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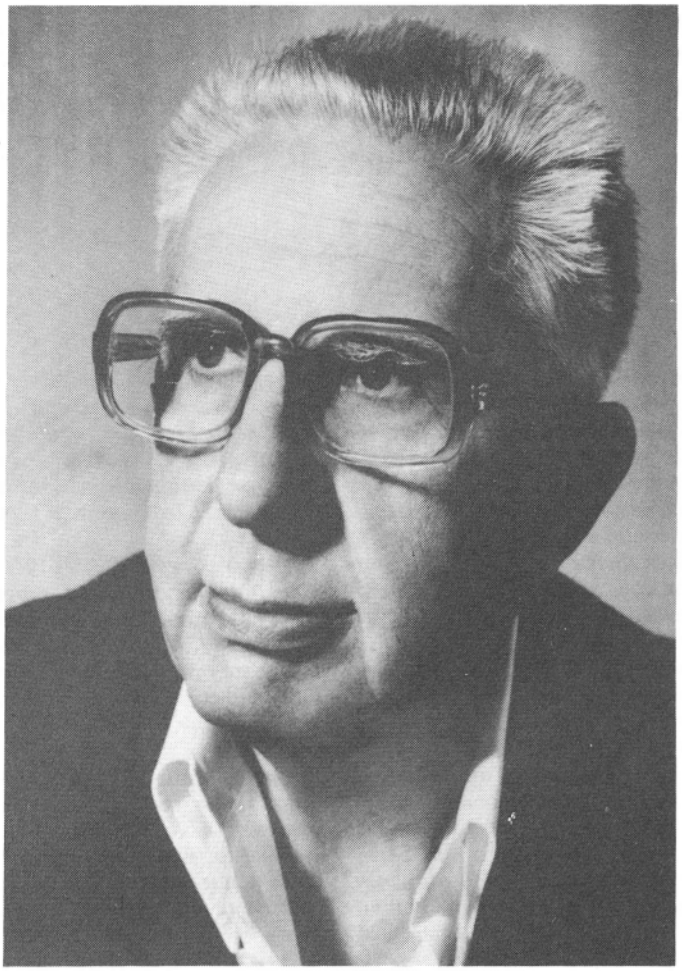
ISSUE OF PEDIATRIC DEPARTMENT OF ENDOCRINOLOGY, DIABETES AND METABOLIC DISEASES IN LJUBLJANA

ŠTEVILKA KLINIKE ZA ENDOKRINOLOGIJO, DIABETES IN PRESNOVNE BOLEZNI PEDIATRIČNE KLINIKE V LJUBLJANI



DEDICATION

This issue of the journal has been dedicated to prof. dr. Leo Matajc (1914–1990), the pioneer of pediatric endocrinology in Slovenia.

POSVETILO

To številko posvečamo prof. dr. Leu Matajcu (1914–1990), pionirju pediatrične endokrinologije v Sloveniji.

Leading article/Uvodnik

THIRTY YEARS (1963–1993) OF PEDIATRIC DEPARTMENT OF ENDOCRINOLOGY, DIABETES AND METABOLIC DISEASES LJUBLJANA – SLOVENIA

Ciril Kržišnik

Due to the fact that the same endocrine disorders in children and adolescents could have quite different signs, symptoms and course than in adults, pediatric endocrinology was separated from general endocrinology almost sixty years ago. The first pediatrician who established pediatric endocrinology in Europe was Prof. Dr. Andrea Prader from University Department of Pediatrics in Zürich, born in 1919.

In Slovenia Prof. Dr. Leo Matajc (1914–1990) was the one who started to treat pediatric and adolescent endocrinopathies in early sixties. Following his visit to Professor Henry Lestradet in the Centre d'Etudes sur le Diabète et la Nutrition chez l'Enfant at Herold Hospital Paris in 1960, Professor Matajc introduced the modern concept of regular diet for diabetic children in Slovenia, as well as self-monitoring based on measuring glycosuria and later on glycaemia using test-strips, and adaptation of insulin doses according to the results of these measurement. Education of newly admitted young diabetics and their parents was organised at the Department of Endocrinology, Diabetes and Metabolic Diseases, Childrens' Hospital of Ljubljana which he officially established in 1963 (1). At that time he met with considerable opposition from diabetologists treating adult patients who were convinced that young diabetics needed nothing but a strict diet and fixed doses of insulin. Most of these conflicts have been resolved in last 15 years since modern treatment of insulin dependent diabetes based on blood glucose monitoring has been generally accepted. Nowadays, children and adolescents are treated only with human insulin, the majority with pen-injector devices. Dr. Brus has worked very hard to determine in the appropriate care for young diabetics. Since 1967, summer camps have been organised for diabetic children at the Adriatic coast, and since 1971, also financial support in order to provide the more expensive food. In addition, a bulletin for young diabetic patients »Sladkorčki« (candies) has been issued since 1971. In 1977, a »Club for parents of diabetic children and adolescents« was founded, which organises an annual meeting attended by several hundred diabetic adolescents and their parents. In 1974, Prof. Matajc was one of the pediatric diabetologists who met in Paris and founded International Study Group of Diabetes in Children and Adolescents – ISGD which was transformed into the society ISPAD in 1993.

The 15th annual meeting of the ISGD was organised in Bled by Department of Pediatrics, University Medical Centre Ljubljana in 1989. The scientific meeting in which diabetic ketoacidosis, diabetes in adolescence and recent results of IDDM investigations were the main topics was attended by more than 200 participants from all continents. In the same year, the department got involved in the epidemiological and aetiological research project on IDDM, sponsored by European community called EURODIAB (2).

In 1992, the project was extended to numerous substudies, including clinical, genetic and immunological aspects of IDDM, with the final goal to intervene in the proper time of prediabetes in affected subjects. Since 1990, the epidemiological data on IDDM have been incorporated into the WHO sponsored project DIAMOND with the official seat in USA (3). The members of ISGD from Slovenia were actively involved in preparation of IDF-WHO sponsored guidelines of treatment and care of diabetic children and adolescents in the framework of St Vincent Declaration (4). There were not only epidemiological – aetiological studies (5); the research of the department in the field of diabetology was also devoted to adrenal cyrcadian activity in diabetic children, the value of glycosylated haemoglobin measurement in erythrocytes of different ages (6), the effect of nicotinamide on thyroid function in Hashimoto disease in children, and hearing loss in young diabetic patients with limited joint mobility (7).

The department, which has 23 beds for patients, has taken care of children and adolescents with endocrinopathies and metabolic diseases from all Slovenia. In the last years, there were on average 700 admissions per year, and about 4,000 patients were treated on outpatient-basis. Of five doctors at the department, three achieved M. Sc. and one Ph. degree. All have been engaged in teaching medical students, residents, nurses and medical technicians with in the framework of the teaching programme of Medical Faculty, University of Ljubljana.

In Slovenia, screening for phenylketonuria started in 1979; and for hypothyroidism partly in 1981, while the whole republic of Slovenia was covered in 1985. The organisation of the screening programme was based on the Swiss research conducted by Prof. Ruth Illig, who also took part in the »International Conference on Congenital Hypothyroidism« in Ljubljana in 1987, which was chaired by Prof. Varl and Prof. Matajc from the University Medical Center Ljubljana. The initial programme and follow-up of patients was adapted after 10 years based on the experience and recent recommendations of the European Society for Pediatric Endocrinology (8).

Children suffering from pituitary dwarfism have been treated with growth hormone (GH) extracted from pituitary glands since 1969. Since 1987, only synthetic GH has been used. Our department has been actively involved in clinical trials of GH produced by genetic engineering in patients with GH defficiency and short stature due to Turner syndrome, and those of synthetic IGF-I in patients suffering from Laron syndrome – GH insensitivity (9, 10). The data of patients treated with GH have been followed and analysed in the scope of collaborative study of the International Study Group on Growth Disorders-KIGS, which has the official seat in Sweden (11).

Growth disorders, diabetes mellitus and precocious puberty were also the main topics of the First International Symposium on Recent Advances in Pediatric and Adolescent Endocrinology in 1988 at which Prof. Laron from Sackler University, Tel Aviv, Israel and Prof. Rogol from the University of Virginia, Charlotville, USA were the main speakers.

A part of collaborative research work of the Department and the Institute of Oncology, Ljubljana, was devoted also to endocrinopathies in patients who were treated for cancer in childhood (12, 13, 14). Doctors from the department have been involved in joint research and clinical work with the Institute of Pediatric and Adolescent Endocrinology in Tel Aviv, Israel, and the departments of pediatric endocrinology at Hôpital Debré, Paris, France and Loyola University Medical Center in Chichago, USA.

In late eighties, the Department of Endocrinology, Diabetes and Metabolic Diseases, Ljubljana, started to collaborate in the research projects with pediatricians endocrinologists from Alps-Adria region, which consists of the neighbouring areas of Italy, Croatia, Hungary and Austria. In 1990, the Alps-Adria Study Group of Pediatric Endocrinology and Diabetes – AASGPED was officially established in Trieste, Italy.

The Fourth Annual Meeting of the group which took place in Ljubljana in October 1993 was partly joined with the Second International Symposium on Recent Advances in Pediatric and Adolescent Endocrinology celebrating the 30th anniversary of the Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases Ljubljana. Most of the presentations from this international symposium are published in this issue of the leading Slovenian medical journal »Zdravniški vestnik«.

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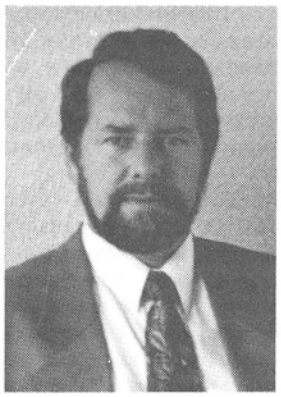
Ciril Kržišnik was born on 5 July 1944 in Ljubljana, Slovenia, where he graduated at Medical Faculty, University of Ljubljana, in 1969. He passed speciality board examination in paediatrics in 1976. In 1980, he achieved M. Sc. degree, and in 1986, Ph. D. degree in the field of paediatric endocrinology. In 1978, he was elected teaching assistant at the chair of paediatrics, Medical Faculty, University of Ljubljana, in 1987, he became assistant Professor, in 1992, Professor of paediatrics, and since 1993, he has also been the head of the chair of paediatrics. During the same period he has been the head of Paediatric Department of Endocrinology, Diabetes and Metabolic Diseases since 1982, since 1986, also deputy director, and since 1994, director of the Children's Hospital in Ljubljana. His research was



focused on glycosylated haemoglobin in diabetic children, clinical value of insulin, growth hormone and IGF-I synthesized by genetic engineering, and endocrine disorders in patients treated for malignant diseases in childhood. He has been the representative of Slovenia in International Growth Study Project KIGS with the seat in Stockholm, in European Diabetological Epidemiological Project EURODIAB with the seat in Odense, and global WHO supported project DIAMOND with the seat in Pittsburgh. He has been a member of many endocrinological and diabetological study groups and societies, including ISGD-ISPAD, ADA, IDF, EASD and AASGPED. He was a member of the group which introduced screening of congenital hypothyroidism in Slovenia. He has been the editor of the postgraduate series »Selected Topics in Paediatrics« published by Medical Faculty, University of Ljubljana.

Together with his coworkers, he published more than 120 professional and scientific articles, including five chapters in Slovenian and foreign medical textbooks.

Professional article/Strokovni prispevek

24 YEARS OF GROWTH HORMONE TREATMENT IN SLOVENIA

24 LET ZDRAVLJENJA Z RASTNIM HORMONOM V SLOVENIJI

Ciril Kržišnik

Department of endocrinology, diabetes and metabolic diseases, Medical centre Ljubljana, Department of paediatrics, Vrazov trg 1, 61104 Ljubljana, Slovenia

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Key words: *pituitary dwarfism; growth hormone; therapy; final height; psychosocial problems; Slovenia*

Abstract – Background. *Treatment of pituitary dwarfism with human growth hormone (GH) started in Slovenia in 1969. The aim of the study was to evaluate the effect of therapy on the first patients treated with GH in the republic.*

Material and methods. *Four out of 17 patients (15 boys, 2 girls) with isolated GH deficiency and 13 with multiple pituitary hormone deficiency were treated with GH and, if necessary, some other hormone – replacement therapy for at least 3 years. The age at the start of therapy was 10.7±3.5 years. All patients were reexamined at the mean age of 23.1±4.5 years, and 12 of them agreed to be interviewed regarding the quality of life. Auxological and some clinical characteristics of patients were analysed.*

Results. *Height velocity 3.7±0.8 cm per year before therapy increased during the first year of treatment to 8.3±1.6 cm and decreased during first three years of therapy to 6.3±1.1 cm. Height SD score at the beginning of therapy – 4.05±1.2 improved significantly ($p < 0.01$) to –1.75±0.81 in adult age. The final height in males was 163.2±5.7 cm, in females 151±4.2 cm. The majority of patients had, despite relatively satisfactory final height, psychological problems like deficiency in self-image, poor adjustment and impaired social relationships.*

Conclusion. *In first 17 patients treated with GH in Slovenia, there were no side effects and the final height improved significantly. In some cases psychological counselling or support had to be arranged.*

Ključne besede: *hipofizna nanosomija; rastni hormon; zdravljenje; končna višina; psihosocialni problemi; Slovenija*

Izvleček – Izhodišča. *Zdravljenje hipofizne nanosomije z rastnim hormonom se je pričelo leta 1957, v Sloveniji pa 12 let kasneje. Namen študije je bil analizirati učinkovitost zdravljenja pri prvih 17 bolnikih, ki so bili zdravljeni z rastnim hormonom vsaj 3 leta.*

Material in metode. *V skupini 17 bolnikov (15 dečkov, 2 deklici) je bilo pri 4 diagnosticirano izolirano pomanjkanje rastnega hormona, pri 13 pa multipli deficit hipofiznih hormonov vključno z rastnim. Zdravljenje z rastnim hormonom se je pričelo v starosti 10,7±3,5 leta. Nadomestno zdravljenje hipotireoze je bilo začeto v starosti 8,1±3,7 leta, hipogonadizem pri 12 dečkih v starosti 17,1±0,8 leta pri eni deklici v starosti 16,8 leta. Vsi bolniki so bili ponovno pregledani v odrasli dobi v starosti 23,1±4,5 leta. Pri 12, ki so soglašali, smo ob pogovoru poskusili oceniti kvaliteto njihovega življenja. Analizirali smo oksološke in nekatere klinične značilnosti zdravljenih bolnikov.*

Rezultati. *Letni prirastek 3,7±0,8 cm pred zdravljenjem se je povečal v prvem letu zdravljenja na 8,3±1,6 cm. V prvih treh letih zdravljenja je znašal 6,3±1,1 cm in med celotnim 6,1±2,8-letnim zdravljenjem 5,5±1,2 cm. Deficit v višini (height SD score) v začetku zdravljenja –4,05±1,2 se je po zdravljenju do odrasle dobe statistično pomembno znižal ($p < 0,01$) na –1,75±0,82. Končna višina pri bolnikih moškega spola je znašala 163,2±5,7 cm, pri ženskah 151±4,2 cm. Večina je imela kljub relativno zadovoljivi končni višini različne psihološke probleme kot motnje v samopodobi, slabo prilagodljivost in zmanjšano sposobnost socialnih kontaktov.*

Zaključek. *Pri 17 bolnikih s hipofizno nanosomijo, ki so bili prvi zdravljeni v Sloveniji z rastnim hormonom, je bilo zdravljenje učinkovito. Dosegli so pomembno večjo končno višino, kot bi jo brez zdravljenja. Stranskih učinkov zdravljenja ni bilo. V posameznih primerih je bila potrebna zaradi psiholoških problemov ustrezna pomoč.*

Introduction

The adult height of growth hormone (GH) deficient patients usually did not exceed 135 cm until Raben commenced treating pituitary dwarfs with human GH in 1957 (1). In Slovenia, professor Matajc started a successful treatment of two GH deficient patients with GH extracted from human pituitary glands, which he collected in different departments of pathology in Slovenia in 1969 (2). The aim of this overview is to analyse some clinical characteristics and effects of GH therapy on the first 17 Slovenian patients who have been treated.

Patients and methods

A total of 17 GH deficient patients (15 boys, 2 girls) for whom the GH therapy of at least 3-year duration started between the years 1969 and 1983 were included in the study. The mean age at the start of therapy was 10.7±3.5 years (range 4.3 to 16.8). None of the patients showed any sign of puberty. For the majority, the data of perinatal history and parenteral heights were available and analysed. The diagnosis was based on clinical characteristics of pituitary dwarfism (Figure 1), the height below 2 standard deviations (SD), retarded bone age (BA) and failure of GH secretion (<10 μg/L) following two provocation tests: insulin 0.1 U per kg b.w., glucagon 0.03 mg per kg b.w. or L-DOPA 125 mg at weight < 15 kg, 250 mg at weight 15 to 30 kg and 500 mg > 30 kg (3). Height, weight and height velocity were measured by standard techniques. Height SD scores at the start of therapy and in adulthood, and target heights were calculated using Kabi Vitrum Growth Computer Mannes (4). BA was estimated by the Greulich-Pyle method (5). GH was measured by RIA. Apart from TSH, LH, FSH, ACTH measurements in all patients, thyroid, adrenal and gonadal functions were studied, and hormone replacement therapy was started if necessary.

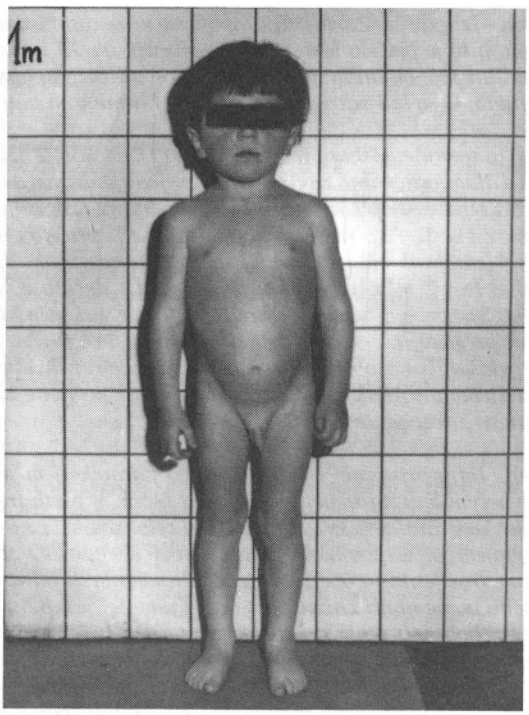

Fig. 1. *Case number 5 at the age of 7, measuring 103 cm (SD – 3.21), weighing 16 kg with typical clinical characteristics of GH deficiency: round head, short and broad face, prominent frontal bone, saddle-shaped nose, underdeveloped chin, short neck, heigh-pitched voice, accumulation of fat in the belly region and acromicria.*

Sl. 1. *Bolnik številka 5 v starosti 7 let, ko je meril 103 cm (SD– 3,21) in tehtal 16 kg, s tipičnimi kliničnimi znaki pomanjkanja rastnega hormona: okroglo glavo, kratkim in širokim obrazom, štrlečim čelom, sedlastim nosom, hipoplastično brado, kratkim vratom, visokim glasom, prekomernim kopičenjem maščevja v predelu trebuha in kratkimi dlanmi in stopali.*

Between 1969 and 1982, the patients were treated with human GH 2U intramuscularly every second day for two weeks followed by breaks of a fortnight. Since 1983 there have been no intervals in therapeutical regimen but therapy with human GH had to be terminated in Slovenia in 1985, like in many other countries, due to reports of Creutzfeld – Jakob disease in patients who had been treated with unpurified pituitary GH (6), contaminated with slow-acting agents. Since 1987, all our patients have been treated with GH produced by recombinant DNA technology using standard therapy of 2 U per m^2 per day, injected subcutaneously in the evening, which imitated the increased secretion of GH during night. Unfortunately, in many patients there were frequent interruptions of therapy due to lack of GH supply.

Hypothyroidism was treated with thyroxin 100 mcg per m^2 while hypocorticism was not treated, except temporarily during stress situations. In cases of hypogonadotropic hypogonadism, puberty was induced in males with testosterone enanthate intramuscularly 50 mg per month the 1st year, followed by 125 mg the 2nd year and 250 mg permanently thereafter. In hypogonadotropic females, ethinil estradiol was used in the dosage of 0.1 mg for the first two years, followed by an increased dose and thereafter by cyclic application of estrogen-progesterone drugs. All 17 patients were reexamined in adulthood after the age of 18 years, and with some of them who agreed, the interview regarding the quality of life was performed.

Results

Some clinical characteristics and effects of treatment on GH deficient patients, as well as data on quality of life are presented in Tables 1 and 2. In the group studied, isolated GH deficiency was stated in 4 (23.5%); combination of GH, TSH deficiency in 1 (5.9%); GH, TSH, LH, FSH deficiency in 9 (52.9%); GH, LH, FSH deficiency in 1 (5.9%); and GH, TSH, LH, FSH, ACTH deficiency in 2 (11.8%) cases. In 10 cases, pituitary dysfunction was classified as idiopathic, while in 7 patients organic aetiology was established; perinatal asphyxia in cases 2, 5, 7, 14, neonatal cytomegaly in case 13, meningitis at 10 months in case 12 and intracranial haermorrhagia due to trombocitopenia at 10 years in case 10. Peak values of stimulated GH did not exceed 3 μg/L. Thyroxin replacement started at 8.1±3.7 years (range 2 to 15.5), GH therapy at 10.7±3.5 years (range 4.3 to 16.8). Height velocity 3.7±0.8 cm (range 2.3 to 4.2) per year before therapy, it increased during the first year of treatment to 8.3±1.6 cm (range 6.7 to 10.7) and decreased during first three years of therapy to 6.3±1.1 cm (range 5.8 to 8.7) per year. The mean duration of GH treatment was 6.1±2.8 years (range 3 to 11.2). During that period the height velocity was 5.5±1.2 cm (range 4.2 to 8.7) per year. Height SD score at the start of therapy, which was 4.05±1.2 (range –2.16 to –6.61), improved significantly ($p < -0.01$) to –1.75±0.82 (range –0.27 to –2.81) in adult age. Spontaneous puberty occured in 3 male patients (case 1, 3, 11) with isolated GH deficiency in the age range from 14 to 16 years and at 17.5 years in a female patient (case 17). Puberty was induced in 12 male patients at the mean age 17.1±0.5 year (range 16 to 20) and in one female patient at 16.8 years.

When reexamined in adulthood, 17 male patients mean aged 23±4.5 years (range 18.6 to 33), had 163.2±5.7 cm (range 156 to 172.9). Two female patients when examined at the age of 20 and 21 measured 154 and 148 cm respectively. The majority of 12 GH deficient patients who agreed to be interviewed declared to have at least some psychological problems. None of them were married, only two males had sexual partners. Four patients with multiple pituitary hormone deficiency (cases 6, 7, 9, 12) revealed that they have never shaved. Three completed only elementary school, two of them worked as unqualified workers, two were unemployed. Nine patients completed secondary or technical school, two of them entered a faculty. More data on employment and psychological problems are evident from the Table 2.

Discussion

Although the majority of our patients started the treatment rather late, for a relatively short period due to lack of GH, and with

Tab. 1. *Some clinical and laboratory characteristics of the first patients with pituitary dwarfism treated with growth hormone in Slovenia.*

Tab. 1. *Nekatere klinične in laboratorijske značilnosti bolnikov s hipofizno nanosomijo, ki so bili prvi zdravljeni z rastnim hormonom v Sloveniji.*

Case number – year of birth	Sex M – male F – female	Hormones deficiency	GH peak response to provocation µg/L	Age at start of GH therapy – years	Bone age – years	Height in cm and SD score in brackets at start of therapy	Height velocity in cm per year				Duration of GH treatment years	The onset of puberty: S (spontaneous) I (induced) – years	Target height cm	Age at reexamination – years	Adult height in cm and SD score in brackets
							Before therapy	During 1st year	During 4 years	During the GH treatment					
Številka bolnika – letnica rojstva	Spol M – moški Ž – ženski	Pomanjkanje hormonov	Maks. porast rastnega hormona ob stim. µg/L	Starost ob začetku zdravljenja z rastnim hormonom – leta	Kostna starost – leta	Višina v cm in v oklepaju SD ob začetku zdravljenja	Letni prirastek v cm				Trajanje zdravljenja z rastnim hormonom I leta	Začetek pubertete: S (spontane) I (inducirane) leta	Ciljna višina – cm	Starost ob ponovnem pregledu – leta	Višina v odrasli dobi v cm, v oklepaju SD
							Pred zdravljenjem	Med 1. letom zdr.	Med 3-letnim zdr.	Med celotnim zdr. z rast. hormonom					
1–1958	M	GH	2.3	11.1	6.5	120 (–3.51)	3.4	10.7	8.7	8.7	3	S – 14	168	33	161.5 (–1.98)
2–1959	M	GH, TSH, LH, FSH	1.5	11.4	6	107.5 (–5.63)	3.5	9.8	7.7	5.2	6.8	I – 19	–	22	160.0 (–2.21)
3–1961	M	GH	1.9	15.5	10	127 (–6.61)	3.9	9.7	7.1	7.1	3	S – 16	167.5	30.1	157.5 (–2.59)
4–1961	M	GH, LH, FSH	2.3	7.9	3	98.5 (–4.74)	3.0	9.1	7.05	6.3	8.2	I – 16	–	30	165.1 (–1.44)
5–1969	M	GH, LH, FSH	2.3	4.3	2	86 (–4.01)	3.5	7.8	6.4	4.6	11.2	I – 16.6	179.1	22	159.5 (–2.29)
6–1967	M	GH, TSH, LH, FSH	1.6	8.0	3	103.5 (–2.16)	3.1	8.1	6.4	4.9	8.5	I – 16.5	170.5	24.1	160.4 (–2.15)
7–1966	M	GH, TSH, LH, FSH	2.4	15.3	7	130.2 (–6.06)	3.2	7.3	5.1	4.3	4	I – 18.4	171.5	23.4	158.5 (–2.44)
8–1964	M	GH, ACTH, TSH, LH, FSH	2.5	10.0	6	106.3 (–4.94)	4.1	10.0	6.5	5.1	9	I – 20	181.5	27.1	167.1 (–1.14)
9–1960	M	GH, ACTH, TSH, LH, FSH	0.8	12.9	8	131.7 (–3.06)	3.9	7.1	5.8	5.8	3	I – 19.8	177	26.7	172.9 (–0.27)
10–1962	M	GH, TSH, LH, FSH	1.0	16.8	11	150 (–3.61)	4.5	8.0	5.2	4.2	4	I – 19.7	180	24.1	172 (–0.41)
11–1968	M	GH	2.7	11.6	7.5	117.5 (–4.25)	4.0	9.8	7.0	6.3	5	S – 15	177.5	22.9	157.5 (–2.59)
12–1973	M	GH, TSH, LH, FSH	2.3	10.5	7.5	120.5 (–2.21)	4.2	10.4	7.8	6.6	6	I – 16	178	20	172.5 (–0.33)
13–1973	M	GH, TSH, LH, FSH	1.0	8.2	5	112.5 (–2.60)	4.0	6.7	5.5	4.6	10	I – 16.5	167.5	19.8	163.5 (–1.76)
14–1973	M	GH, TSH, LH, FSH	1.0	8.9	5	115 (–2.74)	3.1	7.6	6.1	5.4	5.5	I – 17	174.5	20	164 (–1.61)
15–1975	M	GH, TSH, LH, FSH	2.5	4.7	2	90 (–3.50)	3.5	7.1	6.1	6.0	11	I – 17	163.5	18.6	156 (–2.81)
16–1972	F	GH, LH, FSH	1.4	12.9	9.6	131 (–4.25)	3.4	6	4.4	4.8	4	I – 16.8	165	20	154 (–1.37)
17–1969	F	GH	0.4	12.0	10	122 (–4.62)	3.1	5.8	4.7	4.2	4	S – 17.5	–	21	148 (–2.37)

frequent intervals in some cases, it can be stated that their final height significantly improved compared to their height SD score before treatment and to patients who were never treated with GH. Actually, in 1990 we had the opportunity to examine 5 adult patients aged from 49 to 68 years suffering from hereditary multiple pituitary deficiency, including GH, in whom the height in males ranged from 132 to 139 cm, while one female measured 120 cm. Significantly increased height velocity during the first year of GH treatment and progressive decrease of growth during prolonged treatment observed in our patients is well known (7, 8). Although the patients treated did not reach the target height, it is evident that they benefited from therapy. Similar final heights were described by other authors. Burns et al. reported final heights ranging from 158.1 to 168.1 cm in boys and 142.5 cm to 154.4 cm in girls with idiopathic GH deficiency (9). Similar results were reported by Hibi et al., who observed final heights of 151.8±6.6 cm and 141.4±7.4 cm in boys and girls with isolated GH deficiency respectively. Children with associated hypogonadotropism had somewhat better final heights 163.7±3.9 cm in boys and 151.0±5.1 cm in girls (10). Such differences in final height were observed also in our group of patients. They could be due to late induction of puberty, which was started in some cases at the age of 20 (11). Three male patients with relatively good final height, which exceeded 170 cm, had eunuchoid proportions, probably due to late induction of puberty, which was the reason for psychological problems in some cases. The majority of our patients was shy, had poor adjustment, impaired social relationships and, on our estimation, poorer quality of life then general population. Similar observations have been published by other authors (12). Formerly it was assumed that the difficulties of adult hipopituitary patients in every-day life were mainly related to their small stature. After successfull achievement of normal height by modern GH therapy, many difficulties persist for these patients, which could not be explained properly. Blizzard et al. (13) and Dean et al. (14) reported that the rate of unemployment turned out to be three times higher, while the relation of married to unmarried subjects was five times lower than in the normal population. Deficiencies in self-image, including social isolation, powerlessness, incompetence, low self-esteem and the lack of agression expression were reported by several authors (15, 16). In our patients many of these characteristics were registered, therefore, in some cases, psychosocial counselling had to be arranged.

Conclusion

In general, it can be assumed that the GH deficient patients who started the treatment more than 24 years ago in Slovenia benefited from therapy and until now no side effects were observed. It is expected that with more experience and the new strategy of replacement therapy which has been used for the last 6 years, and includes daily injections of recombinant GH, constant adapting of the doses to height velocity effect and starting the therapy as soon as possible, the final height will still improve. Induction of puberty at the bone age of 14 years in boys and 13 years in girls with multiple hormone deficiencies, imitating normal sexual development in healthy peers, will diminish many psychological problems in these patients. In some GH deficient patients psychosocial counselling and organisational support groups are planned to be established.

Tab. 2. *Some data on education, current life and problems of adult GH deficient patients who agreed to be interviewd.*

Tab. 2. *Nekaj podatkov o izobrazbi, trenutnem življenju in problemih odraslih bolnikov s pomanjkanjem rastnega hormona, ki so pristali na pogovor.*

Case number Številka bolnika	Education level completed Izobrazba	Employment Zaposlitev	Age at interview years Starost ob pogovoru – leta	Impression about his–her general health Bolnikova ocena svojega zdr. stanja	Main problems presented at interview (remarks) Glavne težave, navedene ob pogovoru (opombe)
1	Vocational school Poklicna šola	electrician električar	33	excellent odlično	No special problems, well adapted, unmarried Brez posebnih problemov, dobro prilagojen okolju, neporočen
3	Vocational school Poklicna šola	electrician električar	30.1	fair zadovoljivo	Introverted, often depressed, impaired social contacts, muscular weakness Introvertiran, pogosto depresiven, navaja težave pri socialnih kontaktih in mišično oslabelost
5	Elementary school Osnovna šola	unqualified worker nekvalificiran delavec	22	good dobro	Some problems in social contacts, complains about low income (permanent sexual partner) Navaja težave pri vzpostavljanju socialnih kontaktov in zaradi nizkih dohodkov (ima stalnega spolnega partnerja)
6	Secondary school Gimnazija	clerk uradnik	24.1	fair zadovoljivo	Labile mood, fair adapted to environment (he has never shaved) Labilno razpoloženje, zadovoljivo adaptiran v okolju (se ne brije)
7	Vocational school Poklicna šola	trade vocation orodjar	23.4	poor slabo	Shy, poor adjustment, social isolation, feels different from peers, also due to eunuchoid proportions (he has never shaved) Plah, slabo prilagodljiv in osamljen, moti ga različnost od vrstnikov zaradi evnuhoidnih proporcij (se ne brije)
8	Vocational school Poklicna šola	electrician električar	27.1	fair zadovoljivo	Some deficiency in self-image, lack of agressivenes expression if necessary (permanent sexual partner) Navaja motnje v samopodobi in izražanju čustev ter agresivnosti, kolikor je to potrebno (ima stalnega spolnega partnerja)
9	Elementary school Osnovna šola	unqualified worker – unemployed nekvalificiran delavec – nezaposlen	26.7	poor slabo	Labile mood, frequent depression, incompetence, muscular weakness (eunuchoid proportions) Labilno razpoloženje, pogoste depresije, občutek manjvrednosti, mišične slabosti (evnuhoidne proporcije)
11	Vocational school Poklicna šola	trade vocation ostrilec	22.9	fair zadovoljivo	Shy, memory difficulties and irritability, impaired social relationships Plašen, motnje spomina in razdražljivost, težave pri socialnih kontaktih
12	Secondary school Gimnazija	entered a faculty vpisan na fakulteto	20 20	good dobro	No interest in social activities, still overprotected by parents, self-conscious, complaining of eunuchoid proportions Ne vzpostavlja socialnih kontaktov, je nesproščen, še vedno prekomerno zaščiten s strani staršev, moti ga svoj evnuhoidni izgled
13	Technical school Srednja tehnična šola	entered a faculty vpisan na fakulteto	20 20	very good zelo dobro	Feels different from peers due to short stature and delayed puberty, but on general very well adjusted to environment Kljub različnosti od vrstnikov zaradi nizke rasti in kasne pubertete zelo dobro prilagojen okolju
14	Elementary school Osnovna šola	unemployed nezaposlen	20	poor slabo	Deficiency in self-esteem, powerlessness, social isolation Nezadostno samospoštovanje, nemočnost, osamljenost
17	Secondary school Srednja adm. šola	secretary tajnica	21	fair zadovoljivo	Shy, poor adjustment, feels different from others due to short stature Plahost, slaba prilagodljivost, občutek manjvrednosti od vrstnic, predvsem zaradi nizke rasti

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Professional article/Strokovni prispevek

NATURAL CYTOTOXICITY AND GROWTH HORMONE TREATMENT

NARAVNA CITOTOKSIČNOST IN ZDRAVLJENJE Z RASTNIM HORMONOM

Mauro Bozzola, Antonia Moretta, Anice Valtorta, Isabella Biscaldi, Mariangela Cisternino, Francesco Torcetta, Renata Lorini, Francesca Severi

Department of Pediatrics, University of Pavia, IRCCS Policlinico San Matteo, 27100 Pavia, Italy.

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Key words: *short stature; children; cytotoxicity; growth hormone treatment*

Abstract – Background and methods. *We previously studied cytotoxicity evaluated as Natural Killer (NK) activity in 16 prepubertal GH-deficient children, age range 5.2–15.8 yrs, before and after 3, 6 and 9 months of GH therapy (0.6 U/kg s.c. divided in 3 or 4 doses per week). The low NK cell function found in these patients before treatment was restored by long-term GH therapy. In the present study, we evaluated the effect of biosynthetic GH treatment on NK activity of 9 short children without GH deficiency who participated in a multicentric clinical trial of GH therapy in short non-GH-deficient children. These patients, age range 7.9–13.5 yrs, were treated with biosynthetic GH (0.6 U/kg s.c. divided in 3 or 4 doses per week) for 12 months. NK activity was evaluated before and after 6 and 12 months of treatment.*

Results and conclusion. *No difference was found between NK activity of short non-GH-deficient children before treatment and that of age-matched controls. Moreover, no changes in NK activity during treatment were observed in these patients. Our preliminary data suggest that NK cell function in non-GH-deficient children is normal and not affected by long-term GH therapy.*

Ključne besede: *nizka rast; otroci; citotoksičnost; zdravljenje z rastnim hormonom*

Izvleček – Izhodišča. *Rastni hormon (RH) vpliva direktno na imunski sistem, saj so dokazani RH receptorji na humanih limfocitih. Eksperimentalne študije na živalih kažejo na možnost vpliva RH na naravno citotoksično aktivnost, ki jo posredujejo celice ubijalke. Podatki o vplivu RH na naravno citotoksičnost pri ljudeh so različni. Ugotovljeno je bilo, da je pri pomanjkanju RH motena funkcija celic ubijalk in da nanje ne vpliva kratko, pač pa dolgotrajno zdravljenje z rastnim hormonom. V okviru predhodne študije smo pri 16 predpubertetnih otrocih s pomanjkanjem RH, starih 5,2 do 15,8 leta analizirali citotoksičnost, opredeljeno kot naravno aktivnost celic ubijalk. Pri bolnikih, ki so bili zdravljeni z biosintetičnim RH v dozi 0,6 enote/kg telesne teže dnevno v 3 do 4 odmerkih tedensko, so bile meritve izvedene pred, po 3, 6 in 9 mesecih zdravljenja z RH. Med dolgotrajnim zdravljenjem se je nizka začetna citotoksičnost normalizirala.*

Preiskovanci in metode. *V tokratni študiji smo analizirali učinek biosintetskega RH na citotoksičnost pri 9 otrocih z nizko rastjo brez deficita RH, ki so bili zdravljeni z RH v okviru multicentrične klinične študije. Bolniki, stari od 7,9 do 13,5 leta so bili zdravljeni z biosintetskim RH v dozi 0,6 enote/kg telesne teže subkutano dnevno, v 3 do 4 odmerkih tedensko 12 mesecev. Naravna citotoksičnost je bila določena pred, po 6 in 12 mesecih zdravljenja z RH.*

Rezultati in zaključek. *Pri preiskovanih otrocih z nizko rastjo brez deficita rastnega hormona in kontrolni skupini nismo našli razlik v naravni citotoksičnosti. Med aplikacijo rastnega hormona nismo našli nikakršnih sprememb v naravni citotoksičnosti, tako da kaže, da RH ne vpliva na tovrstne spremembe pri aplikaciji otrokom z nizko rastjo brez pomanjkanja rastnega hormona.*

Introduction

Increasing evidence indicates that growth hormone (GH) interacts directly with the immune system, since specific GH receptors have been demonstrated on human cultured lymphocytes (1, 2) and peripheral circulating mononuclear cells (3, 4). Experimental studies in animals (5, 6) suggested that GH may influence natural cytotoxic activity mediated by Natural Killer (NK) cells which are lymphocytes involved in immune surveillance for viral infections and neoplastic diseases (7, 8, 9, 10). In humans, the influence of GH on natural cytotoxicity is still controversial. Impaired NK cell function has been found in patients with GH deficiency. Short-term administration of GH failed to normalize NK activity in GH-deficient children (11). On the other hand, the impaired NK activity of GH-deficient children was restored by long-term GH therapy (12). Accordingly, Crist et al. reported that daily exogenous GH treatment normalized NK cell function in adults with GH deficiency (13). More recently, supplemental GH administration has been suggested to increase natural cytotoxicity also in healthy adults with normal GH secretion (14). The effect of GH treatment on NK activity in non-GH-deficient children is still unknown.

The aim of this work was to study NK cell function in short stature children without GH deficiency during long-term GH therapy.

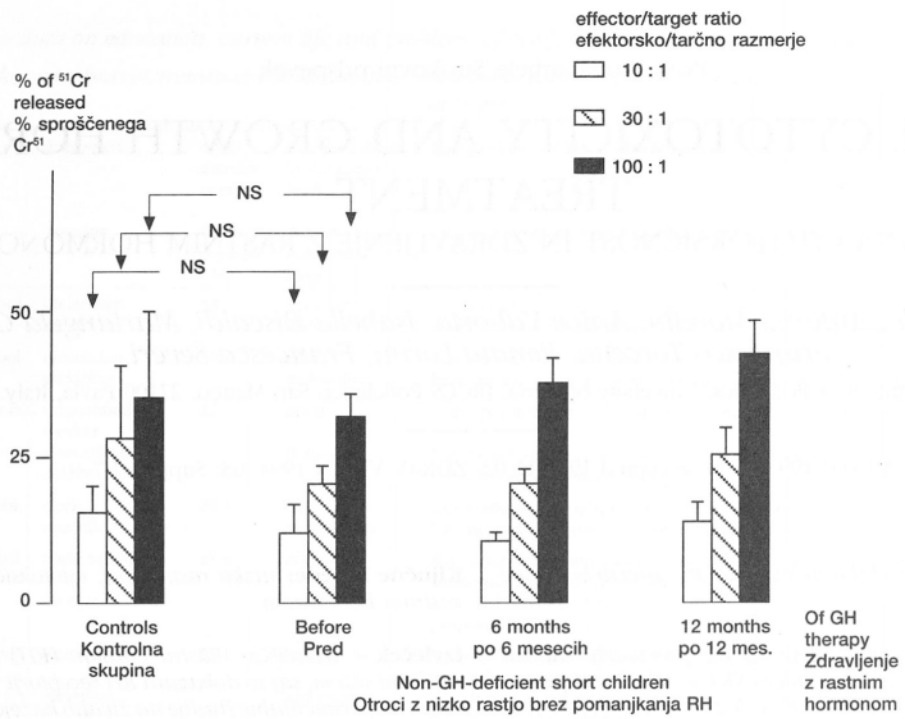

Fig. 1. *NK cell activity as percentage of ^{51}Cr released at effector/target ratios of 10:1, 30:1 and 100:1 in non-GH-deficient short children, before and during long-term GH therapy, and in age-matched control subjects with normal stature.*

Sl. 1. *Aktivnost naravnih celic ubijalk kot procent Cr^{51} sproščenega pri efektor/tarčnih razmerjih 10:1, 30:1 in 100:1 pri otrocih nizke rasti brez pomanjkanja rastnega hormona pred in med dolgotrajno terapijo z rastnim hormonom ter pri vrstnikih iste starosti, normalne rasti.*

Pattern description and methods of analysis

Pattern description: Nine short stature children (5 boys and 4 girls) aged 7.9 to 13.5 years, were investigated. Their heights were –2.86 ± 0.08 SDS below the mean for their age. Bone age was between 5 and 11.5 years. Three patients were prepubertal, whereas in six children the puberty started at the beginning of the treatment period. The mean (± SEM) growth velocity was 4.25 ± 0.44 cm/year less than the 10th percentile for their skeletal age.

All children exhibited a normal GH response (>10 ng/ml) to pharmacological stimuli (insulin, arginine and/or levodopa). None had any other chronic disease or chromosomal disorder.

The subjects were included in a multicentric clinical trial on GH treatment in short stature non-GH-deficient children. They were studied before and after 6 and 12 months of GH therapy (Genotropin from Kabi Pharmacia, Stockholm, Sweden; 0.6 U/kg administered in three or four subcutaneous injections weekly). Blood samples were obtained at the time of routine examinations, 48 hours after the last GH injection.

Four age-matched children without GH deficiency but with height > 2.5 SD and growth velocity ranging between the 25th and the 50th percentile were studied as controls.

Informed consent was obtained from the children's parents and the study was approved by the local ethic committee.

Methods of analysis: Cytotoxic activity was studied as NK cell function and quantified as cellular lysis against the K562 cell line (effector/target ratios of 10:1, 30:1, 100:1) by means of a standard ^{51}chromium release assay, as previously described (15).

Statistical analysis of data was performed by the Student t test. A p value < 0.05 was considered a significant difference between the results.

Results

A significant increase in growth velocity (from 4.25 ± 0.44 to 7.02 ± 0.41 cm/year, $p < 0.0001$) was observed after 12 months of GH treatment.

No difference in NK activity was found between non-GH-deficient patients before GH treatment and age-matched control children (fig. 1). No significant variations in NK cell function were observed in our patients after 6 and 12 months of GH therapy (fig. 1).

Discussion

We previously demonstrated that the impaired NK cell activity of GH-deficient children was restored by long-term GH therapy (12). Like in adults with GH deficiency, an increase in NK function after exogenous GH treatment was observed (13). Taken together, these reports point to some relationship between GH and the cells involved in the surveillance against viral infections and neoplastic diseases. More recently, a GH-induced increase of NK activity was established also in healthy adults with normal GH secretion (14), which suggested that the hormone had an effect on natural cytotoxicity of normal subjects. In contrast, we observed normal NK function and no changes in NK activity during long-term GH therapy in short stature children with normal GH secretion.

The discrepancy between the results obtained in normal children and adults is evident. The dosage of the hormone in healthy adults was within the therapeutic range used to treat GH-deficient children (14). The hypothesis of greater sensitivity of adults' immunoresponsiveness to GH administration could be formulated.

Since exogenous GH administered at conventional dosage increases NK cell function of children with GH-deficiency, but not

of those with normal secretion, we can assume that immunomodulatory action of GH is related to endogenous secretory status.

Conclusions

The lack of variation in NK cell activity in short stature children without GH-deficiency indicates that GH treatment does not affect the natural cytotoxicity of children with normal GH secretion.

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Professional article/Strokovni prispevek

CONGENITAL HYPOPITUITARISM PRESENTING AS CHOLESTASIS

PRIROJENO ZMANJŠANO DELOVANJE HIPOFIZE IZRAŽENO S HOLESTAZO

C. Ellaway, Martin Silink

Ray Williams Institute of Paediatric Endocrinology, Diabetes and Metabolism, Royal Alexandra Hospital for Children, PO Box 34, Camperdown, NSW 2050, Australia

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Key words: *congenital hypopituitarism; hypoglycaemia; cholestatic jaundice*

Ključne besede: *kongenitalni hipopituitarizem; hipoglikemija; holestazna zlatenica*

Abstract – *Congenital hypopituitarism is a well-known cause of recurrent neonatal hypoglycaemia, secondary thyroid and adrenocortical insufficiency, and growth hormone deficiency. Clinical clues to the diagnosis include the presence of midline facial abnormalities, microphallus in boys, optic hypoplasia and, rarely, cholestatic jaundice. We report a further patient with congenital hypopituitarism presenting with cholestatic jaundice. Congenital hypopituitarism should be considered in the differential diagnosis of a neonate presenting with obstructive jaundice.*

Izvleček – *Prirojeno zmanjšano delovanje hipofize je dobro znan vzrok ponavljajoče se hipoglikemije, sekundarne insuficience ščitnice ali skorje nadledvične žleze. Klinični znaki so med drugim medialne abnormalnosti obraza, mikrofalus pri dečkih, optična hipoplazija in redko tudi holestazna zlatenica. V članku je predstavljen bolnik s prirojenim zmanjšanim delovanjem hipofize izraženim s holestazno zlatenico. Prirojeno zmanjšano delovanje hipofize moramo upoštevati pri diferencialni diagnozi novorojencev z obstruktivno zlatenico.*

Congenital hypopituitarism may present in the neonatal period with clinical features of thyroid, adrenal and gonadal deficiencies (1, 2). Whilst recurrent hypoglycaemia is a well-known complication (3), it is not as widely appreciated that cholestatic jaundice may also be caused by the hormonal deficiencies (4–6). This report presents a case history of a neonate with congenital hypopituitarism and cholestatic jaundice.

Case report

Patient M. C. was born after an uncomplicated pregnancy at 42 weeks gestation by an induction forceps delivery, with Apgar scores of 7 at 1 minute and 9 at 5 minutes. Her birth weight was 3900 gms. She was initially breast fed, then commenced on Enfalac because of poor maternal supply. On day 2, the baby became jaundiced and was treated with phototherapy for 3 days. On the same day, she had a cyanotic episode associated with hypothermia, which required monitoring in ICU. After discharge from hospital, the jaundice gradually resolved over the following three weeks.

M. C. presented at 9 weeks of age with a three-day history of dark urine, pale stools, and the day prior to admission became jaundiced.

Physical examination disclosed a well-looking, deeply jaundiced infant with wide anterior and posterior fontanelles. Her head circumference length and weight were on the 50th centile. A hoarse cry and puffy face were noted, however, the ankle jerks were not delayed, and there was no significant bradycardia. The genitalia were normal. Urin analysis revealed moderate bilirubinuria but no urobilinogenuria.

Initial investigation revealed a conjugated hyperbilirubinaemia (total bilirubin 209 µmol/l, conjugated bilirubin 129 µmol/l) with normal liver transaminases and an elevated serum alkaline phosphatase (779 U/L). Coagulation studies were intially abnormal, later corrected with Vitamin K. Further investigation excluded infective causes and inborn errors of metabolism. An abdominal ultrasound excluded extrahepatic obstruction. A DISIDA scan (Diisopropyliminodiacetic acid – Tc99m) was suggestive of hepatocellular disease, with poor extraction of the tracer by the liver and delayed excretion.

After 4-hours fasting, the serum glucose was 2.1 mmol/l, and required dextrose intravenously at 3.5 mg/kg/min and oral feeds every four hours to maintain euglycaemia. A Synacthen stimulation test demonstrated a baseline plasma cortisol value of 124.2 nmol and a 60-minute post-Synacthen value of 204.2 nmol/l. This partial response was considered to be consistent with secondary adrenocortical insufficiency. She was therefore commenced on replacement hydrocortisone. Thyroid function tests revealed secondary hypothyroidism with a low free thyroxine of 6.3 pmol/l (NR 10–30), and the TSH of 3.4 mU/l was in the normal range (1–7 mU/l). Thyroxine was commenced at 25 mcg per day. At the time of hypoglycaemia, plasma growth hormone was 1 mU/l, and an arginine stimulation test confirmed growth hormone deficiency, with none of the levels rising above 1 mU/l.

Despite replacemet hydrocortisone hypoglycaemia recurred within 7.5 hours of fasting, she was unable to remain fasting for a period greater than 8 hours due to hypoglycaemia. The baby was therefore commenced on growth hormone and was able to tolerate an 8-hour fast without hypoglycaemia.

Following hormone replacement therapy, the jaundice gradually resolved over the subsequent weeks after a transient increase in the level of conjugated bilirubinaemia.

Cerebral imaging by ultrasound revealed normal septum pellucidum and corpus callosum, and no evidence of a CNS structural lesion. A skull XRay revealed an extremely small pituitary fossa. An MRI scan is yet to be performed.

Discussion

Congenital hypopituitarism was first described in 1956 by Blizzard and Albert, who reported a newborn presenting with intermittent cyanosis, microphallus and cryptorchidism (1). The baby died within 24 hours and an autopsy revelaed absence of the pituitary, with testicular and adrenal gland hypoplasia. Mosier was the first to describe congenital hypopituitarism in association with cholestatic jaundice, also in 1956 (7). He reported a twin who developed jaundice and seizures shortly after birth, and died within 24 hours. Post-mortem examination revealed hypoplastic adrenals and pituitary, and bile staining of the hepatic cells with bile thrombi in bile canaliculi.

There were similar cases reported by Johnson et al in 1973 (3), and in 1974 Sadegehi-Nejad et al (8) successfully treated a neonate with congenital hypopituitarism with hormone replacement. Whilst the association between congential hypopituitarism and cholestatic jaundice was recognized, it was Herman et al (9) who postulated that the hormonal deficiencies due to congenital hypopituitarism were the cause of the neonatal cholestasis.

The mechanism of the liver dysfunction is still the subject of debate. Leblanc et al (10) suggested in 1981 that cortisol deficiency was important in the aetiology of the cholestasis, after describing five patients with cholestasis and adrenocortical insufficiency. They reviewed studies which revealed decreased bile flow in rats after adrenalectomy and increased bile blow in rats and dogs after hydrocortisone infusion. Drop et al (5) and Giacola et al (6) were unable to confirm this theory by documenting persistent liver dysfunction in several cases despite appropriate cortisol and thyroid replacement, and suggested an aetiological role of growth hormone deficiency in neonatal cholestatis in congenital hypopituitarism. Hypothyroidism has been long recognised as a cause of jaundice in the neonate, this however is unconjugated hyperbilirubinemia.

Sheehan et al in 1992 (2) recognised the work of Copeland (4) demonstrating the partial dependence of hepatic enzymes on growth hormone (eg. ornithine decarboxylase and glucokinase). They postulated that deficiency in one or more of the hormones could predispose to cholestasis by either delaying normal maturation of active transport mechanisms responsible for intestinal hepatic bile acid uptake, or inhibiting bile acid synthesis thereby promoting the accumulation of precursor bile acids with a cholestatic effect (2). They also believed that hormone deficiency could lead to abnormalities in structure and function of bile canaliculi which are essential for bile secretion.

The cholestatic jaundice in our patient resolved gradually with hormonal therapy. It is belived that there is no long-term hepatic function impairment, however, Kaufman described a patient with persistent hepatomegaly with prominent micronodular fibrosis following delay in diagnosis and treatment (11). In our case report the jaundice initially became worse before improving, whilst the serum alkaline phosphatase continued to fall. This is felt to be due to increased enterohepatic circulation, with more bilirubin being presented to the circulatory system, following relief of the obstruction.

In our patient ultrasound evaluation indicated that she had a normal corpus callosum and a normal septum pellucidum. In the series reported by Costello & Gluckman in 1988 from New Zealand (12), there was an increased incidence of congenital hypopituitarism presenting in neonatal period amongst the Polynesian population. They described 17 patients presenting with neonatal cholestasis, of whom 8 patients had at least 1 Maori or Samoan parent. Six of these had septo-optic dysplasia and one had agenesis of the corpus callosum.

In conclusion, congenital hypopituitarism though rare, is a potentially fatal disorder, which is eminently treatable. There are several clinical findings, which may suggest the diagnosis: persistent hypoglycaemia, microphallus in the male (13), optic nerve hypoplasia, and midline facial abnormalities, including cleft lip and palate. We wish to emphasise that cholestatic jaundice should be added to this list, and congentital hypopituitarism considered in the differential diagnosis of a neonate presenting with obstructive jaundice with normal liver transaminases. Failure to recognise these various modes of presentation may result in delayed diagnosis, with risk of death, cerebral damage or prolonged cholestasis progressing to premanent hepatic injury.

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Professional article/Strokovni prispevek

LIMITED JOINT MOBILITY AND HEARING IMPAIRMENT IN DIABETIC CHILDREN AND ADOLESCENTS

OMEJENA GIBLJIVOST SKLEPOV IN OKVARA SLUHA PRI OTROCIH IN MLADOSTNIKIH Z INSULINSKO ODVISNO SLADKORNO BOLEZNIJO

Nevenka Bratanič[1], Ciril Kržišnik[1], Magda Kadrnka-Lovrenčič[2], Jagoda Vatovec[3]

[1] Medical Centre Ljubljana, Department of Pediatrics, Ljubljana, Slovenia
[2] Department of Pediatrics, Sestre Milosrdice, University Hospital, Zagreb, Croatia
[3] Medical Centre Ljubljana, Department of Otolaryngology, Ljubljana, Slovenia

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Key words: *insulin-dependent diabetes mellitus; limited joint mobility; hearing impairment; children; adolescents*

Abstract – Backgrounds and methods. *Limited joint mobility of the hand was present in 39 (16%) out of 244 subjects, aged from 2.9 to 20.3 years, with insulin-dependent diabetes mellitus of duration from 0.7 to 16.5 years. Among 116 healthy controls only 2 (1.7%) had similar limited joint mobility.*

Results. *Limited joint mobility was statistically significantly related to duration of diabetes ($p < 0.01$) and metabolic control expressed as HbA1 ($p < 0.05$). The relation of limited joint mobility in diabetics to gender, age of the onset of the disease and the presence of retinopathy has not been established.*

Conclusions. *The subclinical sensorineural hearing impairment was present in 14 (41.2%) patients with limited joint mobility compared with 6 (16.2%) patients with normal joints. The difference in the prevalence of hearing impairment in groups of diabetic patients with and without joint contractures was statistically significant ($p < 0.05$).*

Ključne besede: *insulinsko odvisna sladkorna bolezen; omejena gibljivost sklepov; okvara sluha; otrok; mladostnik*

Izvleček – Izhodišča in metode. *Med 244 pregledanimi bolniki z insulinsko odvisnim diabetesom, starih 2,9 do 20,3 leta, s trajanjem sladkorne bolezni od 0,7 do 16,5 leta smo ugotovili omejeno gibljivost malih sklepov roke pri 39 (16%) bolnikih. Med 116 otroci in mladostniki iz kontrolne skupine sta imela le 2 (1,7%) spremembe prve stopnje.*

Rezultati. *Omejena gibljivost malih sklepov rok je statistično značilno povezana s trajanjem sladkorne bolezni ($p < 0,01$) in metabolno urejenostjo, opredeljeno z vrednostmi HbA1 ($p < 0,05$). Statistično značilne povezanosti s spolom, s starostjo bolnikov ob začetku bolezni in retinopatijo nismo ugotovili.*

Zaključki. *Senzorinevralno naglušnost smo ugotovili pri 14 (41,2%) sladkornih bolnikih z omejeno gibljivostjo malih sklepov roke in 6 (16,2%) bolnikih brez sprememb na sklepih. Bolniki z omejeno gibljivostjo sklepov roke so imeli statistično značilno pogosteje prisotno okvaro sluha kot bolniki brez sprememb ($p < 0,05$).*

Introduction

Multiple joint contractures, thick and tight skin, and short stature were first described for three insulin-dependent diabetic children with long-standing illness, by Rosenbloom and Frias in 1974 (1). Later studies confirmed that limited joint mobility (LJM) is the earliest clinically apparent long-term complication of diabetes in children and adolescents (2).

Joint stiffness begins typically in the fifth finger, moving radially, affecting interphalangeal, metacarpophalangeal and larger joints, as well as cervical and thoracolumbar spine. The limitation is painless and nondisabling at first. The skin over affected joints in moderate to severe LJM is thick, tight and waxy.

LJM has been reported in 9–58% (3, 4) of patients with insulin-dependent diabetes, which depended on the age of the population, the duration of diabetes and the examination technique.

Different opinions exist regarding whether or not there is a correlation between LJM and factors such as age, duration of diabetes and diabetic control.

It has been suggested that joint contractures may be an early marker of the serious microvascular complications of diabetes, especially retinopathy and nephropathy (5).

According to most authors, diabetics may have an inner-ear affection characterised by slowly, usually bilaterally and symmetrically progressive sensorineural hearing loss, which affects predominantly the higher frequencies in older patients and is difficult to distinguish audiometrically from presbyacusis (6, 7). There has been conflicting results regarding the correlation between hearing impairment and other late diabetic complications (6, 7, 8, 9, 10). We could not find any data in the literature regarding the relationship between limited joint mobility and hearing impairment.

The purpose of our study was to establish the prevalence of LJM in children and adolescents with insulin-dependent diabetes attending our clinics. Audiometry was performed to determine the prevalence of hearing impairment in diabetic patients with LJM and without it.

Patients and methods

Two hundred and forty four patients attending the diabetic clinics of the Pediatric clinics of Ljubljana were studied. There were 108

Fig. 1a. *A handprint of normal hand.*

Sl. 1a. *Normalen odtis dlani.*

Fig. 1b. *A handprint of diabetic patient with LJM stage II.*

Sl. 1b. *Odtis dlani pri OGS II. stopnje.*

females and 136 males. Their age at the time of the study ranged from 2.9 to 20.3 (13.7 ± 4.01) years and duration of diabetes from 0.7 to 16.5 (5.7 ± 3.9) years. The control group consisted of 116 healthy, nonrelated children, 74 females and 42 males of similar age, ranging from 3 to 17.9 (11.7 ± 3.5) years.

The function of statoacoustic apparatus was evaluated in 34 diabetic patients with LJM. The control group consisted of 37 diabetic patients of similar age and diabetes duration without LJM.

To demonstrate joint mobility, the patients attempted to approximate the palmar surfaces of the interphalangeal joints of both hands tightly, with fingers fanned and the wrists maximally dorsi flexed (prayer test) (11). If such approximation was incomplete, the examiner confirmed the limitation by passive extension of the patients' fingers.

Limited joint mobility was more objectively assessed by printing. The patients' fingers and palms were painted with water-soluble paint (Speedball water-soluble ink). The subject then laid a hand palmar surface down onto the sheet of paper and pressed his/her weight firmly onto the distal metacarpal heads and fingers (12). Then the same was repeated with the other hand. On imprint, any fixed flexion deformity of the finger was easily visualised. LJM was classified into LJM 0 – no limitation, LJM I – involvement of one or more joints of one or two fingers, LJM II – involvement of more than two joints on more than two fingers (Figure 1a, b).

Long-term diabetic control was assessed by HbA1 measured by column chromatography, using Bio-Rex 70 columns manufactured by Bio-Rad Laboratories, which has been used in our laboratory since 1985. The normal range of HbA1 in our laboratory is 5–9% (13).

Ophtalmological assessment for the presence of retinopathy has been performed twice a year by different ophtalmologists in the nearest medical centre. It has been done mostly through undilated pupils.

Vestibular function was assessed by examining the patient for spontaneous nystagmus and postural nystagmus, as well as by caloric vestibulometry.

The hearing was determined by pure-tone audiometry using Interacustics Clinical Audiometer AC 30. Audiological examination was performed in a sound proofed room. Hearing was determined for each ear at frequencies of 250–8,000 Hz. Bone and air conduction were tested.

Hearing loss was quantified as "initial" in patients with an auditory treshold up to and above 30 db in one of frequencies from 250–8,000 Hz, and as "mild" with an auditory treshold above 30 db in more than one frequencies in conversational range (250–4,000 Hz).

The statistical significance of the results was evaluated using the chi-square test.

The study was approved by Medical Ethic Committee.

Results

Among the entire population of 244 diabetic patients, 39 (16%) had joint contractures compared to 2 (1.7%) in the control group. Seventy six percent of those affected had joint contractures of Stage I, 24% of Stage II. The prevalence of LJM in the diabetic patients was significantly higher than in control group ($p < 0.01$) (Table 1).

Tab. 1. *Characteristics of subjects assessed for LJM.*

Tab. 1. *Značilnosti preiskovancev na OGS.*

Characteristics Značilnosti	Diabetics Diabetiki (n = 244)	Non-diabetic controls Kontrolna skupina (n = 116)
Sex (F/M) Spol (Ž/M)	108/136	74/42
Age (yrs) Starost (leta)		
Mean (SD) Aritm. sredina (SD)	13.7 (4.0)	11.7 (3.5)
Range Razpon	2.9–20.3	3.0–17.9
Duration DM (yrs) Trajanje (leta)		
Mean (SD) Aritm. sredina (SD)	5.7 (3.9)	–
Range Razpon	0.7–16.9	–
LJM OGS		
No Število	39	2
%	16	1.7

In our study, no significant sex difference was found while some studies showed a slightly higher prevalence in males (14, 15).

There was no statistically significant relationship between occurrence of joint changes and age of onset of diabetes. The LJM prevalence increased with the duration of diabetes; the relation of LJM to duration of diabetes was highly significant ($p < 0.01$) (Figure 2). The prevalence was higher in patients with diabetes duration of 5 or more years. The shortest duration of diabetes among the patients with LJM was one year and a half, and only eight had diabetes for less than 5 years.

In order to relate joint changes with degree of glycaemic control, the mean HbA1 values recorded from 3-monthly clinic visits since 1985 were used, and showed a positive correlation to the LJM prevalence ($p < 0.05$) (Figure 3).

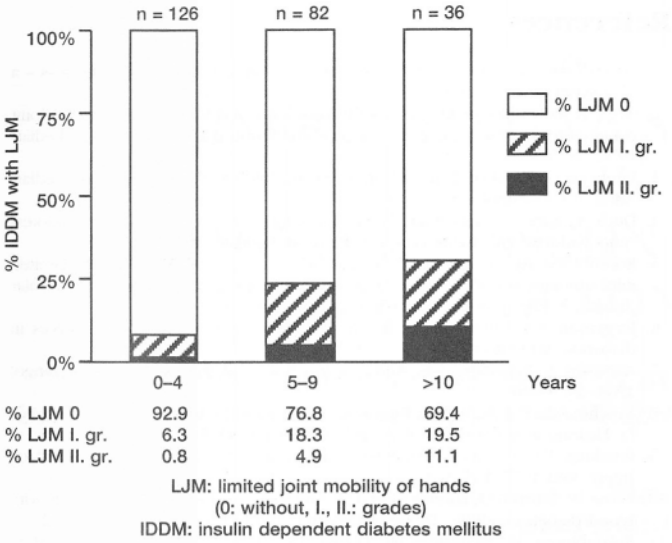

Fig. 2. *LJM prevalence in IDDM by duration of diabetes.*

Sl. 2. *Omejena gibljivost malih sklepov rok pri insulinsko odvisnem diabetesu.*

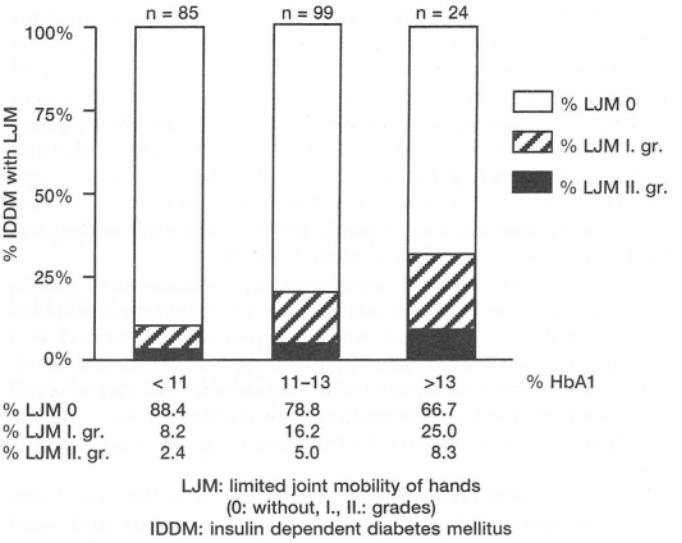

Fig. 3. *Association of LJM and glycaemic control expressed as the mean HbA1 values.*

Sl. 3. *Povezava med omejeno gibljivostjo malih sklepov rok in metabolno urejenostjo, opredeljeno z vrednostmi HbA1.*

The inner-ear function was assessed in 34 patients with LJM and 37 diabetic patients without joint stiffness. There was no history of hearing impairment in family members, no exposure to noise or ototoxic drugs, no neonatal hyperbilirubinaemia, ear disease or craniocerebral trauma.

Tab. 2. *Prevalence of sensorineural hearing impairment and characteristics of patients with LJM or normal joints matched for duration of diabetes.*

Tab. 2. *Prevalenca senzorinevralne naglušnosti in značilnosti sladkornih bolnikov z in brez OGS z enakim trajanjem bolezni.*

Characteristics Značilnosti	LJM OGS (n = 34)	Normal joints Normalni sklepi (n = 37)	Stat. sig. Stat. pom.
Sex (F/M) Spol (Ž/M)	12/22	16/21	
Age (yrs) Starost (leta)			
Mean (SD) Aritm. sredina (SD)	15.6 (2.8)	15.1 (3.1)	
Range Razpon	9.3–19.4	9.7–20.0	
Duration DM (yrs) Trajanje DM (leta)			
Mean (SD) Aritm. sredina (SD)	8.0 (3.7)	7.4 (4.0)	
Range Razpon	1.5–16.6	1.0–16.5	
Sensorineural hearing loss Senzorinevralna naglušnost	14 (41.2%)	6 (16.2%)	$p < 0.05$
– initial – začetna	9 (64.2%)	6 (100.0%)	
– mild – lažja	5 (35.8%)		

Legend:
DM: diabetes mellitus
F: female
LJM: limited joint mobility
M: male
OGS: omejena gibljivost sklepov
SD: standard deviation
Stat. sig.: statistical significance
yrs: years

Vestibular function tests did not reveal involvement of the vestibular part of labyrinth.

The subclinical sensorineural hearing loss was detected in 14 (41.2%) patients with LJM compared to 6 (16.2%) patients with normal joints. The difference was statistically significant ($p < 0.05$). In the group of patients with LJM, initial hearing impairment was found in 9 (64.3%) and mild hearing loss in 5 (35.7%). All the patients in the control group without LJM had initial hearing impairment (Table 2).

Discussion

This study confirms that limited joint mobility, which mainly affects the small joints of the hands, is a common, though often overlooked, manifestation of insulin-dependent diabetes. Previous studies have found a prevalence of 9–58% for this abnormality among diabetic patients (3, 4). The prevalence of 16% in our study is therefore among the lowest observed to date. It could be due to young age of the patients included in the study and printing, more objective and precise method for detection of joint deformity. In other studies where such objective methods were used the prevalence of contractures was also lower (3, 12).

The strong correlation between limited joint mobility and duration of diabetes is in accordance with previous reports (3, 4, 16–19). Limited joint mobility is primarily a metabolic consequence of diabetes. Non-enzymatic glycosylation of periarticular collagen is significantly accelerated in diabetes and results in abnormally cross-linked collagens which are unusually resistant to mechanical and enzymatic degradation (20). The relationship between LJM and metabolic control of diabetes was previously conflicting, whether a score comprising a variety of clinical parameters or glycosylated hemoglobin levels were used (21, 22). However, many of the studies did not show any relationship between HbA1 and LJM because they used a single HbA1 value, which may not be representative of the accumulative effect of glucose control over several years of diabetes (15). In our study, longitudinal HbA1 concentrations from longer period were studied, which showed that LJM was significantly associated with HbA1. Rosenbloom and colleagues have suggested that joint contractures precede the development of microangiopathy and may be of clinical value as a marker for subsequent microvascular complications, especially

retinopathy (5). A correlation between LJM, retinopathy, nephropathy and neuropathy has been studied by many researchers. Most notable was association of LJM of the hand with the presence of proliferative retinopathy (5, 12, 17, 19). A significant association of LJM with neuropathy was found by others (19, 23). Retinopathy has been detected in only 6 patients among entire diabetic population attending our clinics. The reason for such a small number is that the patients included in our study were all very young with short duration of diabetes, and that funduscopy was performed mostly through undilated pupils.

In our study we aimed to determine the association of LJM to hearing-impairment, which is a less known long-term complication of diabetes. A typical hearing-impairment described is a progressive bilateral sensorineural hearing-loss of gradual onset, which affects predominantly higher frequencies but can affect all the other frequencies as well (6). In published surveys, the prevalence of sensorineural hearing impairment varied between 0–80% (7).

There have been conflicting opinions regarding the correlation between hearing impairment and duration, severity and other long-term complications of diabetes. No association between duration of diabetes and hearing-loss was established by most authors (6, 7, 24), but there was a correlation between hearing impairment and the age of the patients (6, 7, 10). Jorgensen and colleagues found a correlation between retinopathy and hearing impairment; proliferative retinopathy was present twice as often in patients with acoustic lesion than without it (6). The correlation between retinopathy and hearing impairment was also established by Krochmalska and Ferrer and coworkers (8, 10). Friedman and colleagues reported subclinical hearing abnormalities in 55% of the diabetic patients with neuropathy (9).

In our study, we analysed the prevalence of hearing-impairment in two subgroups of diabetic patients with and without LJM, matched for sex, age and duration of diabetes to eliminate the possible confounding effects of these characteristics on the relation between these two features. Statistically significant difference in the prevalence of sensorineural hearing-impairment in patients with LJM compared to group of patients without joint contractures was established. Our data revealed that hearing is more often impaired in diabetics with LJM than in those with normal joints.

For further studies it remains to be determined whether subclinical hearing impairment in patients with insulin-dependent diabetes mellitus occurs as a part of neurological abnormalities or as an isolated complication of the disease.

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Professional article/Strokovni prispevek

INCREASED LEVELS OF ANTIBODIES TO ß-LACTOGLOBULIN IN CHILDREN WITH NEWLY DIAGNOSED INSULIN-DEPENDENT DIABETES AND IN THEIR NON-DIABETIC SIBLINGS

ZVIŠANE VREDNOSTI PROTITELES PROTI BETA-LAKTOGLOBULINU PRI OTROCIH Z NOVO ODKRITIM INSULINSKO ODVISNIM DIABETESOM IN NJIHOVIH ZDRAVIH BRATIH IN SESTRAH

Renata Lorini, M. Antonietta Avanzini, Letizia Vitali, Luisella Cortona, Giuseppe D'Annunzio, Patrizia Pignatti, Francesca Severi

Department of Pediatrics, University of Pavia, 27100 Pavia, Italy.

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Key words: *IDDM; ß-lactoglobulin antibodies; milk diet*

Abstract – Background. *It has been a hypothesized that cow's milk may contain a trigger factor for development of insulin-dependent diabetes mellitus (IDDM).*

Subjects and methods. *The aim of our study was to evaluate the prevalence of anti-ß-lactoglobulin (BLG) antibodies in IDDM children, at diagnosis and during 2–5 year follow-up, and in their non-diabetic siblings. IgA and IgG antibodies to BLG, determined by ELISA, were evaluated in 45 newly diagnosed IDDM children, aged 1.2–16.6 years and 46 siblings, aged 3.2–28.2 years.*

Results. *At diagnosis 14/45 patients (31%) had elevated IgA anti-BLG and 4/45 patients (8.8%) elevated IgG anti-BLG. Over the 2–5 year follow-up in 26 patients, we observed a transient increase of IgA anti-BLG in 2 other patients. At the end of follow-up IgA anti-BLG decreased to normal levels in all but 2 girls and IgG anti-BLG in all patients. No correlation was found between anti-BLG antibodies and chronologic age, breast-feeding duration, islet-cell, anti-insulin or other organ and non-organ-specific antibodies and HLA type. Among the 46 siblings increased levels of IgA and IgG anti-BLG were found in 5 (11%) respectively, without difference between breast – or bottle-fed cases.*

Conclusion. *The increase of anti-BLG antibodies at the onset of IDDM is suggested to represent only transient abnormal immunological response, probably unrelated to the beta cells' damage. The increased anti-BLG antibody levels found in healthy siblings could be associated with increased frequency of autoimmune phenomena in these subjects.*

Ključne besede: *insulinsko odvisni diabetes mellitus; protitelesa proti laktoglobulinu; mlečna prehrana*

Izvleček – Izhodišča. *Etiopatogeneza insulinsko odvisnega diabetesa je povezana z interakcijo genetskih faktorjev, vplivov okolja in avtoimunskih procesov. Med hipotetičnimi sprožilnimi faktorji bolezni je tudi zgodnje uvajanje kravjega mleka v prehrano otroka. Pri novoodkritih mladih diabetikih so odkrili protitelesa proti beljakovinam kravjega mleka in beta-laktoglobulina (BLG). Namen študije je bil ugotoviti prevalenco in dinamiko nihanja vrednosti protiteles proti BLG pri diabetičnih otrocih in njihovih zdravih bratih in sestrah.*

Preiskovanci in metode. *Analizirali smo IgA in IgG protitelesa proti BLG z ELISA tehniko pri 45 novoodkritih insulinsko odvisnih diabetikih, starih od 1,2 do 16,6 leta in 46 zdravih bratih in sestrah bolnikov, starih od 3,2 do 28,2 leta. Pri 26 diabetikih so bile vrednosti protiteles, analizirane ob začetku in po 2 do 5 letih trajanja bolezni.*

Rezultati. *Ob začetku bolezni je imelo 14/45 bolnikov (31%) zvišane IgA proti BLG in 4/45 (8,8%) pa tudi IgG proti BLG. Tekom 2 do 5-letnega sledenja so se prehodno zvišale vrednosti IgA proti BLG še pri dveh bolnikih. Ob koncu sledenja so se vrednosti IgG proti BLG znižale pri vseh bolnikih, vrednosti IgA proti BLG pa se niso znižale le pri dveh deklicah. Med protitelesi proti BLG in kronološko starostjo bolnikov, trajanjem dojenja, protitelesi proti beta celicam in insulinu in drugimi organsko specifičnimi in nespecifičnimi protitelesi ter HLA konstelacijo ni bila ugotovljena nikakršna korelacija. Pri 46 bratih in sestrah diabetikov smo ugotovili zvišane vrednosti IgA in IgG proti BLG obakrat v 5 primerih (11%) ne glede na to, ali so bili preiskovanci dojeni ali zalivani.*

Zaključek. *Prehodno zvišanje protiteles proti BLG v začetni fazi insulinsko odvisnega diabetesa kažejo na prehoden patološki imunski odgovor, ki verjetno ni povezan s patogenezo okvare endogenega dela pankreasa. Pozitivne vrednosti protiteles proti BLG pri zdravih bratih in sestrah bolnikov potrjujejo povečano pogostost avtoimunih fenomenov pri teh osebah.*

Introduction

It is a common opinion that etiology of insulin-dependent diabetes mellitus (IDDM) involves interaction of genetic, environmental and immunologic factors (1). Exposure to one or more environmental risk factors seems necessary to convert the HLA-linked genetic susceptibility for IDDM into overt disease (1). It has been observed (2) that in BB rats the evolution of IDDM depends on intact protein intake, which suggests that dietary factors may precipitate the onset of the disease. More recent studies in BB rats and NOD mice have shown reduced incidence of diabetes associated with milk-free diets (3, 4). It has been hypothesized that cow's milk may contain a trigger factor for development of IDDM (5). Breast-feeding seems to have a protective effect on the onset of the disease (5–7), although conflicting data have been reported (8, 9). On the other hand, increased levels of antibodies to cow's milk and ß-lactoglobulin (BLG) were found in children with newly diagnosed IDDM (10–14). The aim of our study was to examine the presence of anti-BLG antibodies in IDDM children, evaluated at diagnosis and during 2–5 year follow-up, as well as in their non-diabetic siblings.

Subjects

Forty-five newly diagnosed IDDM children and adolescents (26 males and 19 females), aged 1.2–16.6 years and 46 of their non-diabetic siblings (16 males and 30 females), aged 3.2–28.2 years were included into this study. Twenty-six patients were further studied over a 2–5 year period. Seventeen out of 45 patients and 16 out of 46 siblings were breast-fed. In all subjects, we evaluated IgA and IgG anti-BLG by ELISA. Total serum IgA, IgG and IgM were normal in all cases. Ninety-seven healthy, age- and sex-matched subjects served as controls. Anti-BLG antibodies are expressed as % of a positive sera pool. The intra- and inter-assay coefficients of variance were 9.8% and 9.6% for IgA, and 10.5% and 12.8% for IgG, respectively. In control subjects IgA and IgG anti-BLG values were skewed and the limit for positivity was set at the 95th centile. In all patients, islet-cell antibodies (ICA), anti-insulin antibodies (IAA) and other organ- and non-organ-specific antibodies were determined, as previously described (15). All patients and siblings were also HLA-typed (16).

Statistical analysis

Statistical analysis was performed using chi-square statistic, Student's t-test, Wilcoxon's non-parametric rank-sum test and Spearm's rank-correlation test.

Results

Patients

In newly diagnosed IDDM patients, IgA anti-BLG levels (85 ± 93%) were significantly higher ($p < 0.005$) than in controls (46.2 ± 23.8%), while no difference was observed for IgG anti-BLG. In particular, 14/45 patients (31%) had elevated (> 95th centile) IgA anti-BLG, and 4/45 patients (8.8%) elevated IgG anti-BLG (Fig. 1). ICA were present in 29/45 (64.4%), and IAA in 7/45 subjects (15.5%).

Over the 2–5 year follow-up, we observed an increase of IgA anti-BLG in 2 other patients. At the end of follow-up IgA decreased to normal levels in all but 2 girls, and IgG in all patients (Fig. 2). No correlation was found between anti-BLG antibody levels and chronologic age, breast-feeding duration, ICA, IAA, other organ- and not-organ-specific antibodies and HLA type. No significant difference was found between breast- and bottle-fed patients. Two of the patients with elevated IgA anti-BLG also presented high serum levels of IgA antigliadin, antireticulin and antiendomysium antibodies. The 2 patients underwent jejunal biopsy and coeliac disease was diagnosed (17).

Siblings

In healthy siblings, IgA anti-BLG levels (64.45 ± 63%) were significantly higher ($p < 0.05$) than in controls (46.2 ± 23.8%), while no difference was observed for IgG anti-BLG. In particular, increased IgA anti-BLG antibody levels were found in 5 siblings (11%), and elevated IgG anti-BLG were observed in other 5 siblings (11%) (Fig. 3).

No correlation was found between anti-BLG antibody levels and chronologic age, breast-feeding duration and HLA type. No significant differences were found between breast- or bottle-fed siblings.

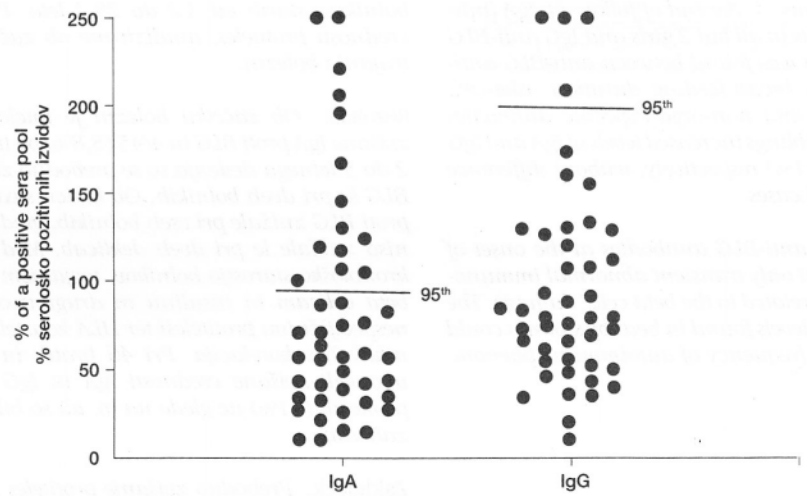

Fig. 1. *IgA anti-BLG and IgG anti-BLG levels in 45 newly diagnosed IDDM patients. The line represents the 95th centile of the values in control subjects.*

Sl. 1. *Vrednosti IgA in IgG protiteles proti BLG pri 45 novoodkritih insulinsko odvisnih diabetikih. Črta predstavlja 95. percentilo vrednosti pri kontrolni skupini.*

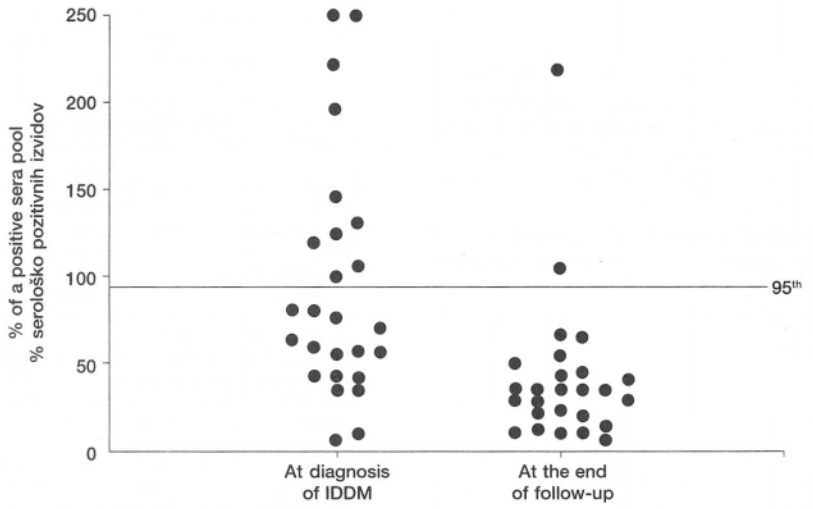

Fig. 2. *IgA anti-BLG levels evaluated at the diagnosis of IDDM and at the end of the 2–5 year follow-up in 26 IDDM children.*

Sl. 2. *Vrednosti IgA protiteles proti BLG pri 26 insulinsko odvisnih diabetičnih otrocih ob začetku in po 2 do 5 letih trajanja bolezni.*

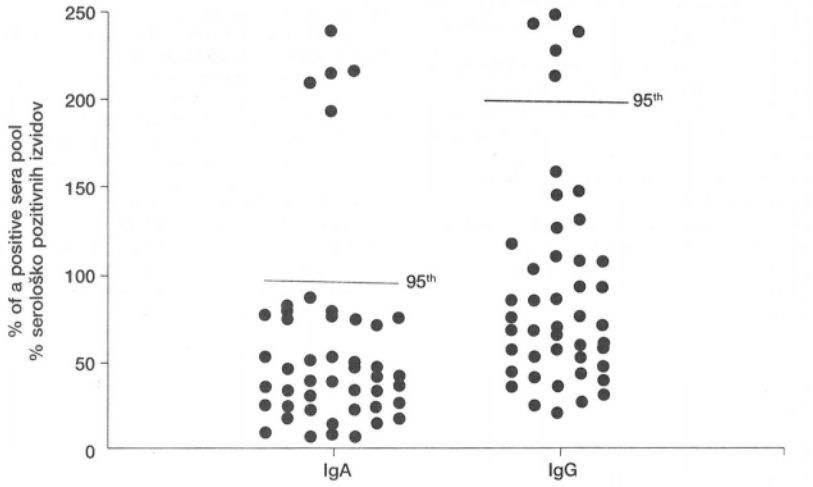

Fig. 3. *IgA anti-BLG and IgG anti-BLG levels in 46 non-diabetic siblings. The line represents the 95th centile of the values in control subjects.*

Sl. 3. *Vrednosti IgA in IgG protiteles proti BLG pri 46 bratih in sestrah diabetikov. Črta predstavlja 95. percentilo vrednosti pri kontrolni skupini.*

Discussion

Savilahti et al. (10) hypothesized that IDDM patients had an altered response and/or an increase of intestinal permeability to cow's milk proteins with an immune reaction in pancreas, which lead to diabetes. Moreover, Dahlquist et al. (14) suggested that early exposure to BLG in genetically susceptible children may lead to the development of Type 1 diabetes. More recently it has been suggested (13) that sensitization and development of immune memory to cow's milk protein is the initial step in the etiology of IDDM in genetically susceptible subjects. These proteins may trigger the autoimmune process which leads to IDDM through a mechanism of molecular mimicry.

Two ecological analyses showed that national consumption of milk proteins was directly related to national IDDM incidence (18, 19). However, contrasting results are reported: a case-control study demonstrated that early introduction of supplementary (non-breast) milk increased IDDM risk (7); whereas Blom et al. (20) and Nigro et al. (8) found no relationship with diabetes status.

In our study, we observed increased anti-BLG antibody levels in children with newly diagnosed IDDM as compared to age-matched non-diabetic controls, but no correlation was found with type or duration of feeding.

A strong antigenic similarity has been observed between human ß-casein and bovin ß-lactoglobulin (21), and the ß-lactoglobulin presence in human milk due to mother's diet has been also confirmed (22). Newly diagnosed IDDM patients were found to have higher IgA levels to mumps and Coxsackie B4 viruses (23), and higher frequency of autoantibodies (24). These observations together with our finding that anti-BLG antibody levels became normal during follow-up suggest that they may represent only a transient abnormal immunological response (25), probably unrelated to the pathogenesis of pancreatic damage.

A high frequency of autoantibodies has been reported in first degree relatives of diabetic patients (26). The increased anti-BLG antibody levels found in healthy siblings of our patients may confirm the increased frequency of autoimmune phenomena, previously reported in these subjects (14, 15, 26).

Conclusions

Many studies in newly-diagnosed IDDM patients have reported a high frequency of antibodies. These observations together with our finding that anti-BLG antibody levels became normal during follow-up suggest that they may represent only a transient abnormal immunological response, probably unrelated to the pathogenesis of pancreatic damage.

A high frequency of autoantibodies has been reported in first degree relatives of diabetic patients. The increased anti-BLG antibody levels found in healthy siblings of our patients may confirm the increased frequency of autoimmune phenomena in these subjects.

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Professional article/Strokovni prispevek

A YEAR OF EXPERIENCE WITH A CUSTOMISED ELECTRONIC DATABASE FOR DIABETIC CHILDREN

ENOLETNE IZKUŠNJE S PRILAGOJENO ELEKTRONSKO BAZO PODATKOV ZA OTROKE S SLADKORNO BOLEZNIJO

Joseph Schwingshandl, Alexander Pilhatsch, Martin Borkenstein

Department of Paediatrics, University of Graz, Austria

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Key words: *diabetes mellitus; computer-assisted data management; follow-up; metabolic control; child; adolescent*

Abstract – Background. *A database for diabetic children which is a part of a database for pediatric endocrinology in general ('OZGROW' by Peter Greenacre, Ray Williams Institute of Pediatric Endocrinology, Diabetes and Metabolism, The Children's Hospital, Sydney, 2050 NSW, Australia) is presented. Examples for data management are given and the use in the day to day routine is discussed.*

Methods. *Data on 175 children and adolescents with type I diabetes (99 males, 76 females, mean age 15.4, SD 5.9, range 1.2 to 32.0 years, and mean duration of diabetes 7.5, SD 5.3, range 0.04 to 26.2 years) were analyzed.*

Results. *The mean HbA_{1c} of the whole group was 8.7 (SD 2.0, range 4.8 to 17.0) %, metabolic control being significantly poorer in girls (9.1, SD 2.1, vs. 8.4, SD 1.8%, p = 0.03). Mean height SDS was +0.1 (SD 1.1, range –3.3 to +2.6) for the whole group. Mean height SDS in the girls was significantly lower if compared with the boys (–0.15, SD 1.3 vs. +0.27, SD 1.0; p = 0.04). Mean weight SDS was +0.3 (SD 0.9, range –2.6 to +2.6). The mean age at diagnosis was 8.1 (SD 4.1, range 0.7 to 20.8) years, the mean interval between the observation of first specific symptoms of diabetes and the diagnosis was 35 (SD 62, range 0 to 365) days. The time between first symptoms and the diagnosis was correlated with the age at diagnosis (r = 0.22, p = 0.04). When data were analyzed separately according to gender, girls on basal-bolus had a significantly higher body weight if compared with girls on conventional insulin substitution (+0.5, SD 0.8 vs. +0.1, SD 0.9 SDS; p = 0.04). Boys on basal-bolus were significantly taller compared to boys on conventional insulin substitution (+0.7, SD 1.0 vs. 0.1, SD 0.9 SDS; p = 0.01).*

Conclusions. *A customised electronic database for children and adolescents with type I diabetes gives easy access to patients data, which brings advantages for the day-to-day routine as well as for scientific analysis.*

Ključne besede: *diabetes mellitus; računalniška obdelava podatkov; metabolna urejenost; rast in razvoj; otrok; mladostnik*

Izvleček – Izhodišča. *Predstavljena je računalniška baza podatkov za bolnike s sladkorno boleznijo, ki je del podatkovne baze za pediatrično endokrinologijo ('OZGROW' – Peter Greenacre, Ray Williams Institute of Pediatric Endocrinology, Diabetes and Metabolism, The Children's Hospital, Sydney, 2050 NSW, Australia). Podani so primeri urejanja podatkov in uporaba v vsakdanji rutini.*

Metode. *Obdelani so podatki 175 otrok in mladostnikov s sladkorno boleznijo tip I (99 fantov, 76 deklic, povprečna starost 15,4, SD 5,9, razpon 1,2 do 32,0 leta, povprečno trajanje sladkorne bolezni 7,5, SD 5,3, razpon od 0,04 do 26,2 leta).*

Rezultati. *Povprečni hemoglobin HbA_{1c} celotne skupine je bil 8,7 (SD 2,0, razpon 4,8 do 17,0) % in statistično značilno (p = 0,03) višji pri dekletih (9,1, SD 2,1%) kot pri fantih (8,4, SD 1,8%). Povprečna ocena standardne deviacije za telesno višino celotne skupine je bila +0,1 (SD 1,1) in statistično značilno (p = 0,04) nižja pri dekletih (–0,15, SD 1,3) kot pri fantih (+0,27, SD 1,0). Povprečna ocena standardne deviacije za telesno težo je bila +0,3 (SD 0,9). Povprečna starost ob postavitvi diagnoze je bila 8,1 (SD 4,1, razpon od 0,7 do 20,8) let in povprečen interval med prvimi specifičnimi znaki bolezni in postavitvijo diagnoze 35 (SD 62, razpon od 0 do 365) dni. Ta časovni interval je bil v korelaciji s starostjo ob postavitvi diagnoze (r = 0,22, p = 0,04). Dekleta z intenzivirano obliko zdravljenja (ena aplikacija dolgo delujočega inzulina in več aplikacij hitro delujočega inzulina) so imela značilno večjo (p = 0,04) telesno težo kot dekleta s konvencionalno obliko zdravljenja. Fantje z intenzivirano obliko zdravljenja so bili značilno višji (p = 0,01) kot fantje s konvencionalno obliko zdravljenja.*

Zaključki. *Prilagojena elektronska baza podatkov za otroke in mladostnike s sladkorno boleznijo tip I omogoča hiter in pregleden dostop do podatkov pri vsakdanjem delu in je hkrati primerna za znanstveno obdelavo podatkov.*

Introduction

Electronic data management has brought along major advantages wherever a great amount of data has to be dealt with. The access to data is quicker, furthermore, data can be analysed and presented in many ways, which facilitates their understanding. In paediatric diabetology, longterm-monitoring of the patient is important. Negative trends in metabolic control as well as in the somatic development should be established as soon as possible. A slight intra-individual change in blood pressure might be an early sign of incipient nephropathy. To extract all this information from a conventional patient's record might be a time-consuming process. The present study investigated the usefulness of a customised electronic database for diabetic children over one year.

Material and methods

The database presented is part of a database for paediatric endocrinology in general ('OZGROW'). It has been developed by Peter Greenacre from the Ray Williams Institute of Paediatric Endocrinology, Diabetes & Metabolism, The Children's Hospital, Sydney, 2050 NSW, Australia (Director Prof. Martin Silink). It is written in the commercially available software CRS (Clinical Reporting systems Pty Ltd, Castle Hill, 2154, Australia). Data to be entered include general information such as patients name, DOB (date of birth), sex, address, names of carers, date of onset of diabetes, date of diagnosis, and date of first referral. Diagnosis codes for the various disturbances of carbohydrate metabolism are provided. At each patients' visit, specific information is obtained and entered in three different tables named 'Growth visit', 'Diabetes-visit', and 'Glycosylated haemoglobin'. The growth visit table includes height and weight expressed as standard deviation score (SDS), pubertal stage, and bone age. The diabetes visit table includes insulin dose and number of injections, numbers of severe hypoglycaemic episodes, episodes of ketosis or hospitalisation for diabetes; systolic and diastolic blood pressure, limited joint mobility, liver size; presence or absence of goitre, clinical retinopathy, cataracts, and clinical peripheral neuropathy; skin manifestations of diabetes such as infections, vitiligo, granuloma annulare; laboratory tests performed at this visit. Data can be retrieved using various search conditions, which can be combined in many ways. New variables such as insulin dose per body-weight, age and duration at a certain date, postpubertal duration of diabetes and so on can be derived. Data can be either seen on screen, printed out, or exported in ASCII-format. For the results presented here data were first exported to a statistical program (SAS) and to a graphic program. Data on 175 children and adolescents with type 1 diabetes (99 males and 76 females), mean age 15.4 (SD 5.9 [range 1.2 to 32.0] years), mean duration of diabetes 7.5 (SD 5.3 [range 0.04–26.2] years) who attended the outpatient department during one year are presented here.

Results

The mean HbA_{1c} of the whole group was 8.7 (SD 2.0 [range 4.8 to 17.0]) %, metabolic control being significantly poorer in girls (9.1 SD 2.1 vs. 8.4 SD 1.8%, P = 0.03) (Fig. 1).

Mean height SDS was +0.1 (SD 1.1 [range –3.3 ± 2.6]) for the whole group. Mean height SDS in the girls was significantly lower if compared with the boys (–0.15 SD 1.3 vs. +0.27 SD 1.0, P = 0.04). A stepwise multiple linear regression model, combining duration of diabetes, HbA_{1c}, and number of daily injections, explained of the 16% variability in height-SDS. A longer duration of diabetes, higher levels of HbA_{1c}, and a lower number of daily injections were associated with lower height. Mean weight (SDS) was +0.3 (SD 0.9 [range –2.6 ± 2.6]). A multiple linear regression model, combining insulin/body-weight and number of daily injection, explained the 6% variability in weight-SDS. Lower doses of insulin and a higher number of injections were associated with higher weight. The mean age at diagnosis was 8.1 (SD 4.1 [range 0.7–20.8]) years, and the mean interval between the observation of first specific symptoms of diabetes and the diagnosis 35 (SD 62 [range 0 to 365]) days. The time interval between the first symptoms and diagnosis was correlated with the age at diagnosis (r = 0.22, P = 0.04) (Fig. 2). When data were analysed separately according to gender, girls on basal-bolus had significantly higher body-weight compared with girls on conventional insulin substitution (+0.5 [SD 0.8] vs. +0.1 [SD 0.9] SDS, P = 0.04). Boys on basal-bolus were significantly taller compared to boys on conventional insulin (+0.7 SD 1.0 vs. 0.1 SD 0.9 SDS, P = 0.01).

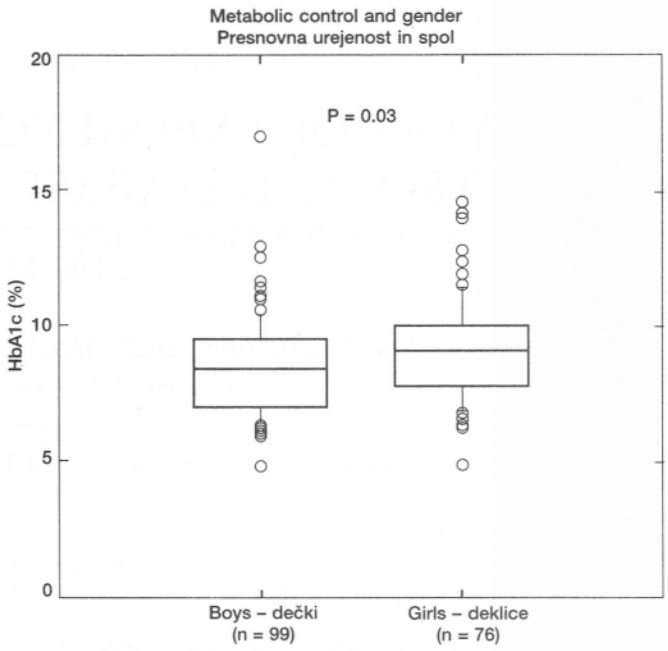

Fig. 1. *Boxplots of HbA_{1c} values for boys and girls. The horizontal lines represent the median, the 25th, and the 75th centile, respectively. Values outside the 10th and 90th centile are given as actual values.*

Sl. 1. *Prikaz vrednosti HbA_{1c} pri fantih in dekletih. Vodoravne črte v pravokotnikih predstavljajo mediano, 25. in 75. centil. Vrednosti nad 90. in pod 10. centilo so podane s posameznimi krožci.*

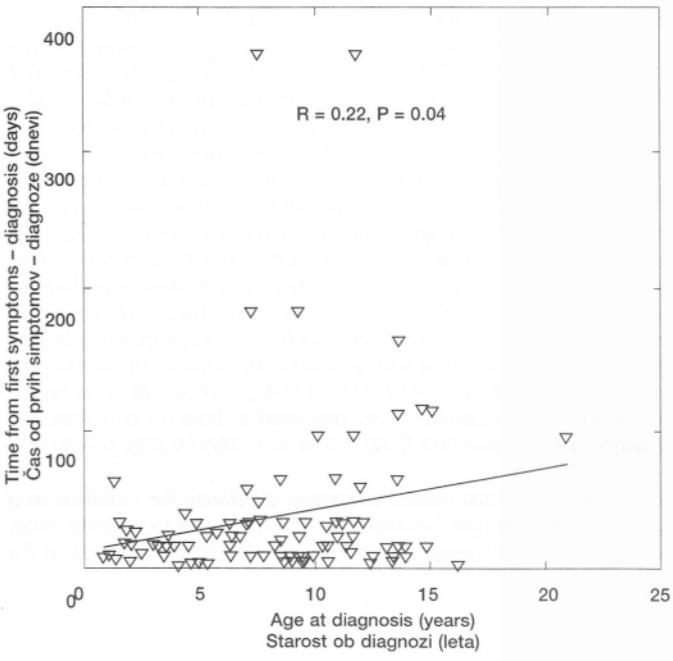

Fig. 2. *The relationship between the age at onset and the time interval between the first specific symptoms and determination of diagnosis.*

Sl. 2. *Odnos med starostjo ob postavitvi diagnoze in časovnim intervalom med prvimi značilnimi znaki bolezni in postavitvijo diagnoze.*

Discussion

The electronic database for diabetic children contains a number of specific information and allows easy access to data. Some examples of statistical analyses confirming reports from the literature are given. Poor metabolic control has been shown to be associated with poor longitudinal growth (1). Continuous monitoring of longitudinal growth as well as of complications of diabetes provide a quality control of the treatment. Diabetes in children younger than four years at onset has been mentioned as a subtype of childhood diabetes. It is characterised by a number of specific features, one of which is the short interval between the onset of symptoms and the diagnosis (2). This has also been confirmed by our data. Multiple injection insulin regimen have been shown to be associated with weight gain (3). Particularly adolescent girls tend to obesity when changed to a basal-bolus insulin routine. Further advantages of this electronic database are ?? that patients can be selected according to various characteristics.

For instance, all patients with poor metabolic control can be selected and included into a specific education program. Changes of metabolic control after intervention can be monitored easily. Export of data and subsequent statistical analysis allows comparison of the outcome of one centre with other centres. If a certain age group of patients should be contacted for a certain activity (such as a camp), address labels of this specific group can be produced. Patients who haven't been to the clinic for longer than 4 months can be contacted by mail and asked to make a new appointment.

The main advantage of the system is, of course, that data can be readily accessed for scientific analysis. The time-consuming process of extracting data from medical records and entering it into a statistical program can be avoided. The options for search conditions are nearly unlimited and can be combined in any fashion.

The way data are presented here is only meant to give some examples of the options included in the database. It should not be mistaken for a study of growth in children with diabetes; too many co-variables have not been taken into account.

In short, the use of a customised electronic database, as presented here, helps the paediatric diabetologist to learn to know his patients better, and to early detect disturbances in somatic development by comparing longitudinal data. Furthermore, data are easily accessible for scientific analysis.

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Professional article/Strokovni prispevek

EARLY DETECTION AND FOLLOW UP OF CHILDREN WITH PHENYLKETONURIA IN SLOVENIA

ZGODNJE ODKRIVANJE IN SPREMLJANJE OTROK S FENILKETONURIJO V SLOVENIJI

Tadej Battelino[1], Ciril Kržišnik[1], Katarina Pavlin[2]

[1] Medical Center Ljubljana, Department of Pediatrics, Vrazov trg 1, 61000 Ljubljana, Slovenia
[2] Medical Center Ljubljana, Department of Nuclear Medicine, Zaloška 7, 61000 Ljubljana, Slovenia

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Key words: *hyperphenylalaninemia; screening; incidence; treatment; maternal phenylketonuria*

Abstract – Background. *In Slovenia, first efforts to start neonatal screening for phenylketonuria (PKU) were made in 1967. The screening has run successfully since 1979. In July 1992, the quantitative fluorometric method was introduced. Results from the 13-year period of Guthrie method application are compared to results from the present period. Additionally, appropriate treatment, duration of therapy and maternal PKU program are discussed.*

Methods. *In all newborns with elevated phenylalanine (PHE) levels detected by neonatal screening performed with a dried blood drop collected from the newborn's heel on a filter paper on days 3 to 5 (cut off level 0.12 mmol/l), serum phenylalanine determination was done. Patients were categorized according to the severity of the PHE elevation. Incidences were calculated and compared by chi-square test.*

Results. *The incidence of classical PKU is 1/8.000. The incidence of mild hyperphenylalaninemia (PHE 0.12–0.59 mmol/L) in the last period, when the quantitative fluorometric screening method is used, is 1/476, which is significantly ($p < 0.0001$) higher than before. One patient with 6-pyruvoyltetrahydrobiopterin synthetase (6-PTS) deficiency was diagnosed.*

Conclusions. *The incidence of classical PKU in Slovenia over the last 13 years is slightly higher than the estimated mean world incidence (1/10,000). Fluorometric screening method enables detection of mild PHE elevations soon after birth. Female patients with serum PHE above 0.24 mmol/l are enrolled in the Maternal PKU program. Patients and their parents are encouraged to stick to the low PHE diet as long as possible. Routine genetic analysis and measurements of PHE hydroxylase activity would be beneficial.*

Ključne besede: *hiperfenilalaninemija; presejalni test; incidenca; zdravljenje; maternalna fenilketonurija*

Izvleček – Izhodišča. *Začetki uvajanja presejalnega testa za hiperfenilalaninemije, kamor sodi fenilketonurija (PKU), segajo v Slovenijo v leto 1967. Presejalno testiranje uspešno poteka od začetka leta 1979; do junija 1992 je potekalo po Guthriejevi metodi, nato pa smo uvedli kvantitativno fluorometrično metodo. Avtorji primerjajo rezultate, dobljene v obdobjih omenjenih dveh metod. Ob tem razpravljajo o sodobnih principih in trajanju zdravljenja ter o programu za preprečevanje maternalne PKU.*

Metode. *Vsem novorojencem se 3. do 5. dan vzame na filter papir kapljico krvi iz pete; tiste, pri katerih je ugotovljen fenilalanin (PHE) 0,12 mmol/l ali višji, se pokliče na določitev PHE v serumu na Pediatrično kliniko v Ljubljani. Bolniki so razdeljeni v kategorije glede na nivo PHE. Podatki so obdelani z metodami opisne in primerjalne statistike.*

Rezultati. *Incidenca klasične PKU je 1/8000. Incidenca mile hiperfenilalaninemije (PHE 0,12–0,59 mmol/L) je v zadnjem obdobju, ko se uporablja kvantitativna fluorometrična presejalna metoda, 1/476 in je značilno višja ($p < 0.0001$) kot prej. Pri enem bolniku smo ugotovili pomanjkanje 6-piruvoiltetrahidrobiopterin sintetaze.*

Zaključki. *Incidenca klasične oblike PKU je v Sloveniji v zadnjih 13-ih letih nekoliko višja, kot je ocenjena povprečna incidenca v svetu (1/10.000). Fluorometrična metoda presejalnega testa omogoča zanesljivo odkrivanje novorojencev s PHE tik nad normalno vrednostjo v prvih dneh po rojstvu. Vse bolnice s PHE, višjim od 0,24 mmol/l, so vključene v program za preprečevanje maternalne PKU. Bolnike in njihove starše spodbujamo, da dieto pri otroku strogo upoštevajo najmanj do konca odraščanja, po možnosti pa vse življenje. Koristne bi bile rutinske genetske preiskave in določanje aktivnosti encima PHE hidroksilaze pri bolnikih s PKU.*

Introduction

Phenylketonuria (PKU) is an inborn error of metabolism secondary to a deficiency of phenylalanine (PHE) hydroxylase, inherited as an autosomal recessive trait. In 1934, A. Folling reported identification of phenylketones in the urine of two siblings with severe mental retardation (1). Twenty years later the PHE hydroxylase was discovered and the low PHE diet preventing extreme increase of PHE in blood and consequently cerebral damage was successfully introduced for the first time. Soon after Guthrie and Susie described the Guthrie method to detect PKU in 1963 (2), newborn screening was started in Massachusetts, USA (3), and subsequently in many other countries. In Slovenia, phenylketonuria (classical clinical manifestations: blond hair, fair skin, blue eyes, seborrheic or eczematoid skin rash, unpleasant odor, mental retardation, hypertonus, seizures, microcephaly, enamel hypopla-

Tab. 1. *Patients with hyperphenylalaninemia in Slovenia born between January 1, 1979 and June 30, 1992. Guthrie screening method was used (n = number of newborns screened; PHE = phenylalanine).*

Tab. 1. *Bolniki s hiperfenilalaninemijo v Sloveniji, rojeni med 1. januarjem 1979 in 30. junijem 1992. Za presejalno določanje fenilalanina je bila uporabljena Guthrijeva metoda (n = število novorojencev, zajetih s presejalnim testom; PHE = fenilalanin).*

	Hyperphenylalaninemia – serum PHE (mmol/L)*				
n = 351,534	0.12–0.23	0.24–0.59	0.60–1.19	> 1.19	
	Mild		Variant	Classical	Pterin
No. of patients (Female/Male)	9 (4/5)	19 (10/9)	20 (11/9)	43 (23/20)	1 (0/1)
No. of pat. on diet (percentage) (Female/Male)	0 0 0	1 (5.3) (1/0)	15 (75.0) (8/7)	43 (100) (23/20)	1=

* highest serum concentrations when on a normal diet (breast fed or milk formula), measured between days 8 and 65
* najvišje serumske koncentracije ob običajni prehrani (dojenje ali prilagojeno mleko), izmerjene med 8. in 65. dnevom
= additional therapy to the diet
= dodatna terapija poleg diete

Tab. 2. *Patients with hyperphenylalaninemia in Slovenia born between July 1, 1992 and April 30, 1993. Quantitative fluorometric screening method was used (n = number of newborns screened; PHE = phenylalanine).*

Tab. 2. *Bolniki s hiperfenilalaninemijo v Sloveniji, rojeni med 1. julijem 1992 in 30. aprilom 1993. Za presejalno določanje fenilalanina je bila uporabljena kvantitativna fluorometrična metoda (n = število novorojencev, zajetih s presejalnim testom; PHE = fenilalanin).*

	Hyperphenylalaninemia – serum PHE (mmol/l)*				
	0.12–0.23	0.24–0.59	0.60–1.19	> 1.19	
	Mild		Variant	Classical	Pterin
No. of patients (Female/Male)	25 (11/14)	12 (3/9)	2 (2/0)	5 (3/2)	0 0
No. of pat. on diet (percentage) (Female/Male)	0 0 0	0 0 0	1 (50.0) (1/0)	5 (100) (3/2)	0

* highest serum concentrations on normal diet (breast fed or milk formula), measured between days 6 and 20
* najvišje serumske koncentracije ob običajni prehrani (dojenje ali prilagojeno mleko), izmerjene med 6. in 20. dnevom

sia, growth retardation) was first described in 1956 (4). Efforts were made to start screening in 1967, and three children with PKU were detected until 1971. Complete neonatal screening for PKU in the whole Slovenia has been performed since 1979 (5). From 1979 until June 1992, screening was performed by the Guthrie test. Since then the quantitative fluorometric method has been used.
All children with detected elevated serum PHE are followed-up in Medical Center Ljubljana, Department of Pediatrics, Department of Endocrinology, Diabetes and Metabolic Diseases.

Patients and methods

Until the end of June 1992 the Guthrie method was used with the semiquantitative cut off level 0.12 mmol/L. From July 1992 on, PKU screening is performed through the quantitative fluorometric method from a dried blood drop from the newborn's heel collected on a filter paper on days 3–5. The cut off level is still 0.12 mmol/L. Measurements of serum PHE are made routinely by the fluorometric method (normal range between 0.085 and 0.11 mmol/L) (6). All newborns delivered in Slovenia from January 1, 1979 with the detected PHE level of 0.12 mmol/L and higher are sent to a second, serum determination of PHE to the Department of Pediatrics, Ljubljana. Categorization of severity of hyperphenylalaninemia is based on the PHE value measured from serum specimen, obtained while children aged from 8 to 65 days (or from 6 to 20 days after July 1, 1992) are on a normal diet (breast fed or milk formula).
Measurements of activity of blood dihydropterin reductase (DHPR) and concentrations of neopterin and biopterin in urine are performed in Kinderspital Zurich, Abteilung für klinische Chemie by Dr. N. Blau.
Psychological development tests adapted for Slovenian population from Brunet-Lezine were used (7).
Incidences were calculated and compared by chi-square test.

Results

From January 1, 1979 until June 30, 1992, 351,534 newborns were screened for hyperphenylalaninemia. Data from this period are shown in Table 1. The incidence of classic PKU was 1.2/10,000 or approximately 1/8,000 newborns. For all hyperphenylalaninemias with serum PHE 0.24 mmol/L and above, the incidence was 2.3/10,000, and for those with serum PHE 0.12 mmol/L and above, 2.9/10,000 or approximately 1/4,000. Data for the last ten months of screening with the quantitative fluorometric method are shown in Table 2. The incidence of mild hyperphenylalaninemia (serum PHE between 0.12 and 0.59 mmol/L) is 1/476, which means it is significantly higher ($p < 0.0001$) compared to the data from the Guthrie screening method period. After the serum concentrations of PHE were established, operational categorization and decisions for further investigations (pterin metabolism) and a low PHE diet were made.
In one patient 6-pyruvoyltetrahydrobiopterin synthetase (6-PTS) deficiency was proven at the age of two years (Table 3).

Tab. 3. *Values of serum phenylalanine (PHE), tyrosine (TYR), blood dihydropteridine reductase activity (DHPR), urine biopterin (B) and neopterin (N), liquor 5-hydroxyindolacetic acid (5-HIAA) and homovanillic acid (HVA) in patient with 6-pyruvoyltetrahydropterin synthetase (6-PTS) deficiency. Serum and urine values are measured basaly and after tetrahydrobiopterin loading (7.5 mg/kg BW p.o.) (BNCR = biopterin – neopterin – creatinine ratio: B/C × B/(B+N) × 100.000).*

Tab. 3. *Vrednosti serumskega fenilalanina (PHE), tirozina (TYR), aktivnosti dihidropteridin reduktaze (DHPR) v krvi, biopterina (B) in neopterina (N) v urinu ter 5-hidroksiindolocetne (5-HIAA) in homovanilične (HVA) kisline v likvorju pri bolniku z motnjo 6-piruvoiltetrahidropterin sintetaze (6-PTS). Vrednosti v serumu in urinu so merjene bazalno in po obremenitvi z tetrahidrobiopterinom (7,5 mg/kg TT p.o.) (BNCR = biopterin – neopterin – kreatinin – razmerje: B/C × B/(B+N) × 100.000).*

	Serum	Blood		Urine			Liquor	
	µmol/L	mU/mg Hb		mmol/mol creat.			nmol/L	
	PHE	TYR	DHPR	N	B	BNCR	5-HIAA	HVA
Basal	1355	62	3.1	11.0	0.17	0.32	9.4	104.2
BH4 loading	263	182	/	17.6	2. 71	36. 1	/	/
Reference R.=	85–110	44–70	2–5	0.2–1.7	0.5–2.7		100–300	400–800

= reference range for the age of 3 years
= normalne frednosti za starost 3 leta

With the exception of the patient with 6-PTS deficiency, all patients detected by neonatal PKU screening are satisfactory, mentally and physically, developed so far, although many discrete deviations are routinely found, especially insufficient motorical coordination and distractibility in preschool children, and slightly lower scores in arithmetic in school children.

Discussion

Hyperphenylalaninemia is a clinically, biochemically and genetically heterogeneous disorder. We assign children with proven elevated serum PHE in three categories: 1) children with classical PKU exhibit blood PHE concentrations of at least 1.2 mmol/L, normal tyrosine concentrations, and an excessive number of PHE metabolites in their urine while on a normal diet, 2) children with variant PKU generally have blood PHE concentrations between 0.6 and 1.2 mmol/L but may not have PHE metabolites present in their urine, unless they ingest excessive amounts of dietary protein: 3) children with benign hyperphenylalaninemia have blood concentrations between 0.12 and 0.6 mmol/L, their urine is usually normal, and their blood tyrosine values are normal. With identification of individual PHE hydroxylase alleles by RFLP haplotype analysis, genetic categorization is possible. Although some differences in the haplotype distribution between different European nations tested exist (8–10), the correlation of the PKU-phenotypes with their RFLP haplotypes is possible. PKU children who have homozygotes for either haplotype 2 or 3 or haplotype 2/3 have classical PKU (11). Other correlations seem to be more complex. In addition, pterin metabolism disorders have their own molecular and genetic background. Thus, analysis of patients with PKU at the molecular level is important, not only in carrier screening and prenatal diagnosis but also in predicting clinical phenotype (12). Tetrahydrobiopterin (BH_4) is required as a cofactor in the hydroxylation of PHE, tyrosine and tryptophan. Inborn errors of BH_4 metabolism may occur in the synthetic pathway or in re-cycling of BH_4 (enzymes: guanosine triphosphate cyclohydrolase, 6-pyruvoyl tetrahydrobiopterin synthetase, dihydropteridine reductase). Patients often present with hyperphenylalaninemia in association with progressive neurological disease, which develops despite early treatment with a low PHE diet. Their neurological disturbance is largely due to a deficiency of catecholamines and serotonin resulting from impaired synthesis of L-dopa and 5-hydroxytryptophan; prenatal damage occurs in some subjects with defect of synthesis. Therefore, early diagnosis and appropriate additional treatment are of special importance.

The incidence of hyperphenylalaninemias depends greatly on the screening technique and the cut off level. The incidence of classical PKU of 1.2/10,000 or 1/8,000 in Slovenia is lower than in Ireland (1/4,000) but slightly higher than the estimated overall incidence which is 1/10,000 (13). In our study, the incidence of mild hyperphenylalaninemia is much higher when the quantitative screening method is used. The cut off level and the recommended age for testing vary greatly among different countries, being from 0.12 to 0.24 mmol/L and between 2 and 14 days respectively (14–16). Because of increasing tendency of early discharge of the newborns from the hospital, the possibility of very early testing of the newborns is more and more advantageous. Therefore the safe cut off level should not be higher than 0.12 mmol/L (17). We use this cut off level since 1979 and no child with PKU has been missed yet. At the same time, the number of children detected with very mild hyperphenylalaninemia (0.12–0.24 mmol/L) is high and represents an additional patient group. If the surum PHE is continuously under 0.24 mmol/L after 3 and 6 months on a normal diet, we discontinue the follow-up. Since many studies have proven that maternal serum PHE as low as 0.36 mmol/L can increase the incidence of fetus malformations (mental retardation, microcephaly, congenital heart disease, birth weight under 2,500 g, spontaneous abortion) (18, 19), all our female patients with serum PHE above 0.24 mmol/L (52 at the moment) are enrolled into an additional follow-up program to be properly educated about the risks of their metabolic status and during late adolescence introduced to a specialist of internal medicine trained in the follow-up of women with hyperphenylalaninemia in their fertile period. This fits the recommendations of the American Committee on Genetics (20). There are no indications so far that children of poorly controlled fathers with PKU are affected (21, 22).

If initial PHE concentration is elevated and especially if it is in a range likely to require intervention, the patient should be seen in a tertiary center (e.g. Department for Pediatrics, Ljubljana) without delay. It seems that the dietary treatment should be started in infants with serum PHE between 0.4 and 0.6 mmol/L, the optimal therapeutic level being within the range of 0.18–0.4 mmol/L (13). Additional investigations (pterin metabolism, serum tyrosine) are mandatory, accompanied by a close follow-up performed by a team consisting of a pediatrician trained in metabolic diseases, a dietitian, a psychologist and a pedagogue.

Many authors have proven that optimal intellectual and academic achievements are obtained by the earliest possible initiation of treatment and strict control of blood PHE levels throughout childhood (23, 24). In addition discontinuation or relaxation of the PHE-restricted diet after early childhood, which used to be a common practice, has proven to have an undoubtedly deteriorating effect on intellectual performance and behavior of children with PKU (25). Many evidences strongly support a recommendation that dietary restriction of PHE should be maintained through adolescence and especially in women throughout their fertile period (26). Continuation of the diet in women is particularly important since the attempts to re-institute the PHE-restricted diet have met with limited success (27). Individuals with PKU, as well as their families, should receive counseling and support from the clinic team to facilitate the maintenance of extended therapy.

Conclusions

Neonatal screening for PKU in Slovenia was started 26 years ago and has been successfully running for the last 14 years. From July 1992 on, the quantitative fluorometric method is used. The incidence of classical PKU (1/8,000) is slightly higher than the estimated mean, the incidence of mild hyperphenylalaninemias being much higher particularly in the last ten months while the quantitative method is used.

After categorization of hyperphenylalaninemia, decisions for pterin metabolism investigations and appropriate treatment are made as soon as possible (within 20 days in the last period). Female patients with PKU 0.24 mmol/L and above are followed-up additionally to be enrolled in the Maternal PKU program on time. Efforts are made to persuade patients and their parents not to discontinue the low PHE diet as long as possible, certainly not before the end of adolescence.

Routine genetic analysis, determination of PHE hydroxylase alleles and determination of the activity of the enzyme would be beneficial and will be one of our future goals.

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Professional article/Strokovni prispevek

RESULTS OF SCREENING FOR CONGENITAL HYPOTHYROIDISM DURING THE TEN-YEAR PERIOD (1981–1991) IN SLOVENIA

REZULTATI PRESEJALNEGA PROGRAMA KONGENITALNEGA HIPOTIREOIDIZMA V DESETLETNEM OBDOBJU (1981–1991) V SLOVENIJI

Ciril Kržišnik[1], Tadej Battelino[1], Nina Bratanič[1], Sergej Hojker[2], Katarina Pavlin[2], Mojca Žerjav-Tanšek[1], Nataša Bratina-Uršič[1], Jana Frelih[3], Barbara Žemva[3], Borut Bratanič[4], Jagoda Vatovec[5], Gorazd Kolar[6]

[1] Department of endocrinology, diabetes and metabolic diseases, Departments of paediatrics, Medical Center, Vrazov trg 1, 61105 Ljubljana
[2] Department of nuclear medicine, Medical Center, Zaloška 7, 61105 Ljubljana
[3] Department of neurology, Departments of paediatrics, Medical Center, Vrazov trg 1, 61105 Ljubljana
[4] Institute of neonatology, Departments of paediatrics, Medical Center, Vrazov trg 1, 61105 Ljubljana
[5] Department of otorinolaryngology, Medical Center, Zaloška 2, 61105 Ljubljana
[6] Department of ophtalmology, Medical Center, Zaloška 2, 61105 Ljubljana

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Key words: *congenital hypothyroidism; screening; children; Slovenia*

Abstract – Background. *Congenital hypothyroidism (CH) is probably the most common preventable cause of mental retardation. The early diagnosis and treatment are crucial to the prevention of severe intellectual deficit. Due to the facts that clinical signs and symptoms of the disease usually become evident after few months when irreversible neurological sequellae occur and that the incidence of CH is relatively high, and the treatment cheap and effective, it is worthwhile to perform the screening for the disease in all newborns.*

Materials and methods. *Screening for CH was started in Slovenia in 1981 by measurement of TSH in filter-paper blood spots by hellprick between the third and fifth day. TSH cut off value has been 20 mU/L for RIA method and 15 mU/L for Delphia method. Patients with elevated TSH levels were placed on thyroxin therapy 100 mcg/m² while the evaluation of the thyroid function was performed at the age of one year after brief interruption of therapy. Patients were examined every 3 to 4 months, while once a year detailed clinical, neurological and psychological exams were done. Since the age of 3 years ophtalmological and audiometric exams were performed as well.*

Results. *Between August 1, 1981 and August 1, 1991, screening for CH was performed in 211,287 newborns. Recall rate was 0.05%. CH with TSH exceeding 20 mU/L until 1989 and 15 mU/L later on was stated in 63 children. The incidence was 1:3,554. In the same period the incidence of transient hypothyroidism was 1:23,476 and that of permanent CH evaluated at the age of one year 1:4,143. The average age in which the therapy was started was 12.3 ± 4.9 days. Two patients were missed during the first two years of screening due to human error. In all children in whom screening was performed properly, except one case of Mb Down, one of cerebral gigantism and two of cerebral palsy due to perinatal anoxia, the psychomotor development was in normal range.*

Ključne besede: *kongenitalni hipotireoidizem; presejalni test; otroci; Slovenija*

Izvleček – Izhodišča. *Kongenitalna hipotireoza (KH) je eden najpogostejših vzrokov duševne subnormalnosti, ki jih je možno preprečiti. Z zgodnjo detekcijo in zdravljenjem se lahko omogoči bolnikom normalen psihomotorični razvoj. Bolezen je možno pravočasno odkriti s hormonskimi analizami s presejalnim testom pri vseh novorojenčkih, saj postanejo značilni klinični znaki bolezni očitni šele po več mesecih, ko se pojavijo ireverzibilne okvare možganovine. Zgodnja diagnoza KH bazira na detekciji zvišanih vrednosti TSH na osnovi mehanizma negativne vzvratne zveze zaradi hipotiroksinemije, ki je posledica ektopije, hipoplazije, aplazije ali dishormogeneze ščitnice, včasih pa tudi nekaterih drugih vzrokov. Presejalni test KH se izvaja navadno hkrati z detekcijo fenilketonurije.*

Material in metode. *Presejalni test KH se izvaja v Sloveniji od leta 1981 z meritvijo TSH iz kapljice posušene krvi na filter-papirju, ki je bila odvzeta pri vbodu pete novorojenčka med 3. in 5. dnevom življenja. Za zgornjo mejo normale (cut off value) je bila vzeta koncentracija 20 mE/l pri RIA metodi in 15 mE/l pri Delphia metodi. Vsi bolniki z zvišanimi vrednostmi TSH so bili zdravljeni s tiroksinom v odmerku 100 mcg/m². V starosti enega leta je bila po kratki prekinitvi terapije izvedena evaluacija funkcije ščitnice. Poleg meritev T4, T3, TSH je bila izvedena ultrazvočna in scintigrafska analiza ščitnice. V primeru prehodne hipotireoze je bilo zdravljenje s tiroksinom prekinjeno. Poleg kliničnega sledenja je bil pri bolniku enkrat letno izveden nevrološki in psihološki pregled, od tretjega leta dalje pa tudi okulistični in otorinolaringološki vključno z audiometrijo.*

Rezultati. *Med 1. 8. 1981 in 1. 8. 1991 je bil izveden presejalni test za KH pri 211.287 novorojencih. Zaradi mejnih ali povišanih vrednosti je bilo treba analizirati serumske vrednosti pri 0,05% otrok. KH z vrednostmi TSH prek 20 mE/l do leta 1989 oziroma 15 mE/l kasneje je bila ugotovljena pri 63 otrocih. Tako je znašala*

Conclusion. *Positive results of neonatal screening for CH in ten-year period in Slovenia urge us to continue the screening programme. The definite evaluation of thyroid function has been done at the age of two years since 1992 in order to minimise the danger of cerebral damage by thyroxin deprivation during the short break of therapy.*

incidenca 1:3554, v istem obdobju je bila incidenca tranzitorne hipotireoze 1:23.476 in permanente KH, ki je bila potrjena ob evaluaciji funkcije ščitnice pri 1 letu, 1:4143. Povprečna starost, v kateri se je začelo zdravljenje, je znašala 12,3 ± 4,9 dneva. Dva bolnika nista bila odkrita pravočasno zaradi napake pri izvedbi testa, ki je bila posledica človeškega faktorja. Pri otrocih, pri katerih je bil izveden test pravilno, poteka razvoj normalno razen pri enem bolniku z Mb Down, enem s cerebralnim gigantizmom in dveh s cerebralno paralizo, ki je posledica perinatalne hipoksije.

Zaključek. *Glede na dejstvo, da je KH relativno pogosta in zdravljenje enostavno, ceneno in učinkovito, je izvajanje presejalnega testa za KH nadvse smiselno. Pozitivni rezultati pri 10-letnem izvajanju testa v Sloveniji so razlog za njegovo nadaljevanje. Na podlagi dosedanjih izkušenj smo program nekoliko modificirali. Od leta 1992 izvajamo definitivno evaluacijo funkcije ščitnice v starosti dveh let, ko kratka prekinitev zdravljenja ne vpliva pomembno na centralni živčni sistem.*

Introduction

Clinical recognition of congenital hypothyroidism (CH) in the newborn is difficult due to minimal or absent signs and symptoms but may be detected biochemically. Classicaly, a low concentration of thyroxine and an increased concentration of thyroid stimulating hormone (TSH) occur in blood. The low thyroxine value is associated with ectopic, hypoplastic, absent, normal in size or even enlarged thyroid gland, in case of thyroid dyshormogenesis and the high TSH due to failure to switch off release by the anterior pituitary. If the treatment is started early, the clinical features (fig. 1) can be prevented and the incidence of mental retardation reduced (1, 2, 3).

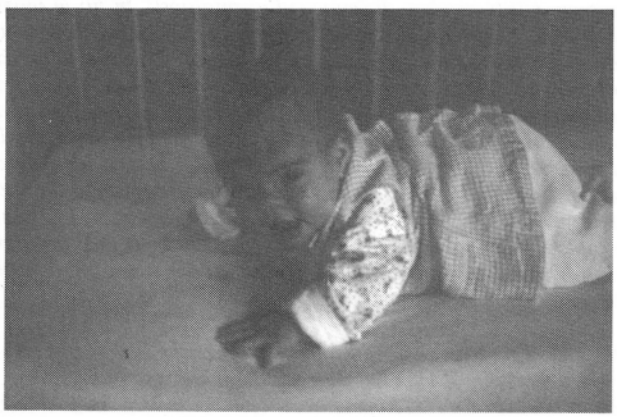

Fig. 1. *Two years old girl, physically and mentaly retarded due to congenital hypothyroidism. She could not sit, stand or talk. Typical facial appearance with scanty hair, hypertelorism, depressed broad nose, macroglossia and coarse voice. As an infant she was obstipated and had poor apetite.*

Sl. 1. *Dve leti stara deklica, duševno in telesno retardirana zaradi kongenitalne hipotireoze. Ne sedi, hodi in govori. Značilen izgled obraza z redkimi lasmi, hipertelorizmom, širokim nosnim korenom, makroglosijo in hripavim glasom. Kot dojenček je bila obstipirana in imela slab apetit.*

In most laboratories screening for CH was incorporated into existing programmes for phenylketonuria using dried blood spots collected from infants during the first week of life. Some centres measured only thyroxine, others TSH, while still others measured both hormones (4).

Material and methods

Newborn screening for CH in Slovenia has been performed by measurement of TSH in filter-paper blood spots by hellprick between the third and the fifth day. TSH was measured by RIA Pharmacia from August 1981 to April 1989. Sensitivity of the method was 10 mU/L. Later on TSH was determined by monoclonal antibodies – Delphia neonatal TSH (LKB Wallace). Sensitivity of this method was 5 mU/L.

While RIA method was used, 20 mU/L were taken as cut-off value. Since introduction of Delphia method, the cut off value changed to 15 mU/L. The values between 15 and 20 mU/L were rechecked on filter-paper. Children with values exceeding 20 mU/L were admitted to Department of paediatrics, Medical centre Ljubljana to measure serum values of T_4, T_3, TSH and to start therapy. L-thyroxin 100 mcg/m^2 was given, later on the dose was adjusted, so that the T_4 serum values were in the upper half of the normal range. Clinical examinations and blood monitoring for T_4, T_3 and TSH were done every two months during the first year, later on three to four times per year.

At the age of one year, evaluation of thyroid function was performed by replacement of L-thyroxine by triiod-thyroxine for two weeks followed by interruption of substitution therapy for one week. In addition to T_4, T_3 and TSH measurements, bone age determination and thyroid scanning with technetium 99 and ultrasonography were done. Clinical examinations including neurological and psychological assessments were also performed. Locomotor, oculomotor, hearing-speech, personal-social parameters were followed according to Brunnette-Lezine. In case of transient neonatal hypothyroidism the therapy was withdrawn. Detailed physical, psychomotor and mental development of all patients was followed every year. Since the age of three years, otolaryngologist and ophtalmologist were consulted and audiometry and visual acuity tests performed.

Results

Between August 1, 1981 and August 1, 1991, screening for CH was performed in 211,287 newborns. Recall rate was 0.05% CH. CH with TSH values exceeding 20 mU/L until 1989, and 15 mU/L later on, was diagnosed in 63 children. The incidence of CH in the ten-year period was 1:3,554. Three children with TSH values exceeding 50 mU/L died of respiratory dystress syndrome soon after birth, therefore, the definite evaluation of thyroid function was not possible. In 9 children at the age of one year, transient hypothyroidism was stated according to normal T_4, T_3, TSH values after withdrawal of thyroxine therapy and normal scan and ultrasono-

graphy of the thyroid gland. The incidence of the transient hypothyroidism in the period observed was 1:23,476. During the first two years, two infants with CH were missed due to human error during the screening process. Evaluating the thyroid function at the age of one year, persisting hypothyroidism was diagnosed in 51 children. The incidence of permanent CH in the ten-year period was 1:4,143. Fourteen (27.5%) had ectopic lingual or sublingual thyroid tissue, sixteen (31.4%) hypoplastic (n = 10) or aplastic (n = 6), while twenty one (41.1%) had normal or enlarged thyroid gland in situ. Four of these patients had siblings with hypothyroidism and normal or enlarged thyroid gland. Majority of these patients were suspected to suffer from thyroid dyshormogenesis while in one case elevated levels of thyroid antibodies indicated hypothyroidism of autoimmune origin. However, more precise assessment of thyroid function abnormality was not carried out in this group of patients. Results of hormone analysis at first admission to the hospital in different groups of patients with CH are presented on table 1.

Tab. 1. *Hormonal values in patients with congenital hypothyroidism at first admission to the hospital.*

Tab. 1. *Hormonske vrednosti pri bolnikih s kongenitalno hipotireozo pri prvem sprejemu v bolnišnico.*

Hormone / Hormon	Normal value in 1st month / Normalne vrednosti v 1. mesecu	Permanent hypothyroidism / Stalna hipotireoza				Transient hypothyroidism / Prehodna hipotireoza
		Ectopic gland / Ektopična žleza	Aplastic gland / Aplazija žleze	Hypoplastic gland / Hipoplazija žleze	Normal or enlarged gland / Normalna ali povečana žleza	
T_4 (nmol/L)	105.5–221.3					
$\bar{x}$		93.5	36.5	53.1	52.7	85.1
SD		54.2	9.9	35.2	43.6	35.4
T_3 (nmol/L)	1.6–3.7					
x		1.9	1.0	1.5	1.6	1.9
SD		0.8	0.2	0.5	1.0	0.6
TSH (mU/L)	0.5–6.5					
x		242.5	354.0	215.1	357.8	73.0
SD		219.2	181.9	199.8	219.5	60.0

The average age at which therapy started was 12.3 ± 4.9 days except for the two missed, for whom it started at the age of 4 and 5 months. In both cases the psychomotor development was retarded as well as in four other cases detected in first three weeks of life, in which besides CH, cerebral gigantism, Mb Down and cerebral palsy, in two cases due to perinatal hypoxia, were registered. In 47 other patients, psychomotor development was in normal range. However, 6 patients had delay in speech, while 5 of them had slight eye-hand dyscoordination, which was not registered any more when they entered school at the age of seven. Seven of the patients had sensineural hypacusis and 3 others conductive hearing loss, which was not clinically significant.

Discussion

The programme of screening for CH in Slovenia, which started in 1981, was based on results and experience of the University department of paediatrics in Zürich, Switzerland (5).

The incidence of CH 1:4,143 in the ten-year period was similar to our previous results in the 7-year period (6) and the incidence rate in other European countries and USA (7, 8), lower than in Scandinavian countries where it does not exceed 1:3,000 and higher than in Austria (9). The proportion of ectopic to dysplastic glands as the cause of CH is similar to that in most European countries while the percentage of thyroid dysfunction with normal or enlarged thyroid gland is surprisinghly high. Although in majority of cases dyshormonogenesis and autoimmune thyroid disorders were suspected to be the etiology of hypothyroidism, further investigations of these groups of patients will be necessary.

In Slovenia, the average age when CH was detected was 12.3 ± 4.9 days, which means it was considerably lower than before the screening of CH. In the period 1968–1982, the patients suffering from CH started to be treated at the mean age of 2.6 years and 60% were mentaly retarded (10).

In some centres, thyroid scanning with J 123 has been performed in newborns with elevated TSH levels detected by screening programme at first admission to the hospital, in order to establish the thyroid disorder as soon as possible. Due to technical reasons, we decided in our center to follow the alternative recommendation of the ESPE (European Society of Pediatric Endocrinology) and since 1992 we re-evaluate the thyroid function by determination of TSH and thyroid hormones in plasma after withdrawal of therapy for two weeks at two years of age, in order to minimise the danger of brain damage by thyroxin deprivation. Apart from the routine clinical follow-up of all patients with CH, we shall continue to perform detailed neurological, psychological, ophtalmological and audiometric exams at the age of 3 and 6 years before entering school, and also later on when necessary. The recent data on psychological follow-up of patients do not prove that the early treatment of CH is the only factor influencing the intelectual outcome (11), since social factors and severity of initial hypothyroidism may play a significant role (12, 13). In spite of that, it is worthwile to start the therapy of patients with CH as soon as possible. In our patients, spontaneous improvement of discrete disturbances of eye-hand coordination was observed before entry to school, while hearing loss, though clinically insignificant, has persisted and will be investigated during follow-up of patients.

Conclusion

The effectiveness of neonatal thyroid screening during the first ten years of application in Slovenia, as regards the prevention of mental retardation, calls for the screening programme to be continued, also for economic reasons.

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Review article/Pregledni prispevek

PREMATURE PUBARCHE: DIFFERENTIAL DIAGNOSIS, AUXOLOGICAL FEATURES AND CLINICAL OUTCOME

PREZGODNJA PUBARHA: DIFERENCIALNA DIAGNOZA, OKSOLOŠKE ZNAČILNOSTI IN KLINIČNI POTEK

Sergio Bernasconi, Lucia Ghizzoni, Alessandra Vottero, Maria Zampolli, Cecilia Volta, Tatiana Onesti, Mauro Costa, Raffaele Virdis

University of Parma, Department of Pediatrics, Via Gramsci 14, 43100 Parma, Italy

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Key words: *premature pubarche; nonclassical congenital adrenal hyperplasia; children; addolescents*

Ključne besede: *prezgodnja pubarha; neklasična kongenitalna adrenalna hiperplazija; otroci; mladostniki*

Abstract – Background. *When occuring before 8 years of age in girls and before 9 years in boys, pubarche is considered premature. The etiology and some clinical characteristics of the disorder are presented. Hypersecretion of a pituary factor stimulating secretion of adrenal androgens, increased sensitivity of the hair follicles to androgens, and premature development of the adrenal zona reticularis with increased activity of the 17.20-desmolase which catalyses the conversion of 17-hydroxyprogesteron to androstendione are presented in this article as possible etiological factors of the so-called idiopathic premature pubarche. Increased incidence of polycystic ovarian syndrome in girls with premature pubarche, and the possibility of adrenal as well as ovarian androgen hypersecretion due to gonadotropin dependent mechanisms are also mentioned. Premature pubarche may also be a sign of nonclassical adrenal hyperplasia due to 21-hydroxylase deficiency. Therefore, the hypersecretion of androstendione, and high frequency of 17-hydroxyprogesterone realease accompanied by relative nocturnal cortisol deficiency are examined as possible causes of precocious puberty.*

Transient acceleration of growth and bone maturation have no effect on the onset or progression of puberty, or final height in children with premature pubarche.

Conclusions. *Though premature pubarche is usually benign and considered a variant of normal development, careful follow-up of these patients is recommended.*

This is the only way for the cases of precocious puberty, and virilising tumors of adrenal or ovarian origin, in all of which premature pubarche is one of the first signs, to be detected and treated in time.

Izvleček – Izhodišča. *Pubarha je poraščenost v genitalnem predelu, ki se pojavlja kot sekundarni spolni znak zaradi delovanja suprarenalnih androgenov. Kolikor nastopi pri deklicah pred osmim letom in pri dečkih pred devetim letom, govorimo o prezgodnji pubarhi. Pogostejša je pri deklicah kot pri dečkih.*

V članku so prikazane nekatere klinične značilnosti otrok z idiopatsko prezgodnjo pubarho in domnevni etiološki faktorji. Med njimi je omenjena možnost hipersekrecije hipofiznega faktorja, ki stimulira izolirano adrenalne androgene, povečana občutljivost lasnih foliklov na androgene in prezgodnji razvoj cone retukularis skorje suprarenalke s povečano aktivnostjo encima 17,20-desmolaze, ki katalizira konverzijo 17-hidroksiprogesterona v moški spolni hormon androstendion. Omenjena je povečana incidenca sindroma policističnih ovarijev pri deklicah s prezgodnjo pubarho in možnost adrenalne pa tudi ovarialne hipersekrecije androgenov zaradi gonadotropinske disfunkcije. Prezgodnja pubarha je tudi klinični znak neklasične oblike kongenitalne adrenalne hiperplazije zaradi pomanjkanja encima 21-hidroksilaze. Pri teh pacientih je možni vzrok prezgodnje pubertete povečana sekrecija androstendiona oziroma povečane frekvence pri sproščanju 17-hidroksiprogesterona, ki jo spremlja relativno zmanjšanje izločanja kortizola v nočnem času.

Prehodno pospešena rast in dozorevanje kosti pri otrocih s prezgodnjo pubarho nima vpliva na začetek pubertete ter na končno telesno višino.

Zaključki. *Čeprav je prezgodnja pubarha benigno stanje in se smatra za varianto normalnega razvoja, je potrebno skrbno sledenje prizadetih otrok. Le tako je možno pravočasno odkriti in ukrepati v primerih, ko je prezgodnja pubarha eden prvih znakov prezgodnje pubertete ali virilizirajočih tumorjev suprarenalnega ali ovarialnega izvora.*

Introduction

The appearance of pubic hair is considered premature whenever it occurs before 8 years of age in females and before 9 in males. It is more frequent in females than in males, and mostly limited to the labia majora in girls. The clinical presentation may also include axillary hair and adult apocrine secretion. Once precocious puberty and virilizing adrenal or gonadal tumors (1) are excluded, differential diagnosis focuses on the idiopathic form of premature pubarche and on the one secondary to adrenal enzymatic defects.

Etiological, clinical and hormonal characteristics of premature pubarche

Idiopathic premature pubarche

The premature appearance of pubic hair in patients with the idiopathic form of the disease is usually accompanied by increased height velocity and slightly advanced bone maturation which is always well correlated with the height age. Testicular and breast size remain at the prepubertal level. Androgens reach levels

commonly occurring in Tanner stage II of pubertal development. Various theories have been proposed, in order to explain the physiological basis of premature pubarche. Some authors have suggested the presence of a specific factor which stimulates only the secretion of androgens and does not affect the synthesis of the glucocorticoid and mineralcorticoic from the zona fasciculata and glomerulosa respectively (2). Until now, however, no clear evidence has been provided to confirm the presence of such a specific factor. Another theory suggests a premature development of the zona reticularis, which would cause an increase in DHEA which would be converted into more powerful androgens resulting in the appearence of pubic hair (3). In line with this hypothesis are the results of a study we carried out with a group of patients affected by premature pubarche and with normal subjects who underwent corticotropin-releasing hormone (CRH) stimulation test (4). Children with premature pubarche showed adrenal steroid responses similar to those of prepubertal children who had already entered the adrenarchal phase. The evaluation of the different enzyme activities through the estimate of product to precursor ratio showed the same behaviour in both prepubertal children and children with premature pubarche. The study also stressed that the children of both groups had shown a decreased 17.20-desmolase activity compared to subjects at a more advanced stage of pubertal development, which means that postnatal maturation of the adrenal glands, and the development of the zona reticularis in particular, are consistent with an increased activity of the 17.20-desmolase which catalyzes the conversion of 17-hydroxyprogesterone (17OHP) into androstenedione (D4A). Therefore, the premature appearance of pubic hair may be either due to an increased sensitivity of the hair follicles to androgens or to a premature development of the zona reticularis.

The use of the ACTH stimulation test to rule out enzymatic defects in patients with premature pubarche is advisable in those children in whom premature pubarche is accompanied by advanced bone maturation, and/or baseline androgen values greater than those compatible with Tanner II, and/or clitoral enlargement, and/or cystic acne. In contrast, in children with isolated premature pubarche the ACTH stimulation test is not strictly necessary, provided that we can garantee a close follow-up of the patients. Idiopathic premature pubarche, in fact, can be considered a paraphysiological condition in which the pubertal development of gonadal function is not accelerated and final height does not seem to be affected by the adrenal hypersecretion. This has been recently demonstrated by the results of a study we carried out in collaboration with Ibanez et al. (5), which provides the first data on the natural history and auxological outcome in premature pubarche from two European populations (Spanish and Italian). The auxological features of 127 girls with premature pubarche, followed longitudinally for several years, were examined. Fourty two of them had menarche and 38 had already reached the final height. The ACTH test had excluded the presence of enzyme deficiencies in all of them. Advanced bone age and tall stature were constant features during the first years of follow-up. These abnormalities subsequently waned, leading to reduction in the chronological age to bone age ratio. The first pubertal signs appeared at age 9.7 ± 0.9 yrs. and menarche at 12 yrs. in accordance with the maternal and population data. Final heights were generally above midparental heights and correlated with height prognosis estimated at diagnosis. Based on these data, it can be concluded that premature pubarche produces a transient acceleration of growth and bone maturation with no negative effects on the onset or progression of puberty, or on final height. Despite the reassuring auxological data, a long-term follow-up of these patients is necessary to define the impact of premature pubarche on future ovarian function. A higher incidence of polycistic ovarian syndrome, in fact, has been reported for peripubertal and postpubertal girls with congenital adrenal hyperplasia, as well as for those with premature pubarche. Adrenal androgen hypersecretion has been suggested by some authors to be a cause of this disorder, although according to others, overproduction of androgens by gonadotropin-dependent mechanisms appears to be the initial event in most cases. The ovarian origin of the hyperandrogenism in postpubertal patients who had premature pubarche in prepubertal age has recently been confirmed by Ibanez et al. (6) in a study examining the postpubertal outcome of 35 such patients. Sixteen of them had hirsutism, oligomenorrhea, and high baseline testosterone/D4A. All patients, as well as a group of girls with regular menses and without hirsutism who served as controls, underwent ovarian stimulation by a LHRH analogue (Leuprolide). The 17OHP and D4A blood levels 24 hours after the stimulus were significantly elevated in all patients with oligomenorrhea, hirsutism, and high baseline testosteron and D4A concentrations. Thus, 45% of patients affected by premature pubarche in childhood showed an altered response to the leuprolide test revealing a functional ovarian hyperandrogenism. Although the high values of 17OHP in response to the stimulus are more frequent in the patients who had exhibited high baseline dehydroepiandrosterone sulfate (DHEA-S) and D4A levels at the time they were diagnosed with premature pubarche, a definite biological marker to predict which of the children with premature pubarche will develop functional ovarian hyperandrogenism is lacking. Therefore, a prolonged and careful follow-up (including clinical examinations and laboratory tests) of females with premature pubarche is necessary.

Premature pubarche secondary to nonclassical 21-hydroxylase deficiency (NCCAH)

The diagnosis of NCCAH is based upon the 17OHP response to ACTH test according to the normograms reported by New et al. The incidence of nonclassical 21-hydroxylase deficiency among children with premature pubarche is extremely variable, depending on the survey examined (7–18). It ranges from 30% reported by Temeck et al. (11) to 0% reported by Morris (12) et al. (Tab. 1). In our series, which includes 120 patients with premature pubarche, the incidence of the 21-hydroxylase defect accounts for approximately 7%, which is as result similar to those of other Italian groups. The outstanding variability of the incidence of adrenal enzymatic defects among children with premature pubarche is to be partially ascribed to the different frequency of the enzyme deficiency in the various populations studied. Askenazi Jews, Hispanics, and the population of former Yugoslavia have shown a disease frequency far greater than Italians (1/27 among Jews and 1/333 among Italians with 1/13 frequency of heterozygotes in the first group and 1/14 in other Caucasian groups) (19). Moreover, the difference in disease frequency is associated with a similarly great phenotypic heterogeneity. In fact, the clinical picture of the nonclassical forms of 21-hydroxylase deficiency can range from the complete absence of symptoms to severe signs of virilization, and comprise mild and isolated symptoms such as premature pubarche, short stature, irregular menstrual cycles, infertility, acne, and hirsutism. This phenotypic heterogeneity is, in turn, associated with a similar genotypic heterogeneity. The mutations so far described causing the nonclassical form of 21-hydroxylase deficiency are Val-281-Leu and Pro-30-Leu (20). The former mutation occurs in nearly all patients who carry the HLA haplotype B14;DR1 and results in an enzyme with 50% of normal activity when 17OHP is the substrate but only 20% of normal activity for progesterone. If we consider that heterozygous carriers of salt-wasting mutations are expected to have about 50% of normal 21-hydroxylase activity, but that they are asymptomatic, it derives that in vivo the enzyme activity in patients with nonclassic 21-hydroxylase deficiency must be less than 50% of normal. This paradox may be explained by the fact that progesterone acts as a powerful competitive inhibitor of the enzyme for its main substrate, 17OHP. It follows that small differences in intraadrenal progesterone concentration may cause the clinical variability that is characteristic of the disease. This inevitably affects peripheral steroid concentrations both upstream

and downhill of the enzyme blockade, as shown by a recent study we carried out in a group of children with premature pubarche secondary to nonclassical congenital adrenal hyperplasia (NCCAH) (21). Both patients and normal age-matched children underwent a 24-hour blood withdrawal according to the Kowarski method for determination of blood cortisol, 17OHP, and D4A levels. The 24-hour profiles analyzed by the Pulsar program showed, not only that a greater amount of 17OHP and D4A is secreted by the patients compared to normal children, but also that the pattern of adrenal steroid secretion is altered in these patients. Specifically, the latter is characterized by a high frequency 17OHP release accompanied by a relative nocturnal cortisol deficiency. Whether such abnormalities of steroid profiles might have practical clinical implications remains to be determined.

Tab. 1. *Premature pubarche due to disorders of steroidogenesis.*

Tab. 1. *Prezgodnja pubarha zaradi motenj v steroidogenezi.*

Authors Avtorji	Subjects Preiskovanci	21-OH deficit Pomanjkanje encima 21-hidroksilaze	3B-HSD deficit Pomanjkanje encima 3-beta hidroksisteroid dehidrogenaze	11B-OH deficit Pomanjkanje encima 11-beta hidroksilaze
August et al.	16 F, 2 M	2		
Rosenfield et al.	4 M	0	0	
Granoff et al.	10 F, 5 M	0		
Klaplowitz et al.	19 F, 2 M	0/3		
Temeck et al.	19 F, 4 M	7 (30%; 6 F, 1 M)	3 (13%; 2 F, 1 M)	
Morris et al.	28 F, 3 M	0	0	0
Rapaport et al.	30 F, 3 M	2 F (6%)	8 (24%; 6 F, 2 M)	
Oberfield et al.	32 F, 2 M	0	4 M, 4 F (12%)	0
Balducci et al.	39 F, 11 M	2 F, 2 M (8%)		
Vasconcelos et al.	19 F	4 F (21%)		
De Sanctis et al.	63 F	3 F (5%)		
Del Balzo et al.	21 F, 5 M	1 (4%)	1 (4%)	
Bernasconi et al. (unpublished) (neobjavljeno)	100 F, 20 M	8 F (7%)		

F = females (ženske)
M = males (moški)

Conclusion

Although isolated premature pubarche either idiopathic or secondary to steroid 21-hydroxylase deficiency is usually a benign condition, a long-term follow-up of these patients is recommended to detect signs and symptoms secondary to the adrenal or ovarian hyperandrogenism that may arise in adult life.

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Professional article/Strokovni prispevek

ULTRASONOGRAPHY IN DIAGNOSIS OF CENTRAL PRECOCIOUS PUBERTY

ULTRAZVOČNA DIAGNOSTIKA PRI OPREDELITVI CENTRALNE PREZGODNJE PUBERTETE

Giorgio Tonini[1], Stefano Marinoni[1], Mariangela Rustico[2], Paola Prampolini[2], Luisella Radillo[3]

[1] Pediatric Department, Children Hospital Burlo Garofolo, Trieste, Italy
[2] Obstetric and Gynecology, Children Hospital Burlo Garofolo, Trieste, Italy
[3] Biochemistry Laboratory, Children Hospital Burlo Garofolo, Trieste, Italy
Via dell'Istria 65/1, 34100 Trieste

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Key words: *precocious puberty; pelvic ultrasonography; gonadotrophins; luteising hormone releasing hormone; analogue*

Abstract – Background. *In children with true precocious puberty, the greatest problems are accelerated growth and bone maturation, short stature due to premature closure of epiphyseal cartillage, and psychological problems due to premature secondary sexual characteristics. The aim of the study was to estimate the value of pelvic ultrasonography in diagnosing precocious puberty, and in the follow-up of patients.*

Methods and results. *16 girls aged 1 to 8.5 years with appearance of pubertal signs before the age of 8, for whom the final diagnosis was true precocious puberty (10 cases), idiopathic precocious telarche (3 cases) and idiopathic precocious pubarche (3 cases), were studied. The ultrasonography of the uterus and gonads in different conditions of bladder refilling, based on the measure of 3 ovary diameters, with the final evaluation of the volume expressed in ml, were performed. The measure of the ovary volume was proven to be the best marker to diagnose a true precocious puberty, since this method showed gonadal increase at the first appearance of pubertal signs, before the changes in hormonal levels, characteristic of pubertal development, appeared (Tanner stage 2–3).*

Conclusion. *It was demonstrated that the pelvic ultrasonography is a good method for early detection of true precocious puberty. It is a practical non-invasive and very sensitive examination, which makes for early treatment of precocious puberty by suppressive therapy with LHRH analogues, and for follow-up of patients.*

Ključne besede: *prezgodnja puberteta; ultrasonografija pelvisa; gonadotropini; analogi luteinizirajoči hormon sproščujoči hormon*

Izvleček – Izhodišče. *Prava prezgodnja puberteta je posledica prezgodnje aktivacije hipotalamusa, pri deklicah pred 8. letom, pri čemer prekomerna pulzatilna sekrecija gonadotropinov poveča ovarialno izločanje estrogenov. Možna je tudi delna prezgodnja puberteta, prezgodnje pojavljanje žleznega tkiva v prsih – prezgodnje telarha, katere etiologija ni jasna. Izoliran prezgodnji pojav poraščenosti v genitalnem predelu – prezgodnje pubarha je posledica povečanega predčasnega izločanja adrenalnih androgenov. Prezgodnja puberteta ima za posledico pospešeno rast in kostno starost, ki jo spremlja predčasno zaprtje epifiznih špranj in posledična nizka rast. Pojavljajo se tudi psihološki problemi predvsem zaradi prehitrega pojava sekundarnih spolnih znakov oziroma različnosti od vrstnikov. S sintetskimi analogi gonadotropinskih pobudnih hormonov je možno zavreti pulzatilno izločanje gonadotropinov in estrogenov. Tako se lahko zaustavi napredovanje sekundarnih spolnih znakov ter dozorevanje kosti, kar vpliva na večjo končno višino od sicer pričakovane. Učinek je tem večji, čim bolj zgodaj se prične zdravljenje. Z analizami hormonov in kostne starosti ni možno opredeliti vedno začetka prave prezgodnje puberteta. Namen študije je bil ugotoviti, ali je možno z ultrazvočno preiskavo gonad in uterusa opredeliti pravo prezgodnjo puberteto, ki jo spremlja povečanje ovarijev, in ugotoviti učinkovitost supresijskega zdravljenja s sledenjem dimenzij gonad.*

Metode in rezultati. *V preiskovalni skupini je bilo 16 deklic, starih od 1 do 8,5 leta, pri katerih so se pojavili sekundarni spolni znaki v starosti pod 8 let. Pri 10 je bila ugotovljena prava prezgodnja puberteta, pri 3 prezgodnje telarha in pri 3 prezgodnje pubarha. Poleg hormonskih analiz in kostne starosti je bila izvedena ultrasonografija uterusa in gonad, ponovljena v različnih fazah praznjenja sečnega mehurja. Izmerjeni so bili longitudinalni (LD), sagitalni (SD) in transverzalni (TD) premer ovarija in izračunan volumen gonade v ml po formuli: 0,5233×LD×SD×TD. Sprememba volumna ovarijev, ugotovljena z navedeno meritvijo, se je izkazala kot najzanesljivejši kazalec začetne prave pubertete, saj se povečajo ovariji pred hormonskimi spremembami, značilnimi za začetni pubertetni razvoj (Tannerjev stadij 2–3).*

Zaključek. *Rezultati študije kažejo, da je pelvična ultrasonografija uporabna za opredelitev prave prezgodnje pubertete. Je praktična, neinvazivna, zelo občutljiva metoda, ki omogoča zgodnje supresijsko zdravljenje prezgodnje pubertete in sledenje učinkovitosti z analizami dinamike spreminjanja dimenzij gonad.*

Introduction

On deciding whether to treat precocious puberty, we must take two factors into account: first, psychological factors depending on the different-developing body shape of the patient, if compared with those of others of the same age; second, the possible bad prognosis for the final height (1–4).
At present the best treatment is use of long-acting luteinising hormone releasing hormone (LHRH) analogue (1, 2, 4, 5, 6, 7, 8), starting when sexual development has begun,(telarche and/or pubarche), and when a gonadotrophin releasing hormone (GnRH) test induces an increase in gonadotropins, especially in LH (2, 3, 5, 7, 9). The hormone levels are not always in pubertal range (3, 6) and the bone age can be quite variable, therefore the ultrasonography of the pelvis be lokely to give more accurate diagnosis of true pubertal precocity (TPP).
We report the preliminary result of an on-going study of patients presenting secondary sexual characteristics, in whom we also performed an ultrasonography of the pelvis.

Pattern description and methods of analysis

The study group consisted of 16 girls (age-range 1.5–8.9 yrs) who developed pubertal signs before the age of 8.
In 10 patients, central puberty (TPP) developed (age-range 1–8.5 yrs); 3 had an idiopathic premature thelarche (IPT) (age-range 1–8.6 yrs); and 3 an idiopathic premature pubarche (IPP) (age-range 6–7.8 yrs).
In all patients with TPP and in some with other pubertal precocity, the following tests were performed: plasma levels of 17-beta oestradiol (17BE2), prolactin (PRL), FSH and LH, basal levels and after i.v. administration of 100 mcg of LHRH.
The bone age was evaluated with the RUS TW2 Mk2 method.
The pelvic ultrasonography was always performed by the same operator with a 5 Mhz probe; the examination was done with a full bladder and with different states of decreasing urine volume until the bladder was completely empty. Three diameters were measured – longitudinal LD, sagittal SD, transversal TD, and the volume was calculated and expressed in ml. For the calculation of the ovary volume, the following formula was used: (0.5233 × LD × SG × TD), assuming the ovary shape to be ellipsoid (12, 13). In different conditions of bladder refilling, the ovary volume varied little, when confirmed the precision of the measure.
The examination of the uterus took into account the uterine corpus/cervix ratio (U Co/Ce R) and the hypoecogenic endometrial line (HEL).

Results

TPP

Table 1 shows hormonal levels, pubertal stages and ultrasonographic results in 10 patients with TPP. The pubertal stage range was 1–2 to 2–3 for pubic hair (PB) and from 1–2 to 3 for breast development (B). Plasma levels of 17BE2 varied, ranging from below 5 pg/ml to a maximum of 26 pg/ml. Higher values were obtained especially for the patients with advanced breast development; a level of 5.8 was found in the girls with stage B3. An increase of FSH and LH over the value of 10 mU/ml was present in 7 and 4 patients respectively, and the inversion of the FSH/LH ratio was observed only in 4 girls, who did not necessarily have breast development beyond the stage B2. It is well known that inversion is typical of the breast stage 2–3. The bone age, expressed in years (in comparison with the chronological age) ranging was advanced in all patients: from +1 to +4 years.

Tab. 1. *Age, pubertal stage, hormonal levels, bone age and pelvic ultrasonography results of patients with TPP.*

Tab. 1. *Starost, pubertetni stadij, hormonske vrednosti, kostna starost in rezultati pelvične ultrasonografije pri bolnikih s pravo prezgodnjo puberteto.*

Age (years) Starost (leta)		Pubertal stage Pubertetni stadij		Peak value Maksimalna vrednost pri stimulaciji				Pelvic ultrasonography Pelvična ultrasonografija			
		PH	B	17BE2 (pg/ml)	FSH (mU/ml)	LH	BA[1]	HEL[2]	U Co/Ce R[3]	ROV (vol. ml)	LOV[4]
1	6	1–2	2–3	26	4.8	1.3	+4	yes – da	1.2	1.4	3
2	8.5	2–3	2–3	10.3	13.2	4.3	+3.8	yes – da	1.1	3.4	2.2
3	6	1–2	2	< 5	11.1	18.8	+4	yes – da	1.3	2.3	2.2
4	8	2	2		8.2	1.3	+0.8	no – ne	1.3	6	3.9
6	8.5	2	1–2	< 5	13.1	2.9	+1.7	–	1	1.6	1.4
7	1.5	1–2	2	< 5	24.6	84.1	+2.1	no – ne	1.2	–	–
8	6.7	1–2	1–2		6	0.45	+1	no – ne	1.2	3.4	2.2
9	6	2–3	2	< 5	11.4	3.2	+1.5	no – ne	1.1	2.7	2.8
10	7.8	2	3	5.8	19	66	+1.9	yes – da	1.1	3.6	2.4

[1] BA: kostna starost
[2] HEL: hipoehogena endometrijska linija
[3] U Co/Ce R: razmerje med uterinim korpusom in cerviksom
[4] ROV: volumen desnega jajčnika
LOV: volumen levega jajčnika

The pelvic ultrasonography showed a constantly increased uterine U Co/Ce R in all patients but one; the right and left ovary volumes (ROV, LOV) were above 2 ml in 7 patients and 8 patients respectively. Patient 7 was also examined but it was impossible to determine the ovary volume, since there were technical problems due to the age; 1.5 years. It was repeated later, after the therapy had started, and the ovary volume exceeded 2 ml.
A hypoechogenic endometrial line, an expression of endometrial activation typical of the first stages of puberty, was present in only 5 subjects.

IPT and IPP

The hormonal levels in these two pubertal precocities (pseudoprecocious puberties) are frequently normal, so we decided not to perform hormonal tests for all patients. When assayed (table 2), 17BE2 was almost undetectable. One patient in each subgroup had a FSH value over 10 mU/ml, but none had a LH level over 10 ng/ml, nor an inversion of FSH/LH ratio. The bone age was only slightly increased except in one, an IPP, for whom it was +3 yrs. As regards the ultrasonographic evaluations, a hypoechogenic endometrial line was never present. The U Co/Ce R exceeded 1 in two girls, one with IPT and one with IPP; ROV and LOV were constantly below 2 ml, and below 1 ml in 4 patients. In patients IPP 1 and 3 the volume was similar to that of the patient TPP 6.

Tab. 2. *Age, pubertal stage, hormonal levels, bone age and pelvic ultrasonography results of the patients with IPT (a) and IPP (b).*

Tab. 2. *Starost, pubertetni stadij, hormonske vrednosti in pelvična ultrasonografija pri bolnikih z idiopatsko prezgodnjo telarbo (a) in idiopatsko prezgodnjo pubarbo (b).*

Age (years) Starost (leta)		Pubertal stage Pubertetni stadij		Peak value Maksimalna vrednost pri stimulaciji				Pelvic ultrasonography Pelvična ultrasonografija			
a) IPT		PH	B	17BE2 (pg/ml)	FSH (mU/ml)	LH	BA	HEL	U Co/Ce R	ROV (vol. ml)	LOV
1	8.3	1	1–2	< 5	15.2	3.4	–0,4	no – ne	1.1	0.8	0.8
2	8.6	1	1–2	< 5	–	–	+0.3	no – ne	1	0.3	0.46
3	1.1	1	1–2	–	–	–	–	no – ne	0.75	0.3	0.5
b) IPP											
1	6	2	1	< 5	25	2.8	+3	no – ne	–	1.8	1.2
2	6.3	2	1	< 5	13.6	3.2	+0.25	no – ne	< 1	0.8	0.6
3	7.8	2	1	< 5	8.4	1.6	–	no – ne	1.2	1.5	1.7

Tab. 3. *Effect of long-acting LHRH analogue on ovary volume and Uterine Body/Neck Ratio in four TPP patients.*

Tab. 3. *Učinek dolgo delujočega LHRH analoga na volumen ovarijev in na razmerje med korpusom in vratom uterusa pri štirih bolnicah s pravo prezgodnjo puberteto.*

Patients Bolniki	ROV and/or LOV reduction Zmanjšanje volumna desnega in/ali levega ovarija	U Co/Ce R reduction Zmanjšanje razmerja med maternicnim korpusom in vratom
3	yes – da	no – ne
4	yes – da	yes – da
5	yes – da	no – ne
7	yes – da	yes – da

The effect of LHRH analogue on U Co/Ce R and ovaries' volume in four TPP patients is presented on table 3, while comparison of hormonal and ultrasonographic results in three different pubertal precocities are shown on table 4.

Discussion

Many authors report a great variation in the hormonal levels in patients presenting precocious signs of pubertal development, such as breasts, pubic hair or both (3, 6). The appearance of the symptoms does not always follow the typical chronology: first thelarche, followed by pubarche (10, 11). Therefore a follow-up is necessary, before starting blood examinations and, if necessary, therapy. Often when the ultrasonography of the pelvis is performed, only two diameters are measured (5, 12), so that it is impossible to determine the precise volume of the gonads. In different conditions of urine content, the bladder compresses the ovaries against the posterior abdominal wall, so that the gonadal parameters are not always constant, and the shape of the ovaries can change, which makes it difficult to evaluate their width and length.

Although one of the aims of the treatment of the TPP is good statural prognosis (8), it must be opportune to start the suppression of the hypothalamic-hypophisal-gonadal axis earlier, in contrast to current habits, avoiding the risk of a very advanced bone maturation, after a too long follow-up of the pubertal development.

In table 4, we report the comparison of the most significant data among the three groups studied.

Bone age is a good index of hormonal secretion, but depends on both the estrogen and androgen secretion, and could be advanced also in IPP (15–17). The HEL, one of the more precocious sonographic signs at the beginning of pubertal development, was detectable only in TPP patients, but was is not always present.

The U Co/Ce R is a good marker of TPP, since it exceeds the value 1 in 9/10 patients, however, it was increased in 1/3 IPT and in 1/3 IPP as well. Moreover, during therapy with LHRH analogue, it did not decrease in some of the treated subjects (Tab. 4). Our findings are not always in accordance with other authors, who consider the modification of uterus shape and size a very good marker of pubertal development (5, 12, 14). However, Salardi et al. demonstrate in an important study of a very large number of cases that the modification, but not necessarily the inversion of U Co/Ce R, occurs also before the development of the first pubertal signs (15).

The volume of at least one ovary had increased to over 2 ml in 8/9 TPP, and in one case was is over 1.5 ml. The IPT and the IPP always have volumes bellow 2 ml. During suppressive therapy there was reduction of the ovary volume in all patients (table 4). Stanhope and other authors also find a significant increase of the ovary volume, above the value of 2 ml in patients with precocious puberty. The ovary volume was higher in their cases, but the patients had a more advanced pubertal stage in comparison with our subjects (16). Tatò et al. consider the measure of the volume a good marker, better than the measure of only two diameters (6). There is complete agreement among all the authors, when the ovary measure is used to monitor the effect of suppressive therapy (5, 6, 12, 16).

Tab. 4. *Comparison of hormonal and ultrasonographyc results in patients with three different pubertal precocities (TPP, IPT, IPP), before (a) and after the supressive therapy (b) with LHRH analogue was started.*

Tab. 4. *Primerjava hormonskih in ultrazvočnih rezultatov pri bolnikih s prezgodnjo pravo puberteto, prezgodnjo telarbo in prezgodnjo pubarbo pred (a) in po uvedbi (b) supresijske terapije z LHRH analogi.*

a)	TPP Prava prezgodnja puberteta	IPT Idiopatska prezgodnja telarha	IPP Idiopatska prezgodnja pubarha
17BEZ2 (increase) (povečan)	3/10	0/3	0/3
LH > FSH	4/10	0/3	0/3
PRL > 10 ng/ml	4/10	0/3	0/3
BA > 1a Kostna starost	9/10	0/3	1/3
HEL (detectable) Pozitivne hipoehogene endometrijske linije	5/9	0/3	0/3
U Co/Ce R > 1 Razmerje med maternicnim korpusom in vratom	9/10	1/3	1/2
ROV > 2 ml 7/9 Volumen desnega ovarija	8/9	0/3	0/3
LOV > 2 ml 8/9 Volumen levega ovarija			
b)			
U Co/Ce R reduction Zmanjšanje razmerja med korpusom in cerviksom uterusa	2/4		
ROV and/or LOV reduction Zmanjšanje volumna desnega in/ali levega ovarija	4/4		

Conclusions

Our preliminary data seem to demonstrate that pelvic sonography is a very good tool for early diagnosis of a TPP, especially when compared with other markers of pubertal development (auxologic and hormonal).

The measure of three diameters, and then of the volume of the ovaries, seems to be more precise and less variable, especially since they take into consideration the different conditions of the bladder during the examination, than the methods which use only two measures.

Rapid variation of volume at the appearance of first pubertal signs becomes evident, soon after the starting of therapy, and often before Gn suppression, suggesting that this practical, non-invasive and sensitive examination is very promising and indication that be started suppressive therapy. Moreover, it is very useful for the follow-up of the effects of the drug during suppressive treatment, in place of hormonal assay, which is more expensive and more invasive.

However, in order to decide exactly when to start LHRH analogue therapy, a larger experience with a greater number of cases is necessary.

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Professional article/Strokovni prispevek

TREATMENT OF CHILDREN WITH CENTRAL PRECOCIOUS PUBERTY BY A SLOW-RELEASE GONADOTROPIN-RELEASING HORMONE AGONIST

ZDRAVLJENJE OTROK S CENTRALNO PREZGODNJO PUBERTETO Z AGONISTOM GONADOTROPINSKIH POBUDNIH HORMONOV

Magda Kadrnka-Lovrenčić[1], Gordana Stipančić[1], Lada Beara-Lasić[1], Dubravka Vignjević[2], Marijan Lovrenčić[3]

[1] Department of pediatrics. "Sestre milosrdnice" Clinical hospital Zagreb, Vinogradska, 41000 Zagreb, Hrvatska
[2] Department of gynecology and obstetrics. "Sestre milosrdnice" Clinical hospital, Zagreb, Vinogradska, 41000 Zagreb, Hrvatska
[3] Department of radiology. "Sestre milosrdnice" Clinical hospital, Zagreb, Vinogradska, 41000 Zagreb, Hrvatska

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Key words: *precocious puberty; growth; gonadotropin-releasing hormone agonist Decapeptyl CR*

Abstract – Background. *The aim of therapy of precocious puberty is to stop the progression of puberty in order to prevent the short final stature and the associated psychosocial problems. In the present study the value of slow-release analogue of gonadotropin-releasing hormone (LHRH), Decapeptyl CR, which can induce a selective and reversible suppression of the pituitary-gonadal axis and thus progression of puberty, was evaluated.*

Methods and results. *Seven girls aged from 4.75 to 9.21 years with central precocious puberty were treated for 6 to 24 months with a slow-release gonadotropin-releasing agonist Decapeptyl CR every 4 weeks intramuscularly in a dose of 60–80 μg/kg. During treatment, the bone maturation slowed, height velocity decreased and predicted adult height improved in all cases except one. In another patient with both growth hormone deficiency and central precocious puberty treated with LHRH agonist and growth hormone, bone maturation was normalised and predicted adult height significantly increased. A rapid decrease in breast enlargement and a halt in pubic hair development were observed. Side effects of Decapeptyl CR were minimal.*

Conclusions. *Treatment with Decapeptyl CR was very effective in the long-term suppression of gonadal activity in children with central precocious puberty. Secondary sex characteristics showed no progression during treatment, and predicted adult height increased in most of patients.*

Ključne besede: *prezgodnja puberteta; rast; agonist gonadotropinskih pobudnih hormonov Decapeptyl CR*

Izvleček – Izhodišča. *Cilj zdravljenja prezgodnje pubertete je zaustaviti napredovanje pubertetnega razvoja in tako preprečiti psihosocialne probleme, ki spremljajo prezgoden spolni razvoj ter relativno majhno končno višino. V prispevku je predstavljena klinična učinkovitost agonista gonadotropinskih pobudnih hormonov, ki omogoča selektivno in reverzibilno supresijo hipofizno-gonadne osi in tako zaustavlja pospešen spolni razvoj.*

Metode in rezultati. *7 deklic s centralno prezgodnjo puberteto, starih 4,75 do 9,21 leta, je bilo zdravljenih 6 do 24 mesecev s počasi delujočim agonistom gonadotropinskih pobudnih hormonov Decapeptylom CR v dozi 60–80 μg/kg vsakih 4 tedne intramuskularno. Med zdravljenjem se je kostno dozorevanje upočasnilo, hitrost rasti zmanjšala in predvidena končna višina povečala v vseh primerih razen enem. Pri bolnici s pomanjkanjem rastnega hormona in centralno prezgodnjo puberteto, ki je bila zdravljena z rastnim hormonom in Decapeptylom CR, se je kostna starost normalizirala in predvidena končna višina pomembno povečala. Pri vseh deklicah se je velikost žleznega tkiva v predelu prsi naglo zmanjšala, pubična poraščenost pa ni napredovala. Stranski učinki zdravljenja so bili minimalni.*

Zaključki. *Uporaba Decapeptyla CR se je izkazala kot zelo učinkovita pri supresiji aktivnosti spolnih žlez otrok s centralno prezgodnjo puberteto. Med zdravljenjem sekundarni spolni znaki niso napredovali in predvidena končna višina se je zvečala pri večini bolnic.*

Introduction

The goal of therapy for children with precocious puberty (PP) is to stop the progression of puberty in order to avoid associated psychosocial problems and to improve the final height. Slow-release analogues of gonadotropin-releasing hormone (LHRH) can induce a selective and reversible suppression of the pituitary-gonadal axis. This treatment has become the therapy of choice for central precocious puberty (CPP) in children (1–5). The treatment has been effective in suppressing clinical and biochemical features of CPP (6).

It has been shown to be effective in decacelerating skeletal maturation. Growth velocity reverts to prepubertal levels.

It is speculated that the final height would improve but up to now there are no sufficient data available. Continued longitudinal studies of final adult stature in CPP patients treated with LHRH agonist are required to characterize the full impact of pituitary-gonadal suppression on growth.

Tab. 1. *Clinical data of patients at the beginning of treatment.*

Tab. 1. *Klinični podatki bolnikov na začetku zdravljenja.*

Patient Bolnik	Age Starost	Height Višina		Bone age Kostna starost		TH	PAH	B	P	Etiology Etiologija
	yr / leta	cm	SD	yr / leta	SD	cm	cm			
B. M.	6.73	129	+2.0	11.5	+6.7	157	144.8	3	2	Cystis arachnoidalis
B. D.	8.33	136	+1.5	11	+3.9	156.5	154	3	2	Idiopathic – Idiopatična CPP
L. M.	8.45	123	+1.2	11.5	+3.9	170.5	151.5	4	2	Hydrocephalus
Z. S.	6.49	139.7	+4.0	9	+3.7	164.1	176.8	3	2	Idiopathic – Idiopatična CPP
M. A.	6.26	134.1	+3.6	12	+7.4	162	158.7	2	2	Idiopathic – Idiopatična CPP
V. An.	9.21	125.5	–1.0	11.5	+3.4	164	140.9	3	2	Glioma piloides
V. A.	4.75	113.5	+1.7	7	+2.9	159	159.4	4	1	Glioma piloides

TH – Target height Ciljna višina PAH – Predicted adult height Predvidena končna višina B – Breast stage Stadij razvoja prsi P – Pubic hair stage Stadij razvoja pubične poraščenosti CPP – Central precocious puberty Centralna prezgodnja puberteta

Tab. 2. *Auxological data during 24-month treatment with Decapeptyl CR for 7 girls.*

Tab. 2. *Podatki o rasti 7 deklic med 24-mesečnim zdravljenjem z Decapeptylom CR.*

Patient Bolnik	At the beginning Začetek				After 6 month Po 6 mesecih			After 12 month Po 12 mesecih			After 18 month Po 18 mesecih			After 24 month Po 24 mesecih		
	TH cm	PAH cm	BA/CA	HV cm/y	PAH cm	BA/CA	HV cm/y	PAH cm	BA/CA	HV cm/y	PAH cm	BA/CA	HV cm/y	PAH cm	BA/CA	HV cm/y
B. M.	157	144.8	1.7	15	151.5	1.6	13	154	1.4	6	156.3	1.5	4.8			
B. D.	156.5	154	1.3	8	156.9	1.2	5	156.5	1.2	5	155.4	1.2	5	153.1	1.2	3
L. M.	170.5	151.5	1.3	1	151.6	1.3	0.8	147.5	1.3	2.4	145.7	1.3	1.6			
Z. S.	164.1	176.8	1.3	11	171.4	1.4	5.6	161.8	1.4	3						
M. A.	162	148.7	1.9	9	151.3	1.7	6									
V. An.	164	140.9	1.2	3	143	1.2	3	144.6	1.1	5	146.8	1.1	8	148.7	1.0	6
V. A.	159	159.4	1.4	7	161.3	1.4	7	160.4	1.3	3	157.7	1.3	5	158.3	1.2	5

TH – Target height Ciljna višina PAH – Predicted adult height Predvidena končna višina BA – Bone age Kostna starost CA – Chronological age Kronološka starost HV – Height velocity Hitrost rasti y – year leto

The LHRH analogues are molecules with amino-acid substitutions of the naturally occurring decapeptide, which were selected because they prolong its half-life. The basis for this treatment is the interference with the episodic secretion of gonadotropins in response to a sudden burst of LHRH, resulting in stimulation of LH and FSH release. The initial response to this stimulation may be gonadotropin release (7). Long-term administration of these agonists leads to pituitary desensitization, and to total refractoriness to further stimulation by natural LHRH. Six agonist analogues have been developed and tested in children with CPP since 1980s. We would like to present our experience with the slow-release LHRH agonist triptorelin [D-Trp-LHRH – Decapeptil controlled release 3.75 (D-CR)] in a group of 7 girls with CPP treated for a period of 6 to 24 months.

Patients and methods

Patients

Seven girls with CPP were treated with D-CR every 4 weeks intramuscularly (60–80 µg/kg). Clinical features at the beginning of treatment are given in table 1.

The diagnosis of CPP was based on visible sexual maturation prior to 8 years of age, advanced bone maturation and pubertal pattern of gonadotropin response to an exogenous bolus of 100 µg synthetic LHRH (gonadorelin hydrochloride) with LH and FSH measured at 0, 10, 20, 30, 60 and 120 minutes (8). Peripheral precocious puberty was excluded by an ovarian ultrasound.

Calculation of the triptorelin dosage was based on the clinical impression of suppression of puberty and on the suppression of the luteinizing hormone response after LHRH administration. LHRH test was performed at the beginning of treatment, 1 week, 1 month and every 6 months after the beginning of treatment.

One of the girls had previously received treatment with a short acting LHRH agonist (Suprefact) for a period of 36 months.

Methods

Height and pubertal stage were assessed every month by the method of Tanner. Height was measured using a wallmounted Harpenden Stadiometer.

X-rays of the left hand were taken every 6 months and the bone age was determined by one observer according to the method of Greulich and Pyle (9). Height percentile and SD score, target height (TH) and predicted adult height (PAH) were calculated on KABI VITRUM Growth Computer Mannes (10).

Results

Sex characteristics

Treatment with LHRH agonist suppressed basal and peak LHRH-stimulated gonadotropin level within the first week. The level of gonadotropins remained suppressed throughout long-term LHRH-agonist therapy.

We observed a rapid decrease in breast enlargement and a stop in pubic hair development. Menses ceased in M. A. postmenarchal girl 2 weeks after the first injection.

Auxological characteristics

Our patients were treated with LHRH-agonists in the period of 6 to 12 months. The patient L. A. was, prior to the treatment with Decapeptyl, treated with Suprefact for 36 months. The patient V. An. developed, apart from CPP, also growth hormone deficiency and hypothyreosis due to TRH deficiency. We started the levothyroxine substitution eleven months before the treatment with Decapeptyl CR and 4 months after Decapeptyl growth hormone substitution was initiated (fig. 1).

Auxological data during the treatment are given in the table 2.

One of the goals of the PP treatment is to improve the final height. Every six months we calculated the adult height prediction. In all

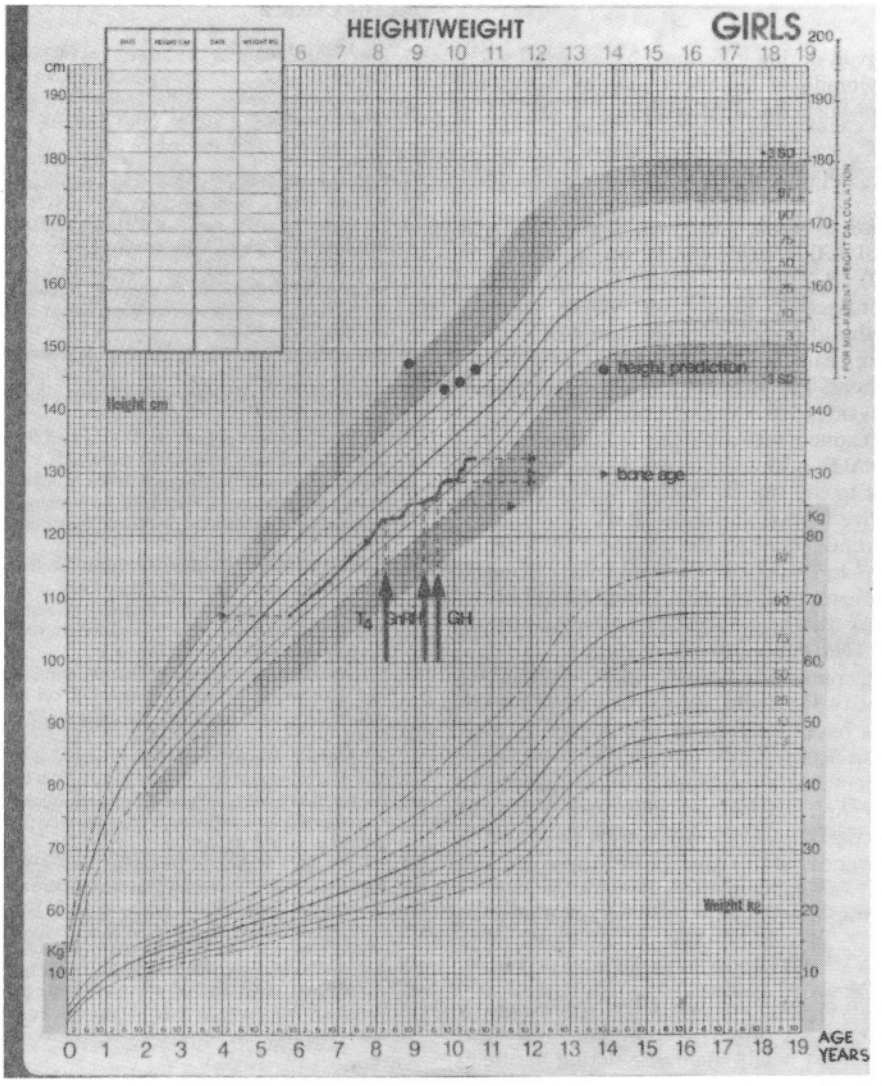

Fig. 1. *Growth chart of patient V. A.*

Sl. 1. *Krivulja rasti bolnice V. A.*

of our patients, a tendency of improvement in adult height prediction was shown, except in patient L. M.

As a measure of bone maturation we used the ratio of bone age to the chronological age (BA/CA). During treatment there was a decrease in BA/CA ratio (fig. 2).

There was also an obvious decrease in height velocity during the first 6 months of therapy in most of our patients (fig. 3).

LHRH agonists proved to be useful in combination with growth hormone in the treatment of patient V. An. who, apart from CPP, also had growth hormone deficiency.

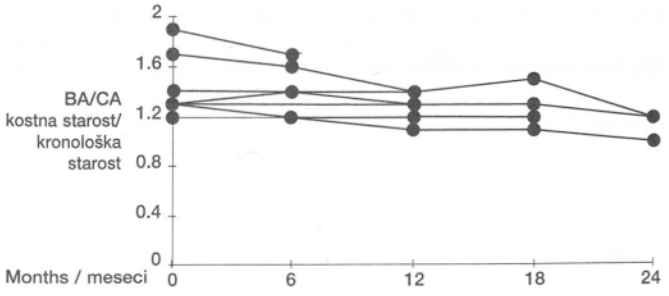

Fig. 2. *Bone age/chronological age (BA/CA) ratio in girls during treatment with Decapeptyl CR.*

Sl. 2. *Razmerje kostne in kronološke starosti deklic med zdravljenjem z Decapeptylom CR.*

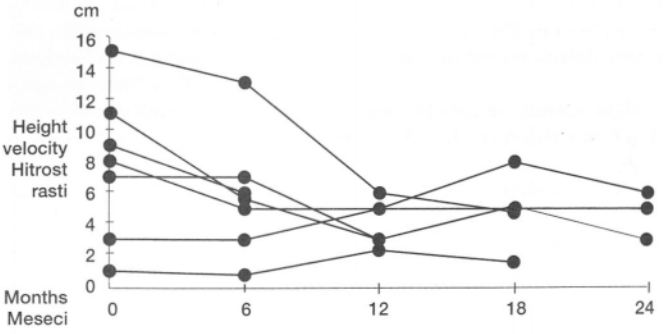

Fig. 3. *Height velocity in girls during treatment with Decapeptyl CR.*

Sl. 3. *Hitrost rasti deklic med zdravljenjem z Decapeptylom CR.*

Side effects

All girls complained of pain during injections, but showed good compliance. At the beginning of treatment, one of them had recurrent episodes of hot flushes and moodiness.

Discussion

Superactive LHRH agonists, synthetic analogues of the amino acid sequence of the natural LHRH decapeptide, are preferred in treatment of true PP (12).

Sexual steroid level returned to prepubertal values and the response to LHRH was almost completely suppressed during the treatment. Secondary sex characteristics showed no progression during prolonged treatment. The successful LHRH-agonist treatment reduced emotional disorders.

One of the goals of the treatment is to attain normal mature height. Predicted adult height (PAH), calculated on height measurements and bone age is difficult to estimate in children with precocious puberty (11). The effective therapy improved PAH.

So far only two studies have reported the achieved final height after treatment with LHRH agonist (13, 14). In seven out of 8 treated girls, final height was greater than predicted adult height at the beginning of therapy (13). The accumulating data on final height in children treated with LHRH agonists are needed.

In our study, only L. M., previously treated with a short-acting LHRH agonist for a period of 36 months, showed a decline in PAH. However, other authors have not noticed any decline in PAH during therapy with short-acting LHRH agonists.

We excluded GH deficiency as a cause of abnormal growth of patient L. M. In patient V. An., who has not only CPP but also GH deficiency, there has been a normalization of BA/CA ratio, and an increase in PAH. Effectiveness of this kind of treatment has also been reported in a study of Cara et al. (16).

Conclusion

Treatment with triptorelin in our study was effective. Secondary sex characteristics showed no progression during prolonged treatment and predicted adult height increased during the treatment in most of our patients.

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Professional article/Strokovni prispevek

ENDOCRINOPATHIES IN PATIENTS AFTER TREATMENT FOR HODGKIN'S DISEASE AND SARCOMAS IN CHILDHOOD

ENDOKRINE MOTNJE KOT POSLEDICA ZDRAVLJENJA HODGKINOVE BOLEZNI IN SARKOMOV V OTROŠTVU

Ciril Kržišnik[1], Berta Jereb[2], Nina Bratanič[1], Gabrijela Petrič-Grabnar[2], Tadej Battelino[1], Mojca Žerjav-Tanšek[1], Jožica Anžič[1]

[1]University Department of Paediatrics, Vrazov trg 1, 61105 Ljubljana, Slovenia
[2]Institute of Oncology, Zaloška 2, 61105 Ljubljana, Slovenia

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Key words: *children; cancer; therapy; endocrionopathies*

Abstract – Background. *In patients treated for Hodgkin's disease (HD) and soft tissue and bone sarcomas (SA) in childhood by surgery, chemotherapy and radiotherapy late sequelae including endocrine dysfunction are common.*

Patients and methods. *Standard endocrionological tests were used to determine endocrine function in 23 patients, 14 (4 females, 10 males) treated for HD and 9 (2 females, 7 males) for SA. In this group, 8 patients were suffering from sarcoma of the soft tissue and one from osteogenic sarcoma. The mean age at diagnosis for the patients with HD was 10.4 years (range 5.0 to 14.0), while for the patients with SA it was 9.5 years (range 5.0 to 13.2).*
The mean age at the time of the follow-up examination for the patients with HD was 22.2 years (range 17.3 to 30) and 18.8 years (range 16 to 22) for the patients with SA.
The evaluation was performed at least 5 years after the treatment, the mean interval being 11.7 years (range 5–21) for the patients with HD, and 9.3 years (range 6–12) for the patients with SA.

Results. *Endocrine dysfunction was found in over 80% of all treated, and it was clinically evident only in 26%. We detected a high prevalence of hypothalamo-pituitary-gonadal dysfunction due to gonadal lesion comprising 11 (78.6%) patients with HD and 7 (77.8%) patients with SA.*
Thyroid dysfunction was found in 7 (30.4%) patients examined, primary latent hypothyroidism in 3 patients with HD and in 2 with SA, secondary hypothyroidism in one patient with HD and in one with SA.
There were 2 cases of adrenal dysfunction, one in a patient with HD and one in a patient with SA.
Pituitary dwarfism with diminished levels of growth hormone and IGF I was found in one patient treated for SA.

Conclusion. *According to the results of our study, for the patients for HD and SA in childhood, the function of the hypothalamo-pituitary-gonadal axis and of the thyroid gland should be assessed at frequent intervals in order to detect endocrionological abnormalities and to introduce a replacement therapy when necessary.*

Ključne besede: *otroci; maligna obolenja; zdravljenje; endokrine motnje*

Izvleček – Izhodišča. *Zdravljenje Hodgkinove bolezni (HB) in sarkomov (SA) v otroštvu s kombinacijo operativnega zdravljenja, kemoterapije in obsevanja ima za posledico okvaro številnih parenhimatoznih organov in tudi endokrinih žlez.*

Bolniki in metode. *Predstavljeni so rezultati endokrinološkega testiranja 23 bolnikov, 14 (4 ženskega spola (Ž), 10 moškega spola (M) s HB in 9 (2 Ž, 7M) s SA.*
Bolniki s HB so bili ob diagnozi stari 10,4 let (razpon 5,0 do 14,0), bolniki s SA pa 9,5 let (razpon 5,0 do 13,2).
Bolniki s HB so bili ob testiranju stari povprečno 22,2 let (razpon 17,3 do 30), bolniki s SA 18,8 let (razpon 16 do 22).
Interval med koncem zdravljenja in testiranjem je bil daljši od 5 let, pri HB povprečno 11 let (razpon 5 do 21), pri bolnikih s SA 9,3 let (razpon 6 do 12).
Za oceno smo uporabili standardne teste za testiranje posameznih endokrinih žlez in hipotalamo-hipofizne osi.

Rezultati. *Endokrina disfunkcija je bila prisotna pri več kot 80% bolnikov, klinično manifestna pri 26%.*
Ugotovili smo visoko prevalenco hipotalamo-hipofizno-gonadne disfunkcije zaradi okvare gonad in sicer pri 78,6% bolnikov s HB in 77,8% bolnikov s SA.
Motnje v delovanju ščitnice smo ugotovili pri 7 (30,4%) bolnikih, primarno latentno hipotireozo pri 3 bolnikih s HB in 2 bolnikih s SA, sekundarno hipotireozo pri enem bolniku s HB in enem s SA.
Disfunckija nadledvičnih žlez je bila prisotna pri 2 bolnikih, enem s HB in enem s SA.
Pri enem bolniku s SA smo ugotovili hipofizno nanosomijo z nizkimi vrednostmi rastnega hormona in rastnega faktorja IGF I.

Zaključki. *Rezultati naše študije kažejo, da so pri bolnikih, zdravljenih zaradi HB in SA v otroštvu, potrebne pogoste analize hipotalamo-hipofizno-gonadne osi in ščitnice zaradi zgodnje ugotovitve možne endokrine disfunkcije in uvedbe nadomestnega zdravljenja, v kolikor je to potrebno.*

Introduction

The combination surgery, chemotherapy and radiotherapy has greatly improved the long-term survival of children with Hodgkin's disease (HD) and sarcoma (SA), but the intensive therapies can lead to different late sequelae including endocrine dysfunctions (1–5).
The aim of our study was to determine endocrine function in our patients who were treated for HD and SA in childhood.

Patients and methods

From 1986 to 1991, 23 patients treated in childhood were studied. There were 14 patients with HD (4 females, 10 males) and 9 with SA, 8 of the soft tissue and one osteogenic (2 females, 7 males). The selection was based on patient's consent, age over 15 years at the time of the follow-up (f/u) examination and the follow-up period exceeding 5 years.
The mean age at diagnosis was 10.4 years (range 5.0–14.0) for the patients with HD, and 9.5 (range 5.0–13.2) for the patients with SA. At the time of f/u the mean age of the patients with HD was 22.2 years (range from 17.3 to 30.0), while that of the patients with SA was 18.8 years (range from 16.0 to 22.0). The post-treatment evaluation for the HD group was performed at the age of 11.7 (range 5.0–21.0); and for the SA patients at 9.3 years (6.0–12.0).
Most patients with HD were treated with 6 cycles of MOPP. In addition, radiation therapy (RT) ranging from 30 to 40 Gy was applied to one of several areas involved, depending on the extent of the HD. One patient treated with the combination of MOPP–ABVD received only 3 cycles RT, and no radiation therapy. Two patients had no chemotherapy.
The patients with SA were treated with surgery, chemotherapy and radiation. Chemotherapy for most of the patients included cyclophosphamide, actinomycin D, vincristine D, vincristine (VAC) +/– adriamycin following the so-called T-2 or T-6 or T-10 protocols of Memorial Hospital, described by Memorial – Sloan Kettering Cancer Center, New York City investigators (6–8). Bleomycin and methotrexate were added in some cases. The doses of radiation varied from 30 to 60 Gy (Tab. 1).
All patients underwent general physical examination. Height, weight and clinical abnormalities were recorded, as well as Tanner stages of pubic hair and genital development, and the onset and the course of puberty.

Endocrine assessment

Thyroid status was assessed by measurement of the serum concentration of T4, T3 (RIA method, SPAC, Mallincrot) and thyroid stimulated hormone (TSH) (DELFIA–LKB) in basal state and 30 minutes after stimulation with 100 mcg of thyrotropin releasing hormone (TRH) i. v. At the same time, basal and stimulated levels of prolactin (DELFIA–LKB) were analysed.
Stimulation test for evaluation of growth hormone (RIA–GH, Pharmacia) consisted of L–DOPA (250 mg orally if body weight was <30 kg, 500 mg when >30 kg) and propranolol (0.75 mg per kg, not exceeding 40 mg). Growth hormone (GH) was measured 60 and 90 minutes after the onset of sleep; deficiency was defined as a GH level <10 mcg/l after stumulation. In some patients only IGF I values were determined by the IRMA–IGF–I, DSL method. Testosterone (RIA, IMUNOTECH) and estradiol (DELFIA–LKB) levels were measured in the basal state. Concentrations of LH and FSH (DELFIA–LKB) were determined before and 10, 20, 30, 60 minutes after i. v. administration of 100 mcg gonadotropin releasing hormone (GnRH). Examination of sperm for azoospermia was done on 2 patients upon their own wish.
Basal and stimulated levels of cortisol in serum (AMERLITE METHOD) 60 minutes after i. v. application of 250 mcg of synthetic ACTH were determined between 7 and 9 a. m.

Results

The abnormalities found at physical examination in the course of puberty and the results of endocrine evaluation are presented in tables 1 and 2.
Growth hormone: GH was evaluated in 17 patients. Subnormal stimulated levels (<10 mcg/l) but normal IGF I values were found in 6 patients (Nos. 5, 7, 9, 10, 15, 19) (HD: n=4, SA: n=2). Pituitary dwarfism with diminished levels of GH and IGF I was found in one patient (No. 18), while for 6 others only IGF I values were determined, which were all in normal range (Nos. 11, 12, 13, 14, 17, 23).
Thyroid: Thyroid dysfunctions were found in 7 patients. Though T4 and T3 levels were normal (some of them close to the low normal range), primary latent hypothyroidism with either normal or elevated basal TSH levels and hyperresponsiveness to TRH was found in 5 patients (Nos. 2, 7, 13, 16, 19) (HD: n=3, SA: n=2). Decreased basal and stimulated TSH levels were found in two patients (Nos. 6, 20) (HD: n=1, SA n=1). Of the 7 patients with HD who had irradiation of the neck with incidental exposure of the thyroid, 3 (Nos. 13, 16, 19) had thyroid dysfunction. Of the 4 patients (Nos. 16, 17, 18, 19) with SA who had irradiation of the neck, 2 (Nos, 16, 19) had thyroid dysfunction. Two patients (Nos. 7 and 20) with thyroid dysfunction had no irradiation of the neck.
Hapothalamo-pituitary-adrenal axis: Low levels of cortisol and exaggerated response to ACTH indicating a hypothalamo-pituitary-adrenal dysfunction were found in one patient treated for HD (No. 5). In one patient treated for SA (No. 21), low basal and normal stimulated levels of cortisol were measured, while another one (No. 22) had high basal and stimulated levels of cortisol.
Hypothalamo-pituitary-gonadal axis: Dysfunctions were found in 11 patients treated for HD (F=2, M=9) (78.6%). Three males had decreased basal levels of testosterone (Nos. 10, 13, 14); two of them were treated with MOPP and irradiation, one received only irradiation (inverted Y). One female (No. 9) had decreased values of estradiol and blunted response of FSH to stimulation. She was considered to be sterile after 3 years of childless marriage. Now, five years after hormonal investigations, she is pregnant. Elevated values of LH in the basal state were found in 7 patients (F=2, M=5) (Nos. 1, 2, 3, 6, 7, 8, 10), while an exaggerated response to Gn–RH was found in 8 (F=2, M=6) (Nos. 1, 2, 3, 4, 7, 8, 10, 12). Increased FSH values in basal and stimulated condition were discovered in 8 patients (F=1, M=7) (Nos. 1, 3, 4, 6, 7, 10, 12, 14) who had MOPP and RT above the diaphragm. In one female and in one male patient (Nos. 9, 13), only basal FSH values were elevated. In those with hypogonadism, infertile marriage was registered in 3 (Nos. 1, 4, 7) and azoospermia was proven in two patients (Nos. 4, 7). Two of the patients were obese; one of the two also exhibited gynecomasty. Puberty occurred late in one case (No. 2).
Out of the 9 patients with SA, primary gonadal dysfunction was found in 7 (F=1, M=6) (77.8%). In two males (Nos. 18, 20), eunuchoid proportions were registered. Increased levels of testosterone were found in one female (No. 22). Increased levels of LH in basal and stimulated conditions were found in 5 male patients (Nos. 15, 16, 18, 20, 23). Exaggerated response of normal basal LH values was found in one female (No. 22) and one male (No. 17). Elevated levels of FSH were observed in 5 patients (F=1, M=4) (Nos. 16, 18, 20, 22, 23). Among the patients treated for SA, one female (No. 19) who had VAC and one male who had T2 (No. 21) and no radiation had hormone levels within the normal range.

Tab. 1. *Age at treatment and study, clinical abnormalities and endocrine dysfunction in patients treated for HD and SA.*

Tab. 1. *Starost bolnikov, zdravljenih zaradi HB in SA ob začetku zdravljenja, v času endokrinološkega testiranja klinične značilnosti in endokrine motnje.*

Pts. no. / Št. primera	Sex / Spol	Age (years) at treat. / Starost (leta) ob začetku zdravlj.	Age (years) at study / v času test.	Years F/U / Trajanje sled.	Diagnosis	Therapy: surgery / kirurško	Therapy: radiation (dose) / Zdravljenje: obsevanje (doze)	Therapy: Cht / kemoterapija	Clinical abnormalities / Klinični znaki
HD									
HB									
1	F	13	23	10	HD III	–	30 Gy neck bil., mediastinum, paraaort.	MOPP	obesity, spontaneous abortion at 22 years, sterility
	Ž	13	23	10	HD III	–	30 Gy vrat obojestr., mediastinum, paraaort. bezg., vranica		debelost, spontan splav pri 22 letih, sterilnost
2	M	12	26	14	HD III	–	30 Gy TNI-spleen	MOPP	ratard puberty, atrophy of soft tissue, hypothyrosis
	M	12	26	14	HD III	–	30 Gy vse bezgavčne regije, vranica		pozna puberteta, atrofija mehkih tkiv, hipotireoza
3	M	13	23	10	HD III	laparatomy	30 Gy TNI + spleen	MOPP	atrophy of neck
	M	13	23	10	HB III	laparatomija	30 Gy, vse bezgavčne regije, vranica	MOPP	atrofija vratu
4	M	10	22	12	HD II	laparatomy	30 Gy, neck bil.	MOPP	astenia, azoospermia
	M	10	22	12	HB II	laparatomija	30 Gy, vrat obojestr.	MOPP	astenija, azoospermia
5	F	13	22	9	HD II	laparatomy	30 Gy, mediastinum, neck bil.	MOPP	obesity, one child
	Ž	13	22	9	HB II	laparatomija	30 Gy, vrat obojestr.	MOPP	debelost, 1 otrok
6	M	5	18	12	HD III	laparatomy	37,5 Gy neck + inverted Y	MOPP	astenia, caries, short stature
	M	5	18	12	HB III	laparatomija	37,5 Gy, vrat, polje obrnjenega Y	MOPP	astenija, karies, nizka rast
7	M	12	24	12	HD I	–	40 Gy, inverted Y	–	bilateral gynecomastia, azoospermia
	M	12	24	12	HB I	–	40 Gy, polje obrnjenega Y	–	obojestr. ginekomastija, azoospermija
8	F	11	22	11	HD III	–	30 Gy, neck	MOPP	atrophy of neck
	Ž	11	22	11	HB III	–	30 Gy, vrat	MOPP	atrofija vratu
9	F	9	24	15	HB III	–	30 Gy mediastinum paraaort. lgl.	MOPP	none-pregnant
	Ž	9	24	15	HB III	–	30 Gy, mediastinum paraaort. bezgavke	MOPP	noseča
10	M	12	21	9	HD III	–	30 Gy mediastinum paraaort. lgl.	MOPP	none
	M	12	21	9	HB III	–	30 Gy, mediastinum paraaort. bezgavke	MOPP	–
11	M	14	19	5	HD II	–	–	MOPP, ABVD	none
	M	14	19	5	HB II	–	–	MOPP, ABVD	–
12	M	5	8	13	HD III	–	30 Gy TNI	MOPP	soft tissue atrophy, short stature
	M	5	8	13	HB III	–	30 Gy, vse bezgavčne regije	MOPP	atrofija mehkih tkiv, nizka rast
13	M	6	17	10	HD II	laparatomy	36 Gy neck bil.	MOPP	none
	M	6	17	10	HB II	laparatomija	36 Gy vrat obojestr.	MOPP	–
14	M	9	30	21	HD II	–	40 Gy inverted Y	–	left fem. nerve paresis, muscle atrophy of the left leg
	M	9	30	21	HB II	–	40 Gy inverzni Y	–	pareza levega femoralnega ž., mišična atrofija leve noge
SA									
SA									
15	M	5	16	11	liposarcoma orbitae dex.	extenteration	50 Gy	–	asymetry of face and orbit, occurence of the third dentition
	M	5	16	11	liposarkom desne orbite	izprazn. celotne orbite	50 Gy	–	asimetrija obraza in orbit, tretja denticija
16	M	10	22	12	ERMS* auriculare sin., lymph node metast.	excicion, lymphadenectomy	60 Gy	VAC	atrophy of the left side of the face, epilepsy (treated by Tegretol)
	M	10	22	12	ERMS* levega uhlja, metastaze v bezgavkah	odstranitev tumorja in priz. bezgavk	60 Gy	VAC	atrofija leve polovice obraza, epilepsija (th. s. Tegretolom)
17	M	13	19	6	ERMS* epipharyngis	–	46 Gy	T_6+T^2	soft tissue and bone atrophy of the right side of face
	M	13	19	6	ERMS* epifaringsa	–	46 Gy	T_6+T^2	atrofija mehkih delov in kosti desne polovice obraza
18	M	9	19	10	ERMS* epipharyngis lymph node metast.	–	50 Gy	VAC	blindness, bilateral atrophy of the face, eunuchoid skeletal proportions, pituitary dwarfism
	M	9	19	10	ERMS* epifaringsa z metastazami v bezgavkah	–	50 Gy	VAC	slepota, atrofija obraza, nizka rast z evnuhoidnim videzom, hipofizna nanosomija
19	F	13	16	2	ERMS* lymph node metast., cheek	extirpation	40 Gy	VAC	atrophy of the neck
	Ž	13	16	2	ERMS* lica z metastazami v bezg.	odstranitev	40 Gy	VAC	atrofija vratu
20	M	11	21	10	ERMS* paratesticular	orchiectomy lymphadenectomy	–	VAC	eunuchoid skeletal proportion
	M	11	21	10	ERMS* ob testisu	odstranitev testisa in bezgavk	–	VAC	evnuhoidni videz
21	M	6	16	10	synovioma right knee	excision (2XX)	–	T_2	none (champion in riding)
	M	6	16	10	synoviom desnega kolena	odstranitev (2XX)	–	T_2	– (prvak v jahanju)
22	F	12	18	6	femur osteosarcoma	AK amputation	–	T_{10}	prothesis
	Ž	12	18	6	osteosarkom stegnenice	amputacija	–	T_{10}	proteza
23	M	6	17	11	ERMS* left scapular region	excision	36 Gy	VAC	muscular atrophy in the left scapular region
	M	6	17	11	ERMS* podr. leve lopatice	odstranitev	36 Gy	VAC	atrofija mišic v področju leve lopatice

TNI = total nodal irradiation
obsevanje vseh bezgavk

ERMS* = embryonal rhabdomyosarcoma
embrionalni rabdomiosarkom

Tab. 2. *Results of endocrine investigations in patients treated for HD (a) and SA (b).*

Tab. 2. *Rezultati endokrinoloških preiskav pri bolnikih, zdravljenih zaradi HB (a) in SA (b).*

Case Bolnik	T4 (nmol/l)	TSH (mU/l) basal baz. vred.	TSH maximal max. vred.	Cortisol (nmol/l) basal baz. vred.	Cortisol maximal max. vred.	Testosteron (nmol/l)	Estradiol (nmol/l)	LH (mU/l) basal baz. vred.	LH maximal max. vred.	FHS (mU/i) basal baz. vred.	FHS maximal max. vred.	GH (mog/l) rastni hormon basal baz. vred.	GH maximal max. vred.	IGFI (U/l)
(a)														
1	111	2.70	19.20	534	1020	2.45	0.13	14.5	28.7	19.6	29.6	1.5	13.9	1490
2	81	4.40	25.90	348	959	17.90	0.03	15.6	26.1	9.2	8.9	1.1	43.1	1530
3	102	1.60	9.30	690	954	24.00	0.11	16.8	103.0	15.6	31.7	0.4	56.2	–
4	96	2.12	10.60	434	1443	23.50	0.09	6.9	64.3	17.9	39.9	1.0	1.7	–
5	120	1.31	18.39	172	630	2.20	0.36	3.1	15.4	8.2	12.4	0.9	5.1	2266
6	103	0.94	0.76	401.2	603.5	29.90	0.05	9.5	12.1	24.6	25.0	0.2	50.1	1979
7	135	4.14	33.84	534	949	17.30	0.10	14.7	89.2	32.2	67.1	0.8	1.7	990
8	109	1.60	11.50	282	756	1.72	0.43	17.2	58.5	11.3	15.3	0.7	13.0	840
9	104	1.54	15.40	316	660	2.50	0.06	6.2	12.2	4.6	5.1	0.3	3.8	1394
10	74	1.01	7.86	421	796	10.40	0.16	19.7	81.7	32.1	68.1	0.06	6.6	2864
11	134	2.32	14.00	636	1346	18.20	0.02	1.6	23.3	8.9	21.8	–	–	779
12	114	1.77	15.22	398	652	17.20	0.25	3.8	35.2	12.6	27.9	–	–	1501
13	89	4.62	53.20	441	1269	10.60	0.07	6.0	24.1	15.5	21.5	–	–	2732
14	105	1.60	11.80	626	946	9.20	0.10	4.9	22.0	21.0	35.2	–	–	870
(b)														
15	108	2.00	11.80	361	804	28.12	0.03	10.5	24.1	8.5	12.1	0.9	6.6	900
16	94	2.90	27.10	716	1083	19.63	0.14	31.3	105.0	67.7	91.1	2.1	23.2	480
17	123	3.38	23.18	263	478	15.00	0.08	5.8	34.5	9.3	17.6	–	–	970
18	134	2.90	9.30	453	741	13.81	0.08	10.6	36.2	13.0	25.0	0.6	1.8	280
19	114	5.84	37.66	767	1124	1.50	0.29	2.90	11.5	7.2	10.0	0.3	1.1	585
20	81	0.04	3.35	542	844	15.30	0.24	14.7	53.7	10.6	16.7	0.1	13.6	2986
21	94	2.90	15.30	180	387	14.40	0.04	8.3	–	9.1	–	0.7	54.7	430
22	95	2.43	14.90	1032	1280	4.40	0.97	6.9	64.4	11.5	27.6	1.1	16.9	5238
23	128	1.73	13.17	372	751	16.90	0.14	10.9	72.4	23.2	37.7	–	–	1266
Normal range Normalni razpon 53–182		0.17–25.00 4.05		at 8 a. m. ob 8. uri 200–690		after puberty po puberteti M moški 13.7–35.4 F ženska 1.0–3.0	M moški 0–0.20 F ženska 0.13–0.78	basal bazalna vrednost M moški 1–8.4 F ženska 1.6–9.3 (phollic. phase) (folikularna faza)		basal bazalna vrednost M moški 1–10.5 F ženska 2.6–9.3 (phollic. phase) (folikularna faza)			0.0–5.6	>10	423–3790

Discussion

There was a high prevalence of endocrine dysfunction, exceeding 80%, in our patients treated for Hodgkin's disease and bone and soft tissue sarcomas. Clinically evident somatic abnormalities were caused by endocrinologic deficiency only on 5 cases (22%) (Nos. 1, 2, 7, 18, 20), while all others were due to tumor, radiation therapy or surgery. There was only one case of clinically evident pituitary dwarfism, in patient No. 18 whose height and weight were below the 3rd percentile. Primary hypogonadism and eunuchoid proportions were also found in this patient. He was treated at the age of 9 for nasopharyngeal rhabdomyosarcoma; the value of radiation included the base of the skull, consequently the pituitary gland received 50 cGy. In all cases of blunted GH response to provocative stimuli and normal IGF I values, the patients' height was in the normal range including the patient No. 4 in whom IGF I was not determined. In general, GH measurement would be of clinical value in cases of retarded growth or decreased growth velocity in still growing children and adolescents in order to start GH replacement therapy when necessary. With these facts in mind, repeated stimulation of GH for possible demonstration of impaired GH secretion was not performed.

In the patients studied there were no clinically important adrenal abnormalities. In one case (No. 22) hypercortisolemia could be due to stress. We belive that there is no need to evaluate the adrenal function in patients treated for HD and SA, but on the other hand, the thyroid and especially hypothalamo-pituitary-gonadal axis should be examined estensively due to the high percentage of dysfunction (87%) found in our patients.

Thyroid dysfunction with decreased stimulated TSH levels and with high basal levels and/or exaggerated response to TRH was found in 30.4% of the patients who were clinically euthyreotic. While the role of chemotherapy is uncertain, it is generally accepted that radiotherapy of the neck is an important cause of thyroid dysfunction (9). Out of eleven of our patients who had the thyroid exposed to radiation, 5 had thyroid dysfunction. For the remaining two whose necks were not irradiated, the reason for pathological values could be chemotherapy in one case (No. 20), while for another patient (No. 7), who was irradiated only below the diaphragm, the reason could not be established.

It has been demonstrated that elevation of TSH levels in the presence of radiation-damaged thyroid tissue is carcinogenic and that administration of thyroid hormone depresses TSH levels and decreases the possibility of radiation-induced thyroid carcinoma (9–12). Recent investigations of the relationship between external irradiation of the neck in childhood and thyroid cancer indicated a much higher risk than was reported previously. Maxon (13) reported 109 thyroid cancers among 5614 children exposed to radiation of the thyroid exceeding 100 cGy. This is more than the number expected to occur spontaneously during the mean observation period of 22.2 years. Schneider (14) found as many as 297 cases of cancer among 2958 patients treated with RT applied to the head and neck. These data indicate that the children who were exposed to irradiation of the neck should be followed for life. Check-ups of the structure and thyroid function should be part of follow-up examinations. Since elevated serum thyroglobulin levels are associated with an increased risk of developing thyroid cancer, thyroglobulin measurements could be a useful adjunct to clinical examination (15)

Hypothalamo-pituitary-gonadal dysfunction: Only 2 of the 14 HD patients had sexual hormone levels within normal range; one female (No. 5) treated with MOPP and radiation above the diaphragm and one male who received MOPP in combination with ABVD and no radiation (No. 22). It is well known that chemotherapy of HD by the MOPP combination may cause a gonadal dysfunction. In our series an additional cause for hypogonadism (azoospermia) was irradiation with the inverted Y technique. Patient No. 7 had irradiation on a Co 60 unit without additional shielding of the testes. The doses to the testes by the same inverted Y on a linear accelerator and with additional shielding is significantly lower (1.5% of the applied tumor dose). Additional shielding of the testes is therefore recommended in all cases when radiation is applied to the lower abdomen (16). Because of the high percentage of hypogonadism in children treated for HD with MOPP, it would seem that, for some of them, radiation is the reasonable choice until less toxic chemotherapy regimens are available (17). ABVD has been proven to be less toxic than MOPP, but still not harmless (18, 19). While thyroid hormone deficiency is easy to treat, infertility is a serious complication.

Hypogonadism followed chemotherapy in all except one patient with SA. The girl received the VAC regimen when she was 13; the same regimen produced hypogonadism in all 4 male patients. The regimens T2 and T6 seem to be less harmful to the gonads; possibly because the cumulative dose of cyclophosphamide was lower than in VAC.

All our patients were postpubertal. The shortest intervals between treatment and endocrine investigations were 5.0 and 6.0 years for HD and SA respectively. Some recovery of damaged endocrine glands several months after treatment has been reported (6). We believe that the endocrine sequelae discovered in our patients can be considered permanent because they were all studied more than 5 years after therapy (median 10 years) (20).

In male patients treated with chemotherapy and radiation to testes, testicular germinal epithelium is damaged more often than Leydig cells, and permanent sterility established in the majority of these patients (21–22).

The normal development of puberty in all our patients except one is in accordance with the known less toxic effectis of alkylating agents on the Leydig cells in boys and theca cells in girls. Additionally, irradiation of males is reported to damage, in a dose-dependent manner, first the germinal epithelium and then the Leydig cells. We found hypogonadism to be more common in males than females. More frequent gonadal damage and decline in fertility in boys is in accordance with the observation that the testes are more vulnerable than the ovaries (21, 23, 24). Means of protecting male gonads are therefore needed. The results of rat experiments are encouraging in this regard. Gn-RH analogues (25) and androgens (26) before chemotherapy and irradiation have been found to protect male gonads. It remains to be seen whether this strategy could be a means of preventing gonadal lesions in males treated for malignancies. The results of clinical trials have not been encouraging so far (27). More optimistic were the reports of normal offsprings of adult patients who had been treated for HD and SA in childhood (28).

Conclusions

The results of our study confirm the high prevalence of endocrine dysfunction in patients treated for HD and SA. The function of the thyroid gland and of the hypothalamo-pituitary-gonadal axis should be assessed at frequent intervals in order to detect endocrinological abnormalities and to introduce replacement therapy when necessary. A high proportion of patients treated for cancer in childhood suffer from gonadal dysfunction. It is, therefore, important to search for drugs or pretreatment that could prevent or diminish the late sequelae.

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for Pediatric Endocrinology and Diabetology

International cooperation

ALPS-ADRIA STUDY GROUP FOR PEDIATRIC ENDOCRINOLOGY AND DIABETES – AASGPED

During the International Symposium on Treatment of Children with Short Stature which took place in Trieste in 1989, the idea to meet and collaborate in the Alps-Adria region was unanimously accepted by pediatricians – endocrinologists from Italy, Slovenia and Austria who attended the scientific meeting.

In November 1990, the Alps-Adria Study Group of Pediatric Endocrinology and Diabetes – AASGPED was officially established under the auspices of Pierell-Kabi in Trieste. The first president, secretary general and "spiritus movens" of the group has been Dr. Giorgio Tonini from Trieste. The main purpose of the group has been to promote collaborative clinical and research work among pediatricians subspecialised in endocrinology from the Alps-Adria region, which shares many historical and cultural roots. Since endocrinopathies in childhood are relatively rare and there are few pediatricans who have treated these disorders, the idea was to cooperate and exchange experience in the area which is overpassing the borders in order to be large enough to obtain a great number of clinicians and scientiests with the same interest. At the moment, there are 23 members of the group from Brescia, Padova, Verona, Parma, Pavia, Regio Emilia, Trento, Trieste and Udine in Italy, Ljubljana in Slovenia, Zagreb and Rijeka in Croatia, Pecs and Györ in Hungary and Graz in Austria.

In the official logo of the study group, the Alps and the Adriatic Sea are symbolically presented, as well as the colours of all the states comprised in the AASGPED.

First two annual meetings of the group were organised by Dr. Tonini in Trieste, the third by Dr. Soltesz in Pecs and the fourth by Dr. Kržišnik in Ljubljana. The meeting in 1994 will be organised by Prof. Borkenstein in Graz and the one in 1995 by Prof. Tato in Verona. The group has been leaded by the Board of directors: Prof. Bernasconi – Parma, Prof. Tato – Verona, Prof. Borkenstein – Graz, Prof. Kadrnka-Lovrenčić – Zagreb, Dr. Soltesz – Pecs, Prof. Kržišnik – Ljubljana and the Secretary General Dr. Tonini from Trieste. The organiser of the annual meeting was also the president of the group for that year.

The research work of the group comprises the studies of Hashimoto and coeliac disease in diabetic children and adolescents, final height of patients with Turner syndrome, echocardiographic changes in patients with growth hormone (GH), deficiency during treatment. Studies have been focused also on genetic analysis of Prader-Willy syndrome and to the value of GH bio-assay in children with tall stature.

Some of the AASGPED collaborative studies were presented in different scientific meetings like the World Congress of Pediatric Cardiology in Paris, and IV. Joint Meeting of European Society for Pediatric Endocrinology and Lawson Wilkins Pediatric Endocrine Society in San Francisco, which were both organised in June 1993, and ISGD meeting in Athens in September 1993.

The last annual meeting of the AASGPED in October 1993 in Ljubljana was partly joined with the 2nd International Symposium on Recent Advances in Pediatric and Adolescent Endocrinology, which was organised on the occasion of the 30th anniversary of the Pediatric Department of Endocrinology, Diabetes and Metabolic Diseases in Ljubljana. The members of the group (figure 1) and guests from UK and Slovakia presented selected lectures from the field of pediatric endocrinology. The main topics were growth disorders, diabetes in the young, precoccious puberty and metabolic diseases in childhood.

Fig. 1. *Dr. M. Bozzola from Pavia, Italy.*

Sl. 1. *Dr. M. Bozzola iz Pavie v Italiji.*

Fig. 2. *Camerata Medica Labacensis.*

Sl. 2. *Camerata Medica Labacensis.*

At the opening ceremony of the meeting, the participants were greeted by Prof. Mitja Bartenjev, the Dean of Medical Faculty, University of Ljubljana.

The welcoming speech of the organisers was followed by a short concert of the orchestra of Slovenian doctors called "Camerata Medica Labacensis" (figure 2). The meeting was attended by more than 100 participants from Slovenia, Croatia, Italy, Hungary, Austria, Switzerland, Slovakia and UK (figure 3).

We believe that collaborative activities in the field of pediatric endocrinology conducted by AASGPED were very positive and fruitful, not only in the research but also in the exchange of ideas and clinical experience in treatment and care of patients, and should be continued and spread in a larger area of neighbouring countries in the future.

Prof. Dr. Ciril Kržišnik
President of AASGPED 1993

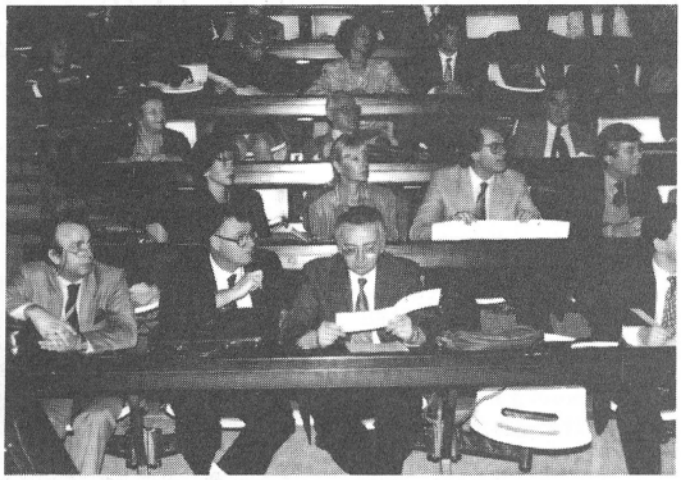

Fig. 3. *Participants of the Joint Annual Meeting of the AASGPED and the International Symposium on Recent Advances in Pediatric and Adolescent Endocrinology organised in October 1993 in the great hall of Medical Faculty, University of Ljubljana.*

Sl. 3. *Udeleženci na letnem srečanju študijske skupine AASGPED in Mednarodnem simpoziju o novostih iz pediatrične in adolescentne endokrinologije, ki sta bila organizirana oktobra 1993 v veliki predavalnici Medicinske fakultete v Ljubljani.*

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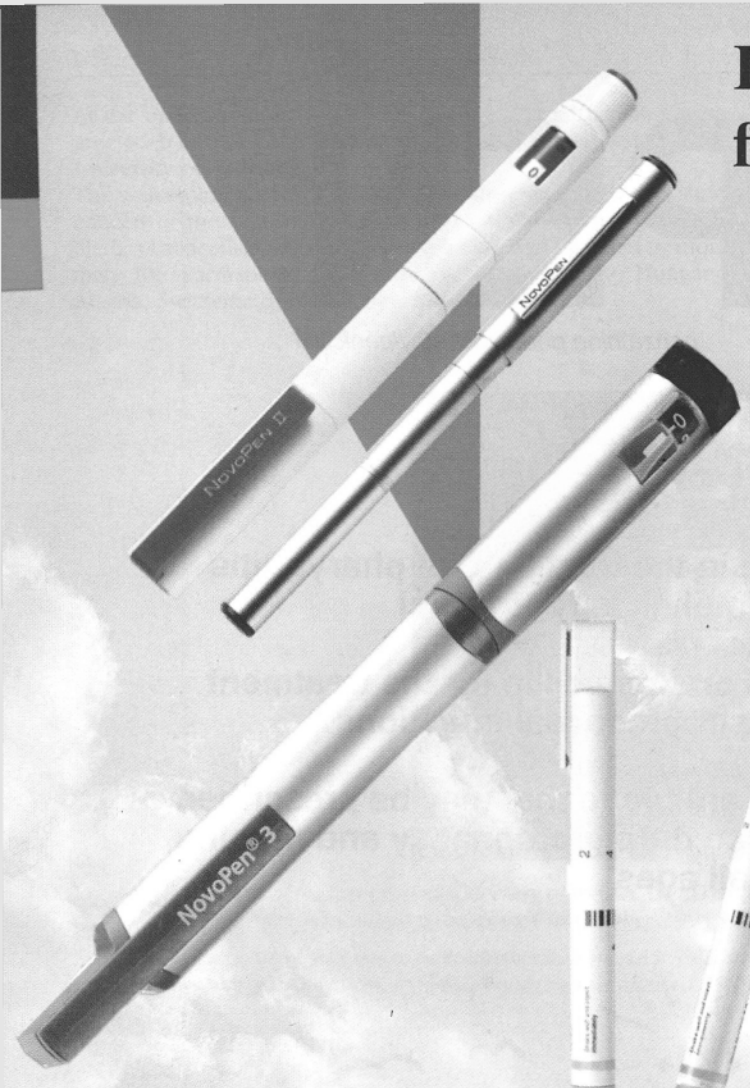

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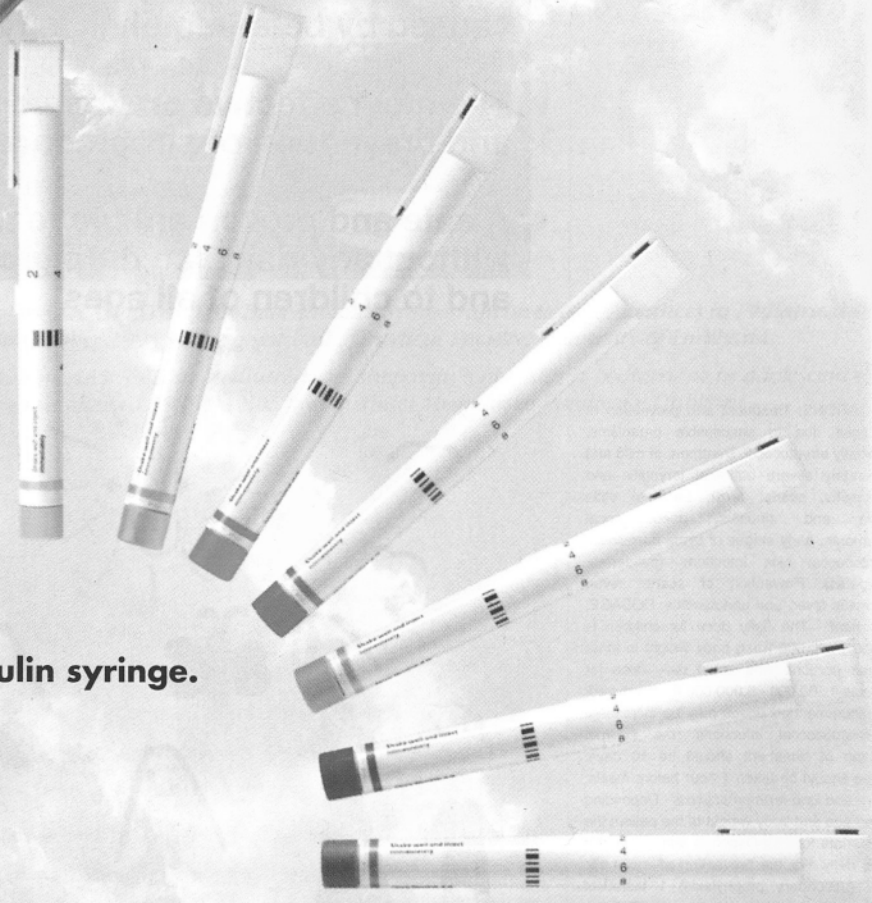

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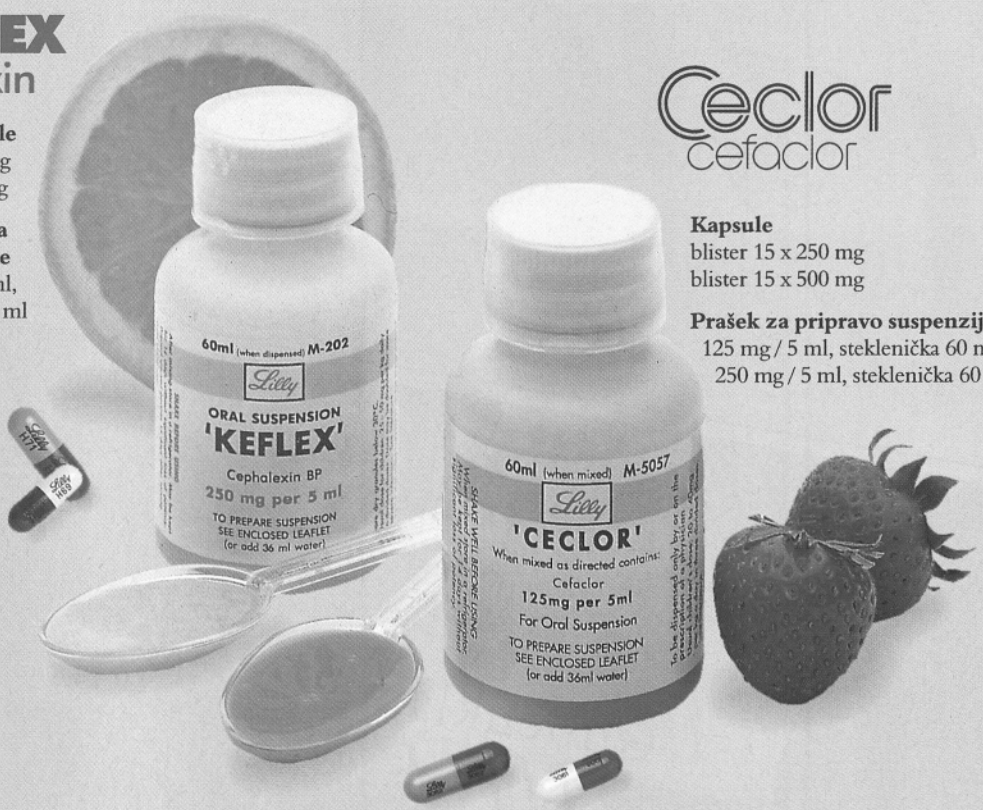

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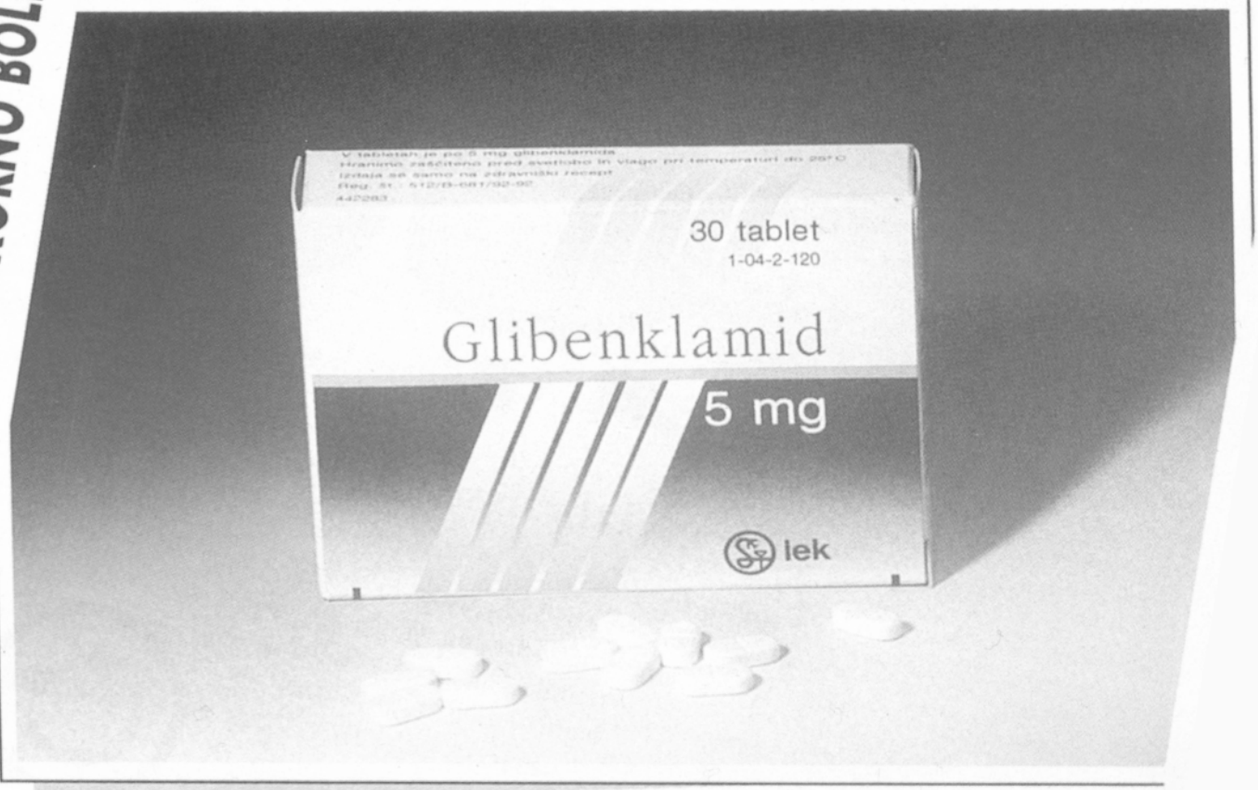

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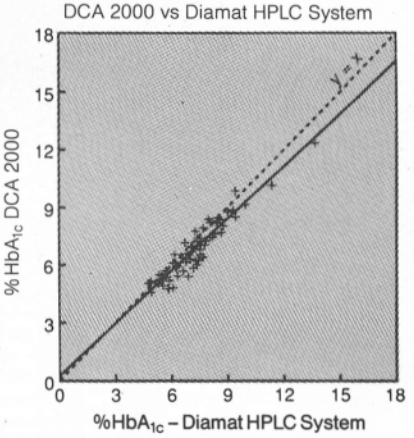

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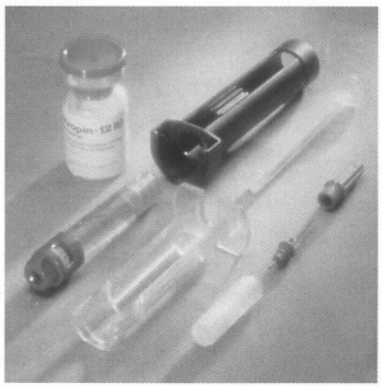

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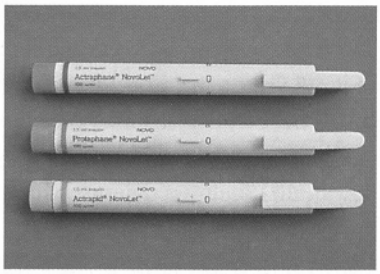

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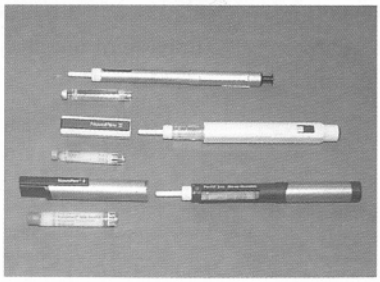

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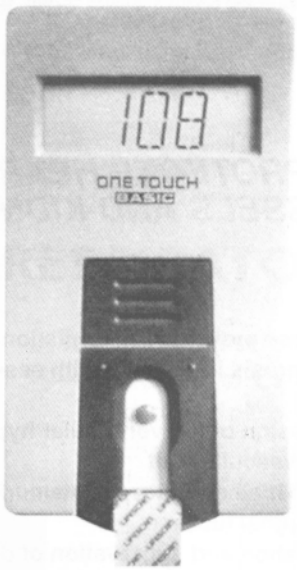

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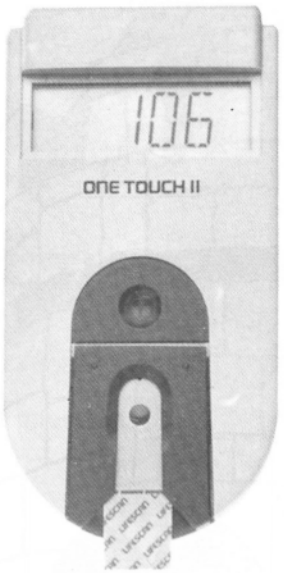

- REZULTAT MERJENJA ŽE PO TREH ENOSTAVNIH KORAKIH
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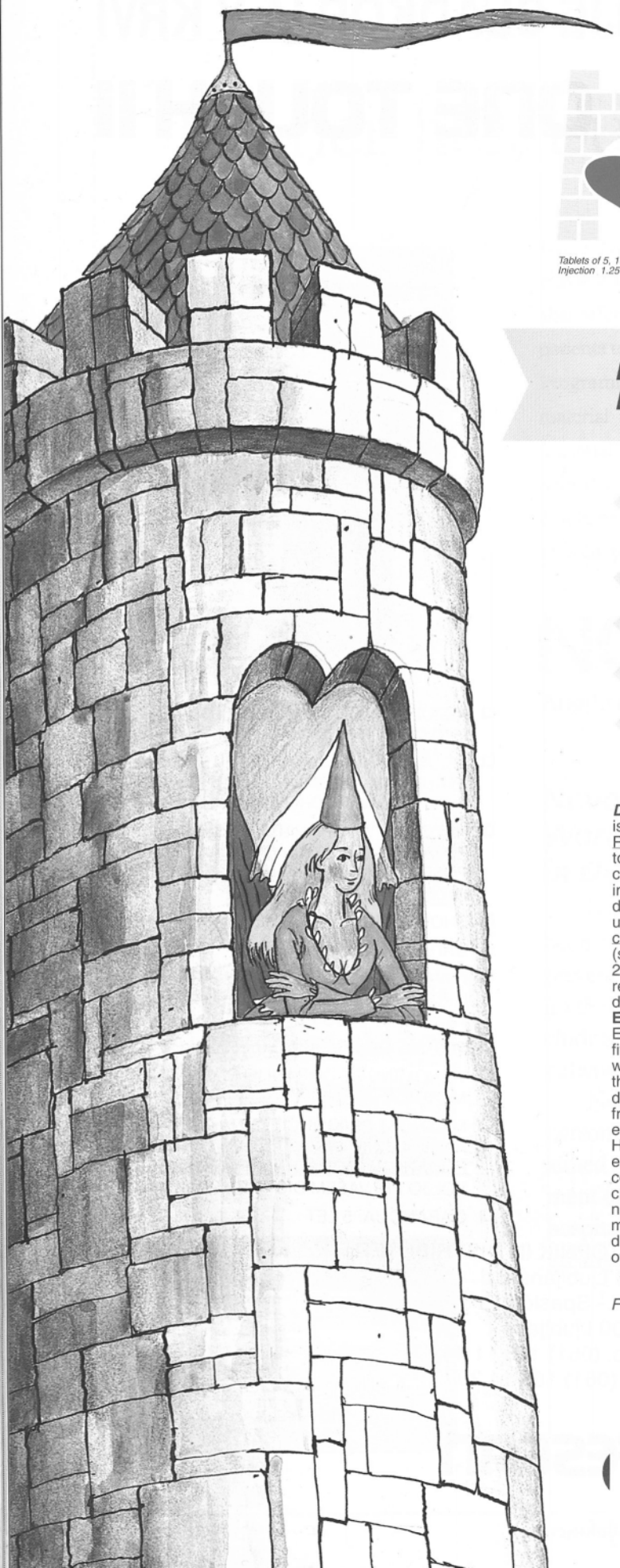

Enap®

Tablets of 5, 10 and 20 mg enalapril maleate
Injection 1.25 mg/ml enalaprilat

enalapril

RELIABLY PROTECTS HEART, BLOOD VESSELS AND KIDNEYS

- **a single daily dose prevents the formation of hypertensive nephroangiosclerosis in patients with essential arterial hypertension**
- **produces regression of left ventricular hypertrophy and hypertrophy of vascular wall**
- **in normotension it also reduces proteinuria and slows the progression of renal disease**
- **it prevents formation and aggravation of diabetic nephropathy**
- **preserves good quality of patients' life**

Dosage and administration: **Enap tablets:** Hypertension: The initial dose is 10 mg daily in a single dose. The usual maintenance dose is 20 mg daily. Renovascular hypertension: The treatment is initiated with doses of 2.5 mg to 5 mg daily and the patient carefully observed. Renal insufficiency: (serum creatinine over 350 μmol/L) initial doses of 2.5 to 5 mg are recommended; interval between each maintenance dose has to be longer and/or the dosage lower. Congestive heart failure: The initial dose is 2.5 to 5 mg. The usual maintenance dose is 10 to 20 mg daily in divided doses. Progressive chronic renal diseases: Treatment of a patient with early renal insufficiency (serum creatinine ca. 200 μmol/L) includes also the use of Enap in doses of 2.5 mg to 5 mg daily if the patient does not suffer from hypertension. It is recommended that before treatment with Enap the dosage of diuretics be decreased and the state of hypovolemia and hyponatremia be avoided. **Enap injection:** Treatment with enalaprilat usually lasts up to 48 hours. Enalaprilat is given intravenously in a dose of 1.25 mg every 6 hours, over a five minute period. In patients who are treated with diuretics and in patients with creatinine clearance lower than 30 ml/min (serum creatinine higher than 265 μmol/L) the initial enalaprilat dose is 0.625 mg (0.5 ml); additional doses of 1.25 mg (1 ml) are then given every 6 hours. When switching over from iv. to the oral therapy, the recommended initial dose is 2.5 to 5 mg enalapril once a day and then increased, if necessary. ***Contraindications:*** Hypersensitivity to the drug, pregnancy, lactation, known angioneurotic edema. ***Side-effects:*** Hypotension, headache, dizziness, fatigue, nausea, cough, rash, muscle spasms. Enap rarely causes an increase of urea and creatinine in the serum. After the treatment is discontinued the values are normalized. ***Interactions:*** An increase in serum potassium concentration may appear with concomitant administration of Enap and potassium-sparing diuretics. ***Supply:*** 20 tablets of Enap 5 mg, 10 mg and 20 mg; 5 ampoules of Enap injection of 1 ml.

For further information, please, contact the manufacturer.

KRKA
SLOVENIA

TARACEF®

Capsules of 250 mg and 500 mg
Suspension 125 mg/5 ml and 250 mg/5 ml

cefaclor

RESPIRATORY TRACT INFECTIONS?

Taracef (cefaclor)

— Excellent efficacy against Haemophilus influenzae and other pathogens producing respiratory tract infections
— Rapid and easy absorption
— May be taken with meals
— Proven in pediatrics

Taracef provides successful treatment of:

— bacterial infections of the ear
— upper and lower respiratory tract infections
— urinary tract infections
— dermal and subdermal infections
— bacterial infections in stomatology and dental surgery

Antimicrobial spectrum: Haemophilus influenzae, staphylococci (including beta-lactamase producing strains), streptococci, Moraxella catarrhalis, E. coli, Proteus mirabilis, Klebsiella spp., Citrobacter diversus, and certain anaerobic bacteria. **Indications:** Upper and lower respiratory tract infections, urinary tract infections, dermal and subdermal infections, infections in stomatology and dental surgery, due to Taracef-susceptible organisms. **Dosage:** *Adults:* 250 — 500 mg every 8 hours. *Children:* 20 mg/kg daily in three divided portions. For more severe infections, the dose may be doubled. The maximum daily dose is 1 g. No dose adjustment is required in patients with reduced renal function. **Contraindications:** Hypersensitivity to the cephalosporin group of antibiotics. **Side effects:** Allergic reactions, nausea, vomiting, diarrhea, dyspepsia, vertigo, headache, and vaginal candidosis; rarely, reversible neutropenia, leukopenia, and occasionally elevation in serum transaminases. **Precautions:** If the usual precautions are followed, Taracef may be administered to patients with allergy to penicillin. There is not enough experience with the use of Taracef in premature infants and neonates, therefore the drug should be used very cautiously. During treatment with Taracef, a positive Coombs' test and a positive reaction for glucose in the urine may occur (not with Tes-Tape test). **Pregnancy and lactation:** There is no evidence of harmful effects during pregnancy. Nursing mothers should discontinue breast-feeding while on treatment. **Supply:** 16 capsules of 250 mg and 500 mg, powder for 60 ml of suspension 125 mg/5 ml and 250 mg/5 ml.

Further information available from the manufacturer.

KRKA
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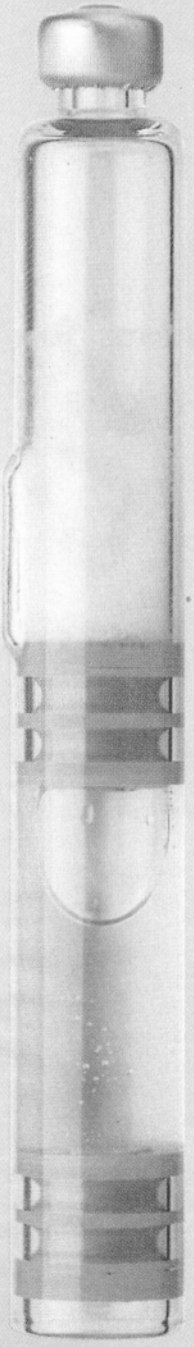

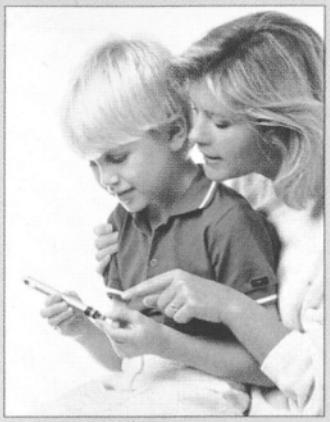

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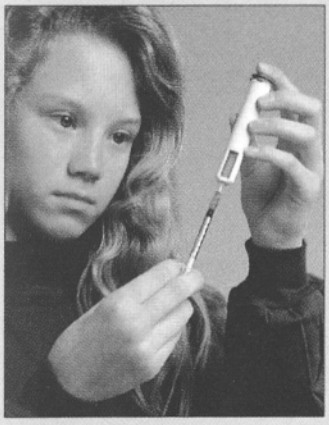
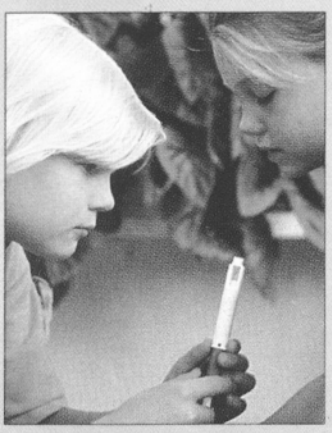
KabiPen



Genotropin® 4 IU
or 16 IU KabiVial



Genotropin® 2 IU, 3 IU
or 4 IU KabiQuick

A range for all children

GROWTH SERVICE
Kabi Pharmacia

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Peptide Hormones
S-112 87 Stockholm.

NAVODILA SODELAVCEM ZDRAVNIŠKEGA VESTNIKA

Zdravniški vestnik (ZV) je glasilo Slovenskega zdravniškega društva. Naslov uredništva je Zdravniški vestnik, Komenskega 4, 61000 Ljubljana, tel. (061) 317-868.

Splošna načela

ZV objavlja le izvirna, še neobjavljena dela. Avtor je odgovoren za vse trditve, ki jih v prispevku navaja. Če ima članek več avtorjev, je treba navesti natančen naslov (s telefonsko številko) tistega avtorja, s katerim bo uredništvo kontaktiralo pri pripravi teksta za objavo, ter kateremu avtorju se pošiljajo zahteve za reprint.

Če prispevek obravnava raziskave na ljudeh, mora biti iz besedila razvidno, da so bile raziskave opravljene v skladu z načeli Kodeksa etike zdravstvenih delavcev SFRJ in Deklaracije iz Helsinkov/Tokia.

Če delo obravnava poizkuse na živalih, mora biti razvidno, da je bilo opravljeno v skladu z etičnimi načeli.

Prispevki bodo razvrščeni v eno od naslednjih rubrik: uvodnik, raziskovalni prispevek, strokovni prispevek, pregledni članek, pismo uredništvu in razgledi.

Raziskovalna poročila morajo biti napisana v angleščini. Dolga naj bodo do 8 tipkanih strani. Slovenski izvleček mora biti razširjen in naj bo dolg do tri tipkane strani. Angleški ne sme biti daljši od 250 besed.

Če besedilo zahteva aktivnejše posege angleškega lektorja, nosi stroške avtor.

Ostali prispevki za objavo morajo biti napisani v slovenščini jedrnato ter strokovno in slogovno neoporečno. Pri raziskovalnih in strokovnih prispevkih morajo biti naslov, izvleček, deskriptorji (ključne besede), tabele in podpisi k tabelam in slikam prevedeni v angleščino.

Članki so lahko dolgi največ 12 tipkanih strani (s tabelami, slikami in literaturo vred).

V besedilu se lahko uporabljajo le enote SI in tiste, ki jih dovoljuje Zakon o merskih enotah in merilih (Uradni list SFRJ št. 13/76).

Spremni dopis

Spremno pismo mora vsebovati: 1. izjavo, da poslano besedilo ali katerikoli del besedila (razen abstrakta) ni bilo poslano v objavo nikomur drugemu; 2. da so vsi soavtorji besedilo prebrali in se strinjajo z njegovo vsebino in navedbami; 3. kdaj je raziskavo odobrila »Etična komisija«; 4. da so preiskovanci dali pisno soglasje k sodelovanju pri raziskavi; 5. pisno dovoljenje za objavo slik, na katerih bi se ev. lahko prepoznala identiteta pacienta; 6. pisno dovoljenje založbe, ki ima avtorske pravice, za ponatis slik, shem ali tabel.

Tipkopis

Prispevki morajo biti poslani v trojniku, tipkani na eni strani boljšega belega pisarniškega papirja formata A4. Med vrsticami mora biti dvojni razmik (po 27 vrstic na stran), na vseh straneh pa mora biti rob širok najmanj 30 mm. Avtorji, ki pišejo besedila s pomočjo PC kompatibilnega računalnika, jih lahko pošljejo uredništvu na 5 1/4 inčnih disketah, formatiranih na 360 Kb ali 1,2 Mb, kar bo pospešilo uredniški postopek. Ko bo le-ta končan, uredništvo diskete vrne. Besedila naj bodo napisana s programom Wordstar ali z drugim besedilnikom, ki hrani zapise v ASCII kodi. V besedilu so dovoljene kratice, ki pa jih je treba pri prvi navedbi razložiti. Že uveljavljenih okrajšav ni treba razlagati (npr. l za liter, mg za miligram itd.).

Naslovna stran članka naj vsebuje slovenski naslov dela, angleški naslov dela, ime in priimek avtorja z natančnim strokovnim in akademskim naslovom, popoln naslov ustanove, kjer je bilo delo opravljeno (če je delo skupinsko, naj bodo navedeni ustrezni podatki za soavtorje). Naslov dela naj jedrnato zajame bistvo vsebine članka. Če je naslov z avtorjevim priimkom in imenom daljši od 90 znakov, je treba navesti še skrajšano verzijo naslova za tekoči naslov. Na naslovni strani naj bo navedenih tudi po pet ključnih besed (uporabljene naj bodo besede, ki natančneje opredeljujejo vsebino prispevka in ne nastopajo v naslovu; v slovenščini in angleščini) ter eventualni financerji raziskave (s številko pogodbe).

Druga stran naj vsebuje slovenski izvleček, ki mora biti strukturiran in naj vsebuje naslednje razdelke in podatke:

Izhodišča (Background): Navesti je treba glavni problem in namen raziskave in glavno hipotezo, ki se preverja.

Metode (Methods): Opisati je treba glavne značilnosti izvedbe raziskave (npr. trajanje), opisati vzorec, ki se ga proučuje (npr. randomizacija, dvojno slepi poizkus, navzkrižno testiranje, testiranje s placebom itd.), standardne vrednosti za teste, časovni odnos (prospektivna, retrospektivna študija).

Navesti je treba način izbora preiskovancev, kriterije vključitve, kriterije izključitve, število preiskovancev, vključenih v raziskavo in koliko jih je vključenih v analizo. Opisati je treba posege, metode, trajanje jemanja posameznega zdravila, kateri preparati se med seboj primerjajo (navesti je treba generično ime preparata in ne tovarniško) itd.

Rezultati (Results): Opisati je treba glavne rezultate študije. Pomembne meritve, ki niso vključene v rezultate študije, je treba omeniti. Pri navedbi rezultatov je treba vedno navesti interval zaupanja in natančno raven statistične značilnosti. Pri primerjalnih študijah se mora interval zaupanja nanašati na razlike med skupinami. Navedene morajo biti absolutne številke.

Zaključki (Conclusions): Navesti je treba le tiste zaključke, ki izhajajo iz podatkov, dobljenih pri raziskavi; treba je navesti ev. klinično uporabnost ugotovitkov. Navesti je treba, kakšne dodatne študije so še potrebne, preden bi se zaključki raziskave klinično uporabili. Enakovredno je treba navesti tako pozitivne kot negativne ugotovitve.

Ker nekateri prispevki (npr. pregledni članki) nimajo niti običajne strukture članka, naj bo pri teh strukturiranost izvlečka ustrezno prilagojena. Dolg naj bo od 50 do 200 besed; na tretji strani naj bodo: angleški naslov članka, ključne besede v angleščini in angleški prevod izvlečka.

Na naslednjih straneh naj sledi besedilo članka, ki naj bo smiselno razdeljeno v poglavja in podpoglavja, kar naj bo razvidno iz načina podčrtavanja naslova oz. podnaslova, morebitna zahvala in literatura. Odstavki morajo biti označeni s spuščeno vrstico. Tabele, podpisi k slikam, prevedeni tudi v angleščino in razlaga v tekstu uporabljenih kratic morajo biti napisani na posebnih listih.

Tabele

Natipkane naj bodo na posebnih listih in zaporedno oštevilčene. Imeti morajo najmanj dva stolpca. Vsebovati morajo: naslov (biti mora dovolj poveden, da razloži, kaj tabela prikazuje, ne da bi bilo treba brati članek; če so v tabeli podatki v odstotkih, je treba v naslovu navesti bazo za računanje odstotka; treba je navesti, od kod so podatki iz tabele, ev. mere, če veljajo za celotno tabelo, razložiti podrobnosti glede vsebine v glavi ali čelu tabele), čelo, glavo, morebitni zbirni stolpec in zbirno vrstico ter opombe ali pa legendo uporabljenih kratic v tabeli. Vsa polja morajo biti izpolnjena in mora biti jasno označeno, če ev. manjkajo podatki.

V besedilu prispevka je treba označiti, kam spada posamična tabela.

Slike

Risbe morajo biti risane s črnim tušem na bel trd papir. Pri velikosti je treba upoštevati, da bodo v ZV pomanjšane na širino stolpca (88 mm) ali kvečjemu na dva stolpca (180 mm). Morebitno besedilo na sliki mora biti izpisano z letraset črkami Helvetica Medium. Treba je upoštevati, da pri pomanjšanju slike za tisk velikost črke ne sme biti manjša od 2 mm. Grafikoni, diagrami in sheme naj bodo uokvirjeni.

Na hrbtni strani vsake slike naj bo s svinčnikom napisano ime in priimek avtorja, naslov članka in zaporedna številka slike. Če je treba, naj bo označeno, kaj je zgoraj in kaj spodaj.

V besedilu prispevka je treba označiti, kam spada posamična slika.

Literatura

Vsako trditev, dognanje ali misel drugih je treba potrditi z referenco. Neobjavljeni podatki ali pa osebno sporočilo ne spada v seznam literature. Navedke v besedilu je treba oštevilčiti po vrstnem redu, v katerem se prvič pojavijo, z arabskimi številkami v oklepaju. Če se pozneje v besedilu znova sklicujemo na že uporabljeni navedek, navedemo številko, ki jo je navedek dobil pri prvi omembi. Navedki, uporabljeni v tabelah in slikah, naj bodo oštevilčeni po vrstnem redu, kakor sodijo tabele ali slike v besedilo. Pri citiranju več del istega avtorja dobi vsak navedek svojo številko, starejša dela je treba navesti prej. Vsi navedki iz besedila morajo biti v seznamu literature.

Literatura naj bo zbrana na koncu članka po zaporednih številkah navedkov. Če je citiran članek napisalo 6 avtorjev ali manj, jih je treba navesti vse; pri 7 ali več je treba navesti prve tri in dodati et al. Če pisec prispevka ni znan, se namesto imena napiše Anon. Naslove revij, iz katerih je navedek, je treba krajšati, kot to določa Index Medicus.

Primeri citiranja

– primer za knjigo:

1. Bohinjec J. Temelji klinične hematologije. Ljubljana: Dopisna delavska univerza Univerzum, 1983: 182–3.

– primer za poglavje iz knjige:

2. Garnick MB, Brenner BM. Tumors of the urinary tract. In: Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS eds. Harrison's principles of internal medicine. 11th ed. Vol 2. New York: McGraw Hill, 1987: 1218–21.

– primer za članek v reviji:

3. Šmid L, Žargi M. Konikotomija – zakaj ne. Med Razgl 1989; 28: 255–61.

– primer za članek iz revije, kjer avtor ni znan:

4. Anon. An enlarging neck mass in a 71-year-old woman. AM J Med 1989; 86: 459–64.

– primer za članek iz revije, kjer je avtor organizacija:

5. American College of Physicians. Clinical ecology. Ann Int Med 1989; 111: 168–78.

– primer za članek iz suplementa revije:

6. Miller GJ. Antithrombotic therapy in the primary prevention of acute myocardial infarction. Am J Cardiol 1989; 64: Suppl 4: 29B–32B.

– primer za članek iz zbornika referatov:

7. Schneider W. Platelet metabolism and membrane function. In: Ulutin ON, Vinazzer H eds. Proceedings of 4th international meeting of Danubian league against thrombosis and haemorrhagic diseases. Istanbul: Goezlem Printing and Publishing Co, 1985: 11–5.

Sodelovanje avtorjev z uredništvom

Prispevke oddajte ali pošljite le na naslov: Uredništvo Zdravniškega vestnika, Komenskega 4, 61000 Ljubljana. Za prejete prispevke izda uredništvo potrdilo. V primeru nejasnosti so uredniki na voljo za posvet, najbolje po poprejšnjem telefonskem dogovoru [tel. (061) 317-868].

Vsak članek daje uredništvo v strokovno recenzijo in jezikovno lekturo. Po končanem redakcijskem postopku, strokovni recenziji in lektoriranju vrnemo prispevek avtorju, da popravke odobri, jih upošteva in oskrbi čistopis, ki ga vrne s popravljenim prvotnim izvirnikom. Med redakcijskim postopkom je zagotovljena tajnost vsebine članka.

Avtor dobi v korekturo prvi krtačni odtis s prošnjo, da na njem označi vse tiskovne pomote. Spreminjanja besedila ob tej priliki uredništvo ne bo upoštevalo. Korekture je treba vrniti v treh dneh, sicer uredništvo meni, da avtor nima pripomb.

Rokopisov in slikovnega materiala uredništvo ne vrača.

Dovoljenje za ponatis slik, objavljenih v ZV, je treba zaprositi od Uredništva Zdravniškega vestnika, Komenskega 4, 61000 Ljubljana.

Navodila za delo recenzentov

Če zaprošeni recenzent prispevka ne more sprejeti v oceno, naj rokopis vrne. Hvaležni bomo, če v tem primeru predlaga drugega primernega recenzenta. Če meni, da poleg njega prosimo za oceno prispevka še enega recenzenta (multidisciplinarna ali mejna tema), naj to navede v svoji oceni in predlaga ustreznega strokovnjaka.

Recenzentovo delo je zelo odgovorno in zahtevno, ker njegovo mnenje največkrat vodi odločitev uredništva o usodi prispevka. S svojimi ocenami in sugestijami recenzenti prispevajo k izboljšanju kakovosti našega časopisa. Po ustaljeni praksi ostane recenzent avtorju neznan in obratno.

Če recenzent meni, da delo ni vredno objave v ZV, prosimo, da navede vse razloge, zaradi katerih delo zasluži negativno oceno. Negativno ocenjen članek po ustaljenem postopku skupaj z recenzijo (seveda anonimno) uredništvo pošlje še enemu recenzentu, kar se ne sme razumeti kot izraz nezaupanja prvemu recenzentu.

Prispevke pošiljajo tudi mladi avtorji, ki žele svoja zapažanja in izdelke prvič objaviti v ZV ter jim je treba pomagati z nasveti, če prispevek le formalno ne ustreza, vsebuje pa pomembna zapažanja in sporočila.

Od recenzenta uredništvo pričakuje, da bo odgovoril na vprašanja na obrazcu ter da bo ugotovil, če je avtor upošteval navodila sodelavcem, ki so objavljena v vsaki številki ZV, in da bo preveril, če so podane trditve in misli verodostojne. Recenzent mora oceniti metodologijo in dokumentacijo ter opozoriti uredništvo na ev. pomanjkljivosti, posebej še v rezultatih.

Ni treba, da se recenzent ukvarja z lektoriranjem in korigiranjem, čeprav ni napak, če opozori na take pomanjkljivosti. Posebej prosimo, da je pozoren na to, ali je naslov dela jasen in koncizen in ali ustreza vsebini; ali izvleček povzema bistvene podatke članka; ali avtor cit isti številki kot ocenjevano delo.

Recenzij ne plačujemo.

Zdravniški vestnik

GLASILO SLOVENSKEGA ZDRAVNIŠKEGA DRUŠTVA ZDRAV VESTN, LETNIK 63, JUNIJ 1994, str. I-1-I-64, SUPPL. I.

ŠTEVILKA KLINIKE ZA ENDOKRINOLOGIJO, DIABETES IN PRESNOVNE BOLEZNI PEDIATRIČNE KLINIKE V LJUBLJANI

VSEBINA