3697	3.3351	1.07	15.60	4.4450
Controls	30	2	6.67%	3.3693	1.6746	0.92	7.10	3.1050
Chronics	18	9	50.00%	7.5233	4.9979	1.90	18.21	6.4150

Normal 1.1-7 $\mu\text{mol/l}$

Table 3. Cholic acid (CA)

Group	No of samples	Results up normal		Mean	SE	CA levels		Median
		n=	%			min	max	
Group 1	20	2	10.0%	0.7695	0.3677	0.22	1.55	0.6700
Group 2	30	7	23.3%	1.0547	0.7133	0.15	3.33	1.0000
Controls	30	2	6.7%	0.7177	0.3122	0.22	1.61	0.5700
Chronics	18	9	50.00%	1.9728	1.4487	0.48	5.64	1.4800

Normal 0.22-1.4 $\mu\text{mol/l}$

Table 4. Chenodeoxycholic acid (CDCA)

Group	No of samples	Results up normal		Mean	SE	CDCA levels		Median
		n=	%			min	max	
Group 1	20	0	0.00%	1.2285	0.7172	0.32	2.79	0.9700
Group 2	30	0	0.00%	1.8820	1.4699	0.36	6.04	1.3900
Controls	30	0	0.00%	1.3513	1.0431	0.18	4.84	1.1400
Chronics	18	0	0.00%	3.8300	3.0610	0.23	9.82	3.7600

Table 5. Analysis of variance - ANOVA

Parameter	ANOVA	t - tests between 2 groups with Bonferroni's level of significance					
	total	controls group 1	controls group 2	controls chronics	group 1 group 2	group 1 chronics	group 2 chronics
TBA	0.0008 xxx	0.9978	0.0047 x	0.0032 x	0.0042 x	0.0031 x	0.1257
CA	0.0019 xx	0.7108	0.0154 +	0.0020 x	0.0758	0.0035 x	0.0230
CDCA	0.0054 xx	0.7941	0.1232	0.0044 x	0.0462	0.0029 x	0.0222
CA / CDCA	0.6653	0.9919	0.6381	0.5914	0.6665	0.5922	0.4182
(CA+CDCA)/(LCA +DCA+UDCA)	0.0049 xx	0.6336	0.0164 +	0.0271	0.4319	0.0150 +	0.0049 x

For abbreviations see text

ANOVA: analysis of variance; x - level of significance 0.05; xx - level of significance 0.01; xxx - level of significance 0.001; + - level of significance 0.12

also between the controls and patients with chronic mild active hepatitis and between the group 1 and chronic group.

Values of cholic acid showed a statistically significant difference between the control group and chronic mild active hepatitis group and between group 1 and chronic mild active hepatitis group. These differences were lower than the 5% level of significance, while there were only suggested differences (under 10% level of significance) between the control group and group 2.

Similar differences between the groups were found also for values of chenodeoxycholic acid. A statistically significant difference ($p=0.05$) was found between the control group and chronic group and between group 1 and the chronic group.

Conventional Liver Tests – Bilirubin, ALT, AST

The values of bilirubin, ALT and AST were obtained and statistically evaluated (Fig. 2-4), and the analysis of variance of these conventional liver tests was performed in all groups using t-test between the individual groups to determine Bonferroni's level of significance of differences (Table 6).

Bilirubin was considerably elevated in group 1 and 2 compared to the control group and chronic hepatitis group, the mean values in the control group and in the chronic hepatitis group ranged in normal limits (11.93 and 13.53 $\mu\text{mol/l}$ respectively), although the bilirubin level exceeded the upper level of the reference range, achieving up to 45 $\mu\text{mol/l}$, in three patients of the chronic group.

No statistically significant difference in bilirubin levels were found between group 1 and group 2, with mean values of 46.85 and 53.6 $\mu\text{mol/l}$, respectively.

The significance of differences between the juvenile hyperbilirubinaemia groups (group 1, 2), control group and chronic group was always under the 1% level.

The ALT and AST parameters ranged within normal values in all individuals of group 1, group 2 and the control group; for ALT, no statistically significant difference was found between any of the four groups under investigation. A statistically significant difference in AST was found between the control group and the chronic hepatitis group and a suggested difference (under 10%

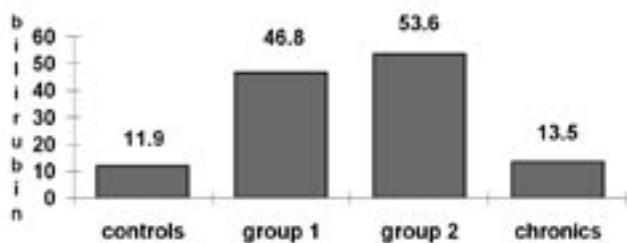


Fig. 2. Mean values of bilirubin ($\mu\text{mol/l}$) and result of ANOVA: $p = 0.0001$.

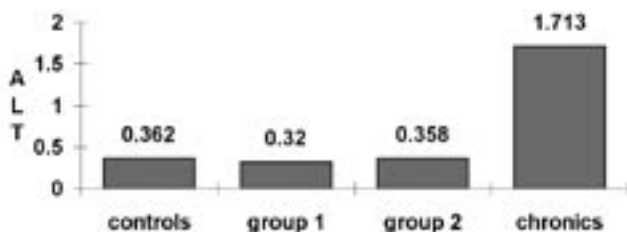


Fig. 3. Mean values of ALT ($\mu\text{kat/l}$) and result of ANOVA: $p = 0.115$.

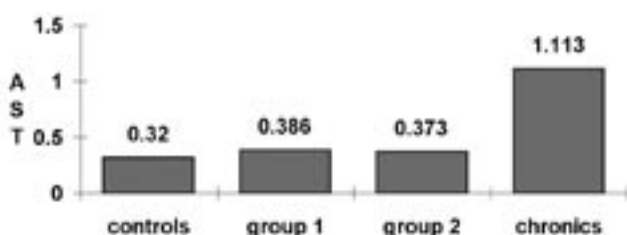


Fig. 4. Mean values of AST ($\mu\text{kat/l}$) and result of ANOVA: $p = 0.008$.

level of significance) was found between group 1 and the chronic hepatitis group and between group 2 and the chronic hepatitis group.

Coefficients of Correlation between the Bile Acids and Conventional Liver Tests

Coefficients of correlations between the TBA and CA, bilirubin, ALT and AST values were calculated (Tables 7, 8).

Table 6. Analysis of variance - ANOVA

Parameter	ANOVA	t - tests between 2 groups with Bonferroni's level of significance					
	total	controls group 1	controls group 2	controls chronics	group 1 group 2	group 1 chronics	group 2 chronics
Bilirubin	0.0001 xxxx	0.0001 xxxx	0.0001 xxxx	0.5410	0.1646	0.0001 xxxx	0.0000 xxxx
ALT	0.1153	0.9003	0.9892	0.0472	0.9099	0.0415	0.0466
AST	0.0076 xx	0.6334	0.0285	0.0076 x	0.6794	0.0132 +	0.0117 +

For legend see Table 5

Table 7. Coefficient of correlation with designed level of significance, total bile acids (TBA) with ALT, AST, ALP, CA, bilirubin

Group	Coefficient of correlation with designed level of significance			
	ALT	AST	CA	bilirubin
Controls n = 30	- 0.225	0.084	0.428 x	- 0.144
Group 1 n = 20	- 0.294	- 0.106	0.655 xx	- 0.423
Group 2 n = 30	- 0.222	0.269	0.699 xx	0.431 x
Chronics n = 18	0.337	0.294	0.901 xx	0.082

For legend see Table 5

Table 8. Coefficient of correlation with designed level of significance, cholic acid (CA) with ALT, AST, bilirubin

Group	Coefficient of correlation with designed level of significance			Critical levels of coefficient of correlation	
	ALT	AST	bilirubin	pro 0.05	pro 0.01
Controls n = 30	- 0.057	- 0.224	- 0.142	0.361	0.463
Group 1 n = 20	- 0.425	- 0.501 x	- 0.467 x	0.444	0.561
Group 2 n = 30	- 0.093	0.280	0.456 x	0.361	0.463
Chronics n = 18	0.114	0.095	- 0.157	0.468	0.590

For legend see Table 5

As expected, the levels of TBA and CA correlated in all study groups; concerning the other parameters, level of bilirubin correlated with TBA in group 2, while ALT and AST failed to correlate with TBA in all 4 study groups.

Similar results were obtained when correlating the levels of CA with conventional liver tests (bilirubin, ALT, AST), where a high positive correlation was found only between the bilirubin and CA levels in group 2 of patients incompatible with Gilbert's syndrome.

Comparison of Results with Reference Values of Bile Acids

We compared a proportion of the patients (in %) with TBA and CA levels increased over the generally accepted norm in the study groups (see Table 2 and 3).

No patient in group 1 exceeded the accepted reference value of total bile acids (7 $\mu\text{mol/l}$) for the given age, while 8 patients from group 2 (26.6%) exceeded this norm; in addition, 2 of the 8 patients exceeded twice the limit for the respective age (14 $\mu\text{mol/l}$). In the control group, 2 patients showed borderline values of TBA max. 7.1 $\mu\text{mol/l}$ and in the mild chronic hepatitis group (group 4) a total of 9 patients (50%) exceeded the limit for the chronic group.

For cholic acid (CA), the limit for the given age (1.4 $\mu\text{mol/l}$) was exceeded by 2 patients from group 1 (10%) and by 7 patients from group 2 (23.3%), whereas the values in group 2 exceeded the limit twice as much in 4 cases reaching up to 3.33 $\mu\text{mol/l}$. In the control group, one patient (3.3%) had a borderline value of 1.55 $\mu\text{mol/l}$; and in the chronic hepatitis group, 50% of individuals achieved elevated levels of CA, where the values exceeded the norm three times (reaching up to 5.64 $\mu\text{mol/l}$) in two cases.

DISCUSSION

The juvenile hyperbilirubinaemia group is heterogeneous in terms of etiology, however, a precise diagnosis within this group is very difficult, especially if a discrimination is needed between familiar hyperbilirubinaemia (Gilbert's syndrome) and conditions after the past liver disorder, or in case of combination of both.

Using the examination of bile acids as an indicator of liver dysfunction, we attempted to obtain an additional parameter, which would help to differentiate more clearly this population group of adolescents with mild liver disease.

In this work we evaluated the results of bile acids with values of conventional liver tests, clinical outcomes during the follow-up of the patients, and with the results of the conventional fasting test, i.e. with the parameters that are used to diagnose Gilbert's syndrome and conditions after past liver disease. We were unable to compare the levels of bile acids in most cases with positive finding of liver biopsy, because this diagnostic procedure is mostly not indicated for use in juvenile hyperbilirubinaemia and longitudinal monitoring of patients is preferred.

Total and Primary Bile Acids in Juvenile Hyperbilirubinaemia

After applying the clinical criteria determined during the longitudinal study (i.e. not during a single examination), the group of 50 patients was split into 2 groups (group 1 and group 2).

In group 1, compatible with Gilbert's syndrome, we found completely normal values of total and primary bile acids; in this group we found a higher number of patients with a positive family history of isolated hyperbilirubinaemia (in 8 patients of group 1 versus 2 patients in group 2). The finding that normal values of total and primary bile acids in group 1 confirms the works from the 1980s, in which total and primary bile acids levels were measured and their values were recommended as one of the criteria of Gilbert's syndrome (8).

The said works were published in 1982 by Roda, who confirmed normal values of total bile acids and cholic acid in patients suffering from Gilbert's syndrome by means of verified and normal finding of liver biopsy and in the conclusion section recommended using of serum bile acid levels to exclude early stages of other hepatobiliary diseases.

Similar conclusions were made in 1982 by Vierling (9), who found the normal values of primary bile acids in all patients suffering from Gilbert's syndrome, irrespective of their classification in one of three groups, according to the elimination of bromsulphalein and indocyanine green.

In 1980, other authors, such as Olsson and Lindstedt (10) compared the results of bile acids with the so-called conventional diagnostic tests and confirmed normal values of bile acids and low specificity and sensitivity of the so-called conventional tests (tests employing nicotinic acid and fasting tests) (11).

In group 2 we confirmed higher levels of total bile acids and cholic acid compared to group 1 and control group at statistically significant level.

Compared to group 1, elevation of cholic acid and total bile acids over the standard values for the given age was found in 25 % of patients, wherein the values reached more than two times the standard value in 2 patients. To explain this result in the absence of cholestasis and upon observation of the basic conditions of sample collection, we must use the model of disorder of clearance capacity of the liver for bile acids accompanied by a dysfunction of liver cells which was not identified by conventional liver tests. The ALT, AST, ALP, and GMT values were normal in all patients during sample collection.

We found no reports in the literature on differentiation of the juvenile hyperbilirubinaemia group using results of bile acids.

The works studying the measurement of serum levels of bile acids in mild liver disease were published by Collazos (12, 13), who compared patients with histologically confirmed mild liver disease (MLD) with patients suffering from chronic active hepatitis. He found increased levels of cholic acid in 10% of patients with mild liver disease and in 53.3% with chronic active hepatitis. In another study, he compared patients with chronic active and chronic persisting hepatitis and revealed an increase by 53.3% for active and by 20% for persisting hepatitis. The results of chronic persisting hepatitis largely correspond with the results obtained from patients in our (high-risk) group 2, where increased total and primary bile acids were confirmed in 25% of patients, and the increase of cholic acid was up to 3.3 $\mu\text{mol/l}$. The level of risk in group 2 following the past liver disease can be seen in the reduced capacity of hepatocytes to adequately respond to stress situations. The disorder of detoxication capabilities of liver cells were described in many works, and the disorder of enzymatic systems of liver cells were confirmed by histochemical analyses. These findings can be further supported by functional isotopic findings of the disorder of the transport mechanisms for organic ions (disorder of clearance of bromsulphaleine and indocyanine green) (14).

Etiological Factors in Juvenile Hyperbilirubinaemia

The etiology of isolated hyperbilirubinaemia in adolescents is variable. In addition to unambiguous genetic factors, external factors play a role bringing certain risks for adolescents, which must be considered in the care provided to these individuals. The study of the impact of occupational setting and external environment should be continued.

1. Gilbert's syndrome – genetic and pathophysiological aspects. Mild isolated non-conjugated hyperbilirubinaemia is caused by Gilbert's syndrome in some adolescents. We know

that Gilbert's syndrome is an autosomal recessive disease. At the molecular level, the disease is caused by a mutation in the promoter region of the gene encoding UDPGT (uridine-diphosphate-glucuronosyl-transferase). The disorder of part of the promoter region of the gene (TATA box) results in the disorder of the start of transcription of the structural gene for the enzyme responsible for the conjugation of bilirubin (3). This molecular disorder is not the only molecular disorder present in Gilbert's syndrome. A disorder of the transport of all organic ions was described in some individuals (disorder of clearance of indocyanine green), which results in the disorder of detoxification function of the liver.

2. Status of post-infectious liver disease. Such diseases can include a disease of the hepatic parenchyma and the alterations described are caused as a result of hepatic damage. The liver damage can be caused by hepatotropic viruses, especially by herpetic infections, which may afflict the liver and trigger a dysfunction of hepatocytes even in the case of the atypical or oligosymptomatic course of disease. With respect thereto, we focused in particular on anti-EBV antibodies. We found a higher share of patients with positive anti-EBV IgG antibodies in the total juvenile hyperbilirubinaemia group; however, in group 2 we found also a substantially higher number of patients, in whom we had detected once or several times during the follow-up period the activation of the EBV infection with positive IgG and IgM against the viral capsid antigen. This finding supports the results obtained by Hamanová et al. (14, 15), who found a higher prevalence of EBV in the group of patients with juvenile hyperbilirubinaemia, thus supporting the theory that EBV infection, and especially its activation, has a role in the development of juvenile hyperbilirubinaemia in some patients. In addition, according to the literature, the presence of anti-EBV IgG antibodies, i.e. the prevalence in adolescent populations of developing countries, is approximately 60 % (16), while it is 88.6% in the juvenile hyperbilirubinaemia group in our work.

3. Influence of drug abuse. Toxic damage to the liver can be another reason for hepatic dysfunction in adolescent patients. We used a target-oriented analysis to exclude the effects of toxic substances (such as abuse of alcohol or contact with hepatotoxic substances at work or during leisure time) in the study patients examined and in the control group. Toxic damage to the liver might be a very important determinant of the current state of juvenile hyperbilirubinaemia in terms of epidemiology. Many published works reporting an increased incidence of hyperbilirubinaemia in the population seem to support this view. A syndrome of risk behavior in adolescents could have an important role in this situation. Abuse of addictive substances often brings a very high risk of liver damage. The extent of this problem in developing countries and in the Czech Republic could have a negative influence on the state of health of the entire adolescent population. There are several thousands of so-called problematic drug users in the Czech Republic; the share of intravenous drug users has been increasing significantly – currently they form more than 60% of problematic users of addictive substances. The extent of so-called experimenting with drugs is several times higher and these are estimates only. Experimenting with drugs can also result in liver damage, including increased bilirubin levels. From this point of view, high-risk behavior includes inhalation of organic

solvents (sniffing) (17) and hepatitis caused by abuse of MDMA (methylenedioxyamfetamin = ecstasy) (18).

4. Wilson's disease and α_1 -antitrypsin deficiency. Other liver diseases, such as Wilson's disease or A1-AT deficiency can manifest during their initial phases by isolated hyperbilirubinaemia only. These diagnoses were excluded by targeted examination of the said markers in all patients of group 1 and 2.

CONCLUSION

In a group of adolescent patients with juvenile hyperbilirubinaemia, we examined serum levels of total, primary, and secondary bile acids. During the 2- to 4-year-follow-up period, we divided 50 patients into 2 groups, based on clinical criteria, as compatible and incompatible with benign hyperbilirubinaemia (Gilbert's syndrome).

Our measurements gave statistically significant differences in the results of total and cholic acid. The percentage of patients with elevated bile acids in the group is higher than in patients with mild liver disease and approximately identical as in patients with chronic persisting hepatitis, as observed in other works.

According to our findings, the examination of serum levels of total and primary bile acids can be used for the detection of liver cells dysfunction in the group of juvenile hyperbilirubinaemias, which is poorly diagnosable causally, to differentiate between the Gilbert's syndrome and conditions after the history of liver disorders or after combination of both.

The near future will probably bring the possibility of using molecular biology approaches for diagnosis of Gilbert's syndrome, which are already quite well developed today. However, what is important for the group of adolescent patients is the functional aspect of the presence or absence of liver dysfunction, as a matter that will decide in the given phase of an adolescent's life, upon choice of professional career, and especially upon the capability of individuals with juvenile hyperbilirubinaemia to perform competitive or professional sports.

When assessing the contribution to scientific knowledge, we believe that the point of this work is in contributing to the differentiation in the juvenile hyperbilirubinaemia group using serum levels of bile acids, which we did not find in the relevant literature dealing with this issue.

The second finding, directly resulting from our work, is the higher presence of antibodies against EBV compared to the general adolescent population or in the control group, and the higher presence of activation of EBV infection in the group incompatible with Gilbert's syndrome, where previous liver damage is a characteristic.

After a liver disorder, adolescents require a sufficient liver-protecting regimen. Undesirable chemical stress and toxic effects of alcohol should be excluded.

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