FIFTY-FIVE YEARS OF PEDIATRIC ENDOCRINOLOGY AND 50 YEARS OF THE DEPARTMENT OF PEDIATRIC ENDOCRINOLOGY, DIABETES AND METABOLIC DISEASES IN SLOVENIA

55 LET PEDIATRIČNE ENDOKRINOLOGIJE IN 50 LET KLINIČNEGA ODDELKA ZA ENDOKRINOLOGIJO, DIABETES IN PRESNOVNE BOLEZNI PEDIATRIČNE KLINIKE

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ABSTRACT

Paediatric endocrinology started its independent development early in the general development of this specialty, with a strong focus on research and clinical excellence. Slovenian paediatric endocrinology was an integral part of the European paediatric endocrinology from its beginnings and a founding member of the first 'International Study Group for Diabetes in Children and Adolescents'. After the pioneering work of Prof. Lev Matajc, Prof. Ciril Kržišnik firmly integrated the Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases at the University Children's Hospital in Ljubljana in the international scientific community. In the last decade, the department participates in cutting-edge research and provides clinical services at highest international standards.

Keywords:
paediatric endocrinology, diabetes, metabolic diseases, research, clinical care, Slovenia

IZVLEČEK

Pediatrična endokrinologija je začela s svojim neodvisnim razvojem zgodaj v splošnem razvoju te specilanosti, z izrazitim poudarkom na raziskovalnemu delu in klinični odličnosti. Slovenska pediatrična endokrinologija je bila že od začetkov evropske pediatrične endokrinologije njen sestavni del, še posebej kot soustanoviteljica prve mednarodne skupine 'International Study Group for Diabetes in Children and Adolescents'. Po pionirskem delu prof. dr. Leva Matajca je prof. dr. Ciril Kržišnik KO za endokrinologijo, diabetes in bolezni presnove Pediatrične klinike v Ljubljani trdno vpel v mednarodno znanstveno skupnost. V zadnjem desetletju KO sodeluje pri vrhunskem raziskovalnem delu in zagotavlja klinično oskrbo po najvišjih mednarodnih standardih.

Ključne besede:
pediatrična endokrinilogija, sladkorna bolezen, bolezni presnove, raziskovalno delo, klinična oskrba, Slovenija

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The field of clinical endocrinology developed parallel to the development of biochemistry, with identification of chemical substances called hormones. Descriptions of first successful treatments of diseases related to hormones appeared at the beginning of the 20th century and, in 1917 'The Association for the Study of Internal Secretions' (now 'The Endocrine Society') was established in the United States, along with its journal 'Endocrinology' (now 'The Journal of Clinical Endocrinology and Metabolism'). In Europe, 'The Society for Endocrinology' was established in the United Kingdom in 1946, followed by other countries like Germany, in 1964, with 'Deutsche Gesellschaft für Endokrinologie'.

The development of paediatric endocrinology was based on a very strong and active research in this field and, in 1962, the 'European Society for Paediatric Endocrinology' (ESPE) was established, preceding any similar internal medicine association in the old continent. In the United States, a very active group of paediatric endocrinologists from Johns Hopkins University formalized in 1964, and transformed into a national association called the 'Lawson Wilkins Pediatric Endocrine Society' in 1972 (now 'The Pediatric Endocrine Society').

In Slovenia, paediatric endocrinology started with the activities of Prof. Lev Matajc. The first children with endocrine diseases were treated at the University Children Hospital in Ljubljana in the late fifties. Prof. Matajc formally established the Section of paediatric endocrinology in 1963 (1, 2), and after visiting Prof. Henry Lestradet in Paris, he introduced self-control for children with diabetes, started with diabetes summer camps in 1967 (they are organized annually since), national childhood diabetes registry in 1970, and a bulletin for young patients and their families in 1971. In 1974, Prof. Matajc was among the founding members of the paediatric diabetes society 'International Study Group for Diabetes' (in childhood and adolescence) (ISGD), established in Paris under the initiative of Prof. Lestradet (and transformed in 1993 into the 'International Society for Pediatric and Adolescent Diabetes' (ISPAD)). Additionally, in 1969, Prof. Matajc was among the first in Europe using human growth hormone extracted from cadaveric pituitaries for treating growth hormone deficiency.

The leadership in Slovenian paediatric endocrinology was forwarded to Prof. Ciril Kržišnik, who took over the Section of pediatric endocrinology, diabetes and metabolic diseases in 1982, and upgraded it to the Department of the University Children's Hospital in 1990. His main legacy was integrating the department and its research activities into the international collaboration (EURODIAB, DIAMOND) and professional societies. By organizing the 15th ISPAD annual congress in 1989 in Slovenia, he settled the department on the map of international centres. He continued with this initiative, and participating in the writing of the first WHO-IDF-ISPAD guidelines, was a founding member of the 'Alpe-Adria Study Group for Paediatric Endocrinology and Diabetes' (AASGPED) in 1990, the 'Middle European Working-group of Paediatric Endocrinology' (MEWPE) in 1994, and the 'Mediterranean Society for Paediatric Endocrinology' (MSPE) in 1995. Increasing research activities and international collaboration of the department enabled him to bring the 42nd annual congress of the 'European Society for Paediatric Endocrinology' to Ljubljana in 2003. In addition to his professional endeavour, he became the medical director of the University Children's Hospital (UCH) in 1996, and conducted the construction of a new, modern building into which the UCH moved in 2009, providing the Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases with the environment and means for further clinical excellence and research.

In the last decade, the Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases at the UCH in Ljubljana, shoulders a central role in conducting cutting-edge research in the field of diabetes (3), endocrinology (4-6) and metabolic diseases (7, 8), and maintains internationally comparable excellence in providing medical care (9, 10). The department has a strong vision to further increase integration into European research structures (it currently participates in two ESPE research grants, in two EU grants, and has three grants from the Slovene National Research Agency), quality assurance programs (it obtained the SWEET-IDF-ISPAD certificate in 2014) and clinical excellence. Finally, tight cooperation with the Laboratory for Medical Genetics at the UCH Ljubljana also fosters applied basic research (11-19).

The current issue of the Slovenian Journal of Public Health (Zdravstveno varstvo) kindly hosts the department's anniversary by publishing articles based on recent research and clinical activities. Our own results were combined with results from a lively international collaboration into an assortment of original data and information.

All these aims and endeavours are driven by the needs of our chronically ill children, adolescents, young adults and their families – our success will be measured by how much we alleviate day-to-day burden of their diseases.

Figure 1. The Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases at the University Children's Hospital Ljubljana in 2014.

REFERENCES

1. Matajc l, Brus L, Kržišnik C. et al. Health protection of diabetic children in Yugoslavia. Diab Croat 1978; 7: 65-8.
2. Kržišnik C. Forty years (1960-2000) of pediatric endocrinology in Slovenia. Slov Pediatr 2000; 7: 91-4.
3. Phillip M, Battelino T, Atlas E, Kordonouri O, Bratina N, Miller S.et al. Nocturnal glucose control with an artificial pancreas at a diabetes camp. N Engl J Med 2013; 368: 824-33.
4. de Schepper J, Rasmussen MH, Gucev Z, Eliakim A, Battelino T. Long-acting pegylated human GH in children with GH deficiency: a single-dose, dose-escalation trial investigating safety, tolerability, pharmacokinetics and pharmacodynamics. Eur J Endocrinol 2011; 165: 401-9.
5. Raivio T, Avbelj M, McCabe MJ, Romero CJ, Dwyer AA, Tommiska J. et al. Genetic overlap in Kallmann syndrome, combined pituitary hormone deficiency, and septo-optic dysplasia. J Clin Endocrinol Metab 2012; 97: E694-9.
6. Kotnik P, Keuper M, Wabitsch M, Fischer-Posovszky P. Interleukin-1ß downregulates RBP4 secretion in human adipocytes. PLoS One 2013; 8: e57796.
7. Sedej K, Kotnik P, Avbelj Stefanija M, Grošelj U, Širca Čampa A, Lusa L. et al. Decreased prevalence of hypercholesterolaemia and stabilisation of obesity trends in 5-year-old children: possible effects of changed public health policies. Eur J Endocrinol 2014; 170: 293-300.
8. Groselj U, Murko S, Zerjav Tansek M, Kovac J, Trampus Bakija A, Repic Lampret B. et al. Comparison of tandem mass spectrometry and amino acid analyzer for phenylalanine and tyrosine monitoring-Implications for clinical management of patients with hyperphenylalaninemia. Clin Biochem 2015; 48: 14-8.
9. Groselj U, Tansek MZ, Smon A, Angelkova N, Anton D, Baric I. et al. Newborn screening in southeastern Europe. Mol Genet Metab 2014; 113: 42-5.
10. Dovc K, Telic SS, Lusa L, Bratanic N, Zerjav-Tansek M, Kotnik P. et al. Improved metabolic control in pediatric patients with type 1 diabetes: a nationwide prospective 12-year time trends analysis. Diabetes Technol Ther 2014; 16: 33-40.
11. Kovač J, Podkrajšek KT, Lukšič MM, Battelino T. Weak association of glyoxalase 1 (GLO1) variants with autism spectrum disorder. Eur Child Adolesc Psychiatry 2015; 24: 75-82.
12. Smigoc Schweiger D, Mendez A, Kunilo Jamnik S, Bratanic N, Bratina N, Battelino T. et al. Genetic risk for co-occurrence of type 1 diabetes and celiac disease is modified by HLA-C and killer immunoglobulin-like receptors. Tissue Antigens 2014; 84: 471-8.
13. Hovnik T, Bratanič N, Podkrajšek KT, Kovač J, Paro D, Podnar T. et al.Severe progressive obstructive cardiomyopathy and renal tubular dysfunction in Donohue syndrome with decreased insulin receptor autophosphorylation due to a novel INSR mutation. Eur J Pediatr 2013; 172: 1125-9.
14. Tansek MZ, Groselj U, Murko S, Kobe H, Lampret BR, Battelino T. Assessment of tetrahydrobiopterin (BH(4))-responsiveness and spontaneous phenylalanine reduction in a phenylalanine hydroxylase deficiency population. Mol Genet Metab 2012; 107: 37-42.
15. Groselj U, Tansek MZ, Kovac J, Hovnik T, Podkrajsek KT, Battelino T. Five novel mutations and two large deletions in a population analysis of the phenylalanine hydroxylase gene. Mol Genet Metab 2012; 106: 142-8.
16. Kotnik P, Debeljak M, Avbelj M, Hovnik T, Ursic Bratina N, Krzisnik C. et al. Lack of association of common allelic variants in the thyroglobulin gene with Hashimoto's thyroiditis in young subjects with type 1 diabetes. Horm Res Paediatr 2010; 73: 244-7.
17. Hovnik T, Dolzan V, Bratina NU, Podkrajsek KT, Battelino T. Genetic polymorphisms in genes encoding antioxidant enzymes are associated with diabetic retinopathy in type 1 diabetes. Diabetes Care 2009; 32: 2258-62.
18. Podkrajsek KT, Milenković T, Odink RJ, Claasen-van der Grinten HL, Bratanic N, Hovnik T. et al. Detection of a complete autoimmune regulator gene deletion and two additional novel mutations in a cohort of patients with atypical phenotypic variants of autoimmune polyglandular syndrome type 1. Eur J Endocrinol 2008; 159: 633-9.
19. Cihakova D, Trebusak K, Heino M, Fadeyev V, Tiulpakov A, Battelino T. et al. Novel AIRE mutations and P450 cytochrome autoantibodies in Central and Eastern European patients with APECED. Hum Mutat 2001; 18: 225-32.

CLINICAL AND MOLECULAR CYTOGENETIC CHARACTERISATION OF CHILDREN WITH DEVELOPMENTAL DELAY AND DYSMORPHIC FEATURES

KLINIČNA IN MOLEKULARNA CITOGENETSKA OBRAVNAVA OTROK Z RAZVOJNIM ZAOSTANKOM IN DISPLASTIČNIMI ZNAKI

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ABSTRACT

Introduction. Developmental delay and dysmorphic features affect 1 - 3 % of paediatric population. In the last few years molecular cytogenetic high resolution techniques (comparative genomic hybridization arrays and single-nucleotide polymorphism arrays) have been proven to be a first-tier choice for clinical diagnostics of developmental delay and dysmorphic features.

Methods and results. In the present article we describe the clinical advantages of molecular cytogenetic approach (comparative genomic hybridization arrays and single nucleotide polymorphism arrays) in the diagnostic procedure of two children with developmental delay, dysmorphic features and additional morphological phenotypes. Additionally, we demonstrate the necessity of fluorescent in situ hybridization utilisation to identify the localisation and underlying mechanism of detected chromosomal rearrangement.

Conclusions. Two types of chromosomal abnormalities were identified and confirmed using different molecular genetic approaches. Comparative genomic hybridization arrays and single nucleotide polymorphism arrays are hereby presented as important methods to identify chromosomal imbalances in patients with developmental delay and dysmorphic features. We emphasize the importance of molecular genetic testing in patients' parents for the demonstration of the origin and clinical importance of the aberrations prior determined in the patients. The results obtained using molecular cytogenetic high resolution techniques methods are the cornerstone for proper genetic counselling to the affected families.

Keywords:
copy number variations, CGH-array, SNP-array, FISH

IZVLEČEK

***Uvod**. Razvojni zaostanek in displastične znake ugotavljamo pri 1-3% otrok. Molekularne citogenetske tehnike z visoko ločljivostjo (CGH- in SNP-mikromreže) so v zadnjih letih postale ključna preiskava v rutinski klinični diagnostiki pri preiskovancih z razvojnim zaostankom, displastičnimi znaki in drugimi nepravilnostmi.*

***Metode in rezultati**. V prispevku želimo prikazati klinične prednosti molekularnega citogenetskega pristopa v diagnostičnem postopku dveh otrok z razvojnim zaostankom, displastičnimi znaki in drugimi nepravilnostmi. Potrditev kromosomske preureditve z metodo FISH je potrebna za opredelitev točne kromosomske lokacije in mehanizma nastanka kromosomske nepravilnosti.*

***Zaključek**. V prispevku predstavljamo dva tipa kromosomskih nepravilnosti, ki smo jih ugotovili in potrdili z različnimi molekularnimi metodami. Poudariti želimo pomen potrjevanja in analize pri starših za opredelitev izvora nastanka kromosomske preureditve. Rezultati genetske preiskave so ključni pri genetskem svetovanju prizadetim posameznikom in njihovim družinam.*

Ključne besede:
variacije v številu kopij, CGH-mikromreže, SNP-mikromreže, FISH

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1 INTRODUCTION

Developmental delay (DD) and dysmorphic features are common in the paediatric practice affecting 1–3 % of children (1). Although the aetiology is heterogeneous, microscopic and submicroscopic copy number variants (CNVs) are among the most common genetic causes (2, 3). It has been shown that molecular karyotyping should be a first-tier clinical diagnostic test for individuals with DD and dysmorphic features (4, 5) as clinically relevant CNVs have been detected in 10–20 % of cases (6). Introduction of comparative genomic hybridization arrays (CGH-array) and single-nucleotide polymorphism arrays (SNP-array) have revolutionized the molecular cytogenetic diagnostics in the past few years (7). CGH- and SNP-arrays allow the mapping of genomic imbalance at submicroscopic level, thereby directly linking disease phenotypes to gene dosage alterations (8). At the same time fluorescent *in situ* hybridization (FISH) analysis remains an important tool for confirmation and chromosomal identification of detected genomic imbalances and their breakpoints.

We report the case of two children with DD, dysmorphic features and additional morphological phenotypes. Additionally, the identification of chromosomal rearrangement using different molecular cytogenetic techniques is described, and the clinical usefulness of molecular cytogenetic approaches in the diagnostic procedure discussed.

2 CLINICAL ASSESSMENT OF PATIENTS

2.1 Case 1

A 12-year-old girl with developmental delay, dysmorphic features, epilepsy, obesity, hypercholesterolemia and hypoplastic posterior corpus callosum is presented. She is treated at the Department for paediatric endocrinology, diabetes and metabolism at University Children's Hospital in Ljubljana, Slovenia. The girl was born at term after uneventful pregnancy to healthy non-consanguineous parents. Birth weight was 2890 g (10-25 P), and the birth length 47 cm (10 P); head circumference (HC) data was not given. She had feeding difficulties in the first few weeks of life. Speech and motor developmental delay was observed in early childhood. At the age of 5 years, epilepsy was diagnosed. Head MR showed hypoplastic posterior corpus callosum. Thereafter, she gained excessive weight and hypercholesterolemia was diagnosed. At 11 years of age, her height was 161 cm (99 P), she weighted 68.75 kg (100 P), HC 52.2 cm (50%), and a marked developmental speech delay was determined. The following facial dysmorphic features were described: thin, long face, high forehead, full cheeks, deep naso-labial sulcus, small mouth, high vaulted palate and thin, long and flat philtrum. The eyes were wide open, while the neck was short with low-set hairline and ears. The nipples were widely spaced.

2.2 Case 2

A 13-year-old boy with developmental delay, dysmorphic features, atrial septal defect and recurrent infections is presented. He is treated at the Institute for Maternal and Child Health-IRCCS 'Burlo Garofolo,' Trieste, Italy. He was born to healthy non-consaguine parents with no diagnosed Mendelian disorders or neurologic disorders in the family. Apart from heart abnormality and frequent infections, no additional relevant findings were determined. Ophtamologic and otorynolaringologic examinations were normal. Aged 7 years, 10 months, the presence of mild dysmorphic features was determined, namely: wide forehead, telecanthus, flat line (malar bone hypoplasia) and thick lips.

3 METHODS

All cytogenetics and molecular-cytogenetics analyses were performed with written and signed informed consent. Cytogenetic postnatal analyses were carried out on peripheral blood lymphocytes from patients and their parents. Genomic DNA was extracted from whole blood, using FlexiGene DNA isolation kit (Qiagene GmbH, Hilden, Germany).

3.1 Cytogenetic and FISH Analysis

Chromosome analysis using GTG banding on metaphases was performed according to standard procedures. Additional FISH experiments were undertaken using locus specific and sub-telomere FISH probes (BlueGnome and Vysis, Abbott). Hybridization and washing was done according to the manufacturer's protocol, and a minimum of 100 interphase cells was analyzed. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole, and images were captured using the CytoVysion Imaging System.

3.2 Comparative Genomic Hybridization Microarray

DNA was hybridized to Agilent 60K human CGH microarray (Agilent Technologies, Inc., Santa Clara, Calif., USA). Discovered copy number variants were identified using Agilent CytoGenomics edition 2.0.6.0 software and interpreted according to the publicly available databases: Database of Genomic Variants (http://projects.tcag.ca/variation/gbrowse/hg19), PubMed and ISCA database (http://www.iscaconsortium.org/).

3.3 Single Nucleotide Polymorphism Microarray

DNA was processed using the Illumina HumanCNV370-Quad/OmniExpress genotyping microarray according to the protocol. Data analysis was performed using the Illumina GenomeStudio v.2011 and Illumina cnvPartition (ver 3.2.0) software program.

4 RESULTS

In the first case, postnatal CGH-array revealed 5.4 ± 0.05 Mb duplication at the cytoband location 17p13.3p13.2 in the absence of additional relevant submicroscopic aberrations. Conventional cytogenetic analysis on metaphases from peripheral blood showed an unbalanced karyotype with an additional chromosomal material on chromosome 20. Partial trisomy of the segment 17p13.2 was confirmed with FISH probe specific for region 17p13.2 (Figure 1). Parental chromosomal analysis showed that derivative chromosome 20 was inherited from the mother, a carrier of apparently balanced reciprocal translocation between chromosomes 17 and 20 (Figure 2).

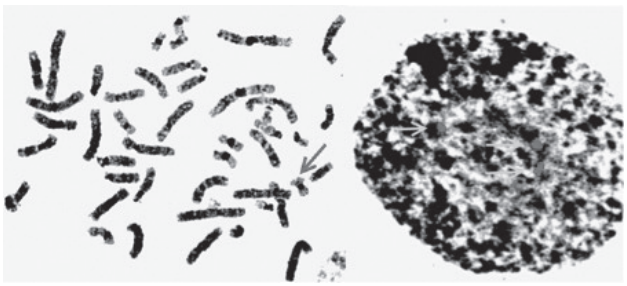

Figure 1. The subject's metaphase (left) and interphase (right) FISH for region 17p13.2 (RP11-104019; Blue Gnome, UK). The red arrow indicates the signal (pink) of the probe on chromosome 20. The yellow arrows indicate three signals (red) in interphase nucleus for region 17p13.2.

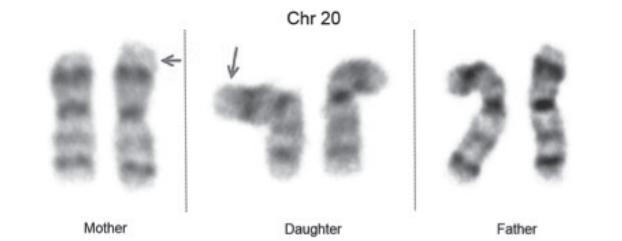

Figure 2. The comparison of mother's, daughter's and father's chromosome 20. The red arrow indicates the slightly enlarged region where the material of chromosome 17 is present.

In the second case, postnatal karyotype analysis carried out on lymphocytes was considered normal (46, XY). Furthermore, SNP array analysis (Figure 3) revealed a duplication of 14.9 Mb in the 4p16.3p15.33 regions and a deletion of 14 Mb in the 4q34.2q35.2 regions. The parents refused to be submitted to analysis.

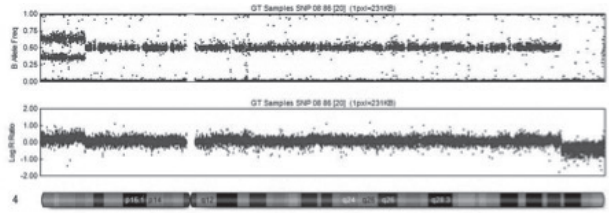

Figure 3. The results of *SNP array [arr 4p16.3p15.33(17,764-15, 392,559)x3,4q34.2q35.2(176,447,305-190,977,969) x1]* revealing the presence of rearranged chromosome 4.

5 DISCUSSION

Two children with developmental delay and dysmorphic features are presented. Using different molecular cytogenetic approaches they were both found to harbour genomic imbalances.

In Case 1, the child inherited the derivative chromosome 20 from the mother, who is the carrier of the apparently balanced translocation between chromosomes 17 and 20. Partial trisomy 17pter is causative for the phenotype of the proband, and is consistent with 17p13 microduplication syndrome (9). The duplication sizes in previously reported cases varied from 0.24 to 4.0 Mb, and their phenotype mainly depends on altered expression of *PAFAH1B1* gene (10). There are nine cases reported in the medical literature with a pure 17p13 microduplication syndrome caused by an interstitial duplication or a terminal duplication of 17p13. These patients had mild to moderate psychomotoric retardation and dysmorphic features, including high forehead, small mouth and nose, together with dysgenesis of corpus callosum (9, 11). The expressed phenotype of our patient may be due to the synergistic effect of 17p13.3 duplication and accompanying translocation that may cause the disruption of the gene at the translocation breakpoints. The short arm of chromosome 17 is particularly prone to submicroscopic rearrangements due to the presence of high density low copy repeats (LCRs) (12). The translocation of a small portion of chromosome 17, with only a p telomeric/subtelomeric cap on chromosome 20, is derived from a presumable mechanism of single-segment exchange (13). It has been suggested that reciprocal translocations are the result of recombination between repetitive sequences, such as between a variable number of tandem repeats or AT-rich regions, as observed in the case of the common recurrent translocation t(11;22) (14). Combining the results of CGH-array and FISH analysis, we can postulate that patients with unbalanced translocation involving 17pter chromosomal region, have similar clinical phenotype compared to pure 17p13 microduplication syndrome. Using CGH-array, the breakpoints in 17p13.2 region were identified, while FISH results revealed exact localization and mechanism of the chromosomal

aberration. Nevertheless, the introduction of array technology into the routine clinical diagnostic procedures revealed a wide variety of new microduplication syndromes (15).

SNP-array in the second case revealed the duplication of 4p and the deletion of 4q, which indicates the presence of recombinant chromosome 4. Both the deletion and the duplication have approximately the same size and similar banding pattern, hence the aberration was not detected by conventional karyotyping. Recombinant chromosomes arise from pericentric inversion of parental origin (16). To date, 11 cases of a similar rearrangements have been reported (17). The recombinant chromosome 4 [rec(4) dup(4p)] is present in 80 % of the viable recombinants (13), since the deletion seems to have more deleterious effect than large duplications (18). Interestingly, all cases in the literature have breakpoints within 4p13 ~ 4p15 and 4q35 implicating a recurrent event between repetitive DNA sequences predisposing pericentric inversion at these hotspots. During meiotic crossing over chromosome with pericentric inversion may give rise to recombinants with duplicated p or duplicated q arm.

The association between specific chromosome 4 rearrangements and clinical features was not recognized within the earliest reports due to variations in the sizes of the 4q deletion, differences in the breakpoints, and variable expression of the partial trisomy of the 4p (18). It has been suggested that rec(4)dup(4p) represents a discrete entity with consistent phenotype of growth retardation, microcephaly, dysmorphic features and genital anomalies. Additionally, 5 of 11 cases present with cardiac defect (19). Although recombinant chromosome 4 is a rare chromosomal anomaly, several genotype-phenotype correlation studies have been conducted, revealing that urogenital and cardiac defects are probably due to the deletion of 4q, whereas other clinical features are likely due to 4p duplication (19). It is now recognized that clinical features of patients with rec(4) are relatively consistent and specific to the regions of duplication or deletion (17). Since our case has similar clinical features compared to other reported cases, we can conclude that recombinant chromosome 4 syndrome can be diagnosed on the basis of clinical features and specific deleted and duplicated chromosomal regions. Using SNP-array and specific clinical features, the breakpoints and their origin can be precisely characterized even without parental analysis

6 CONCLUSION

CGH and SNP-arrays are ideal diagnostic tools to identify chromosomal imbalances, their breakpoints and origin in patients with developmental delay and dysmorphic features (3, 4, 7, 15). In addition, SNP-arrays may offer further information regarding segmental uniparental disomies and absences of heterozygosity when required. On the other hand, FISH analysis is used to confirm the presence of chromosomal aberration and its exact location, revealing underlying mechanism of genomic rearrangement. In addition, follow-up parental studies are recommended to demonstrate the origin of the rearrangement, in order to offer more accurate genetic counselling to the families, while pre- and post-diagnostic genetic counselling should be available to anyone undergoing molecular karyotyping.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Written informed consent was obtained from all patients or/and their parents.

REFERENCES

1. Shevell M, Ashwal S, Donley D, Flint J, Gingold M. et al. Practice parameter: evaluation of the child with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and The Practice Committee of the Child Neurology Society. Neurology 2003; 60: 367-80.
2. Regier DA, Friedman JM, Marra CA. Value for money? Array genomic hybridization for diagnostic testing for genetic causes of intellectual disability. Am J Hum Genet 2010; 86: 765-72.
3. Bartnik M, Nowakowska B, Derwińska K, Wiśniowiecka-Kowalnik B, Kędzior M, Bernaciak J. et al. Application of array comparative genomic hybridization in 256 patients with developmental delay or intellectual disability. J Appl Genet 2014; 55: 125–44.
4. Battaglia A, Doccini V, Bernardini L, Novelli A, Loddo S. et al. Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features. Eur J Paediatr Neurol 2013; 17: 589-99.
5. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR. et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010; 86: 749-64.
6. Koolen DA, Pfundt R, de Leeuw N, Hehir-Kwa JY, Nillesen WM. et al. Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. Hum Mutat 2009; 30: 283-92.
7. Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, Goldstein A. et al. Phenotypic heterogeneity of genomic disorders and rare copy-number variants. N Engl J Med 2012; 367: 1321-31.
8. Vissers LE, Stankiewicz P Microdeletion and microduplication syndromes. Methods Mol Biol 2012; 838: 29-75.
9. Roos L, Jonch AE, Kjaergaard S, Taudorf K, Simonsen H, Hamborg-Petersen B, Brondum-Nielsen K et al. A new micro-duplication syndrome encompassing the region of the Miller-Dieker (17p13 deletion) syndrome. J Med Genet 2007; 46: 703–10.

10. Avela KI, Aktan-Collan K, Horelli-Kuitunen N, Knuutila S, Somer M. A microduplication on chromosome 17p13.1p13.3 including the PAFAH1B1 (LIS1) gene. Am J Med Genet A 2011; 155A: 875-9.
11. Bi W, Sapir T, Schelochkov O, Zhang F, Withers MA, Hunter JV, Levy T. et al. Increased LIS1 expression affects human and mouse brain development. Nat Genet 2009; 41: 168–77.
12. Capra V, Mirabelli-Badenier M, Stagnaro M, Rossi A, Tassano E, Gimelli S, Gimelli G. Identification of a rare 17p13.3 duplication including the BHLHA9 and YWHAE genes in a family with developmental delay and behavioural problems. BMC Med Genet 2012; 13: 93.
13. McKinlay Gardner RJ, Sutherland GR, Shaffer LG. Chromosome abnormalities and genetic counselling. 3rd edition. Oxford: University Press, 2004.
14. Shaffer LG, Lupski JR. Molecular mechanisms for constitutional chromosomal rearrangements in humans. Annu Rev Genet 2000; 34: 297-329.
15. Shinawi M, Cheung SW. The array CGH and its clinical applications. Drug Discov Today. 2008; 13: 760-70.
16. Gersan, Keagl. Principles of clincial cytogenetics. 2nd edition, 2005.
17. Hemmat M, Hemmat O, Anguiano A, Boyar FZ, El Naggar M. et al. Genotype-phenotype analysis of recombinant chromosome 4 syndrome: an array-CGH study and literature review. Mol Cytogenet 2013; 6: 17.
18. Garcia-Heras J, Martin J. A rec(4) dup 4p inherited from a maternal inv(4)(p15q35): case report and review. Am J Med Genet 2002; 109: 226-30.
19. Battaglia A, Brothman AR, Carey JC. Recombinant 4 syndrome due to an unbalanced pericentric inversion of chromosome 4. Am J Med Genet 2002; 112: 103-6.

ASSOCIATION OF AVERAGE TELOMERE LENGTH WITH BODY-MASS INDEX AND VITAMIN D STATUS IN JUVENILE POPULATION WITH TYPE 1 DIABETES

POVEZAVA POVPREČNIH DOLŽIN TELOMEROV Z INDEKSOM TELESNE TEŽE IN VITAMINOM D PRI MLADOSTNIKIH S SLADKORNO BOLEZNIJO TIPA 1

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ABSTRACT

Background. Type 1 diabetes (T1D) is an autoimmune chronic disease where hyperglycemia, increased risk of oxidative stress, advanced glycation end-products and other genetic and environmental factors lead to T1D complications. Shorter telomeres are associated with hyperglycemic levels and lower serum vitamin D levels.

Methods. Average telomere length (ATL) in whole blood DNA samples was assessed with qPCR method in 53 Slovenian T1D children/adolescents (median age 8.7 years, 1:1.3 male/female ratio). Body mass index standard deviation score (BMI-SDS), glycated haemoglobin and serum level of vitamin D metabolite (25-(OH)-D3) and the age at the onset of T1D were collected from the available medical documentation.

Results. Results indicate shorter ATL in subjects with higher BMI-SDS when compared to those with longer ATL (0.455 ± 0.438, -0.63 ± 0.295; p=0.049). Subjects with higher BMI-SDS had lower serum vitamin D levels when compared to those with lower BMI-SDS (40.66 ± 3.07 vs. 52.86 ± 4.85 nmol/L; p=0.045). Vitamin D serum levels did not significantly differ between subjects with longer/shorter ATL.

Conclusion. T1D children/adolescents with shorter ATL tend to have higher BMI-SDS. Lower serum vitamin D levels were associated with higher BMI-SDS, while associations between vitamin D serum levels, age at the onset of T1D, glycated haemoglobin and ATL were not observed. Additional studies with more participants are required to clarify the role of the telomere dynamics in T1D aetiology and development of complications.

Keywords:
type 1 diabetes, juvenile patients, average telomere length, vitamin D, body mass index

IZVLEČEK

Izhodišče. *Sladkorna bolezen tipa 1 (SBT1) je kronična avtoimunska bolezen, pri kateri hiperglikemija ter zvišana raven oksidativnega stresa in končnih produktov glikacije skupaj z genetskimi in okoljskimi dejavniki privedeta do nastanka diabetičnih zapletov. Krajše dolžine telomerov so povezane s hiperglikemičnimi epizodami in nižjimi serumskimi vrednostmi vitamina D.*

Metode. *Z metodo qPCR smo iz vzorcev DNK periferne krvi določili povprečne dolžine telomerov 53 slovenskim bolnikom s SBT1 (povprečna starost 8,7 leta, razmerje med dečki in deklicami 1:1,3). Indeks standardnega odklona indeksa telesne teže (BMI-SDS), vrednosti serumskega metabolita vitamina D - 25-hidroksikalcifediola (25-(OH)-D3), glikiran hemoglobin in starost preiskovancev ob izbruhu bolezni smo pridobili iz razpoložljive medicinske dokumentacije.*

Rezultati. *Rezultati nakazujejo krajše dolžine telomerov pri bolnikih z višjimi vrednostmi BMI-SDS (0,455 ± 0,438, -0,63± 0,295; p=0,049). Preiskovanci z višjimi vrednostmi BMI-SDS so imeli nižje vrednosti 25-(OH)-D3 kot preiskovanci z nižjimi vrednostmi BMI-SDS (40,66 ± 3,07 proti 52,86 ± 4,85 nmol/L; p=0,045). Vrednosti 25-(OH)-D3 niso statistično značilno različne pri preiskovancih z višjimi oziroma nižjimi povprečnimi dolžinami telomerov.*

Zaključki. *Otroci in mladostniki s SBT1 s krajšimi dolžinami telomerov imajo nekoliko višje vrednosti BMI-SDS. Nižje vrednosti 25-(OH)-D3 so povezane z višjim BMI-SDS. Povezav med serumskimi vrednostmi 25-(OH)-D3, starostjo bolnikov ob izbruhu bolezni, glikiranim hemoglobinom in povprečnimi dolžinami telomerov nismo zaznali. Za razjasnitev vloge telomerov v etiologiji, patogenezi in nastanku zapletov SBT1 bodo potrebne nadaljnje raziskave z večjim številom preiskovancev.*

Ključne besede:
sladkorna bolezen tipa 1, otroci, mladostniki, povprečna dolžina telomerov, indeks telesne teže

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1 INTRODUCTION

Telomeres are nucleoprotein structures located at the end of the chromosomes, the role of which is to ensure genomic stability, and prevent chromosomal breaks and fusions. Telomeres are composed of repetitive hexameric DNA sequence TTAGGG, and bounded with proteins of shelterin complex, namely: TRF1, TRF2, TPP1, POT1, RAP1 and TIN2. Their role is the regulation of telomere length, protection, DNA damage repair and control in signalling cascade. At the 3' end of telomere, DNA forms single stranded T loop structure (1) with characteristic secondary quadruplex structures (Figure 1) (2). Telomere length is very variable and can vary between different cell types, chromosomes and even between ends of the same chromosome (3).

Telomere ends are prone to replication shortening due to the inability of the polymerases to completely replicate telomere 3' ends in the process of semiconservative replication (4). The enzyme telomerase reverse transcriptase (TERT) is capable of sustaining telomere stability, but only in steam and germ cells (5, 6), while in other somatic cells, TERT expression level is very low and the telomeres are shortened by every cell cycle for 100-200 bases (1), resulting in a limited number of cell replications. When telomeres reach the critical length (Hayflick limit), the cell enters the senescence, stagnation and finally the apoptosis (4, 7).

The telomere length is also affected by environmental and genomic factors, transcriptional control, oxidative stress, inflammation, immune system, endocrine system, DNA damage and other. Additionally, mutations in TERT genes can lead to the development of dyskeratosis congenita, aplastic anaemia and other diseases associated with telomere dysregulation (8, 9).

Environmental factors, chronic and autoimmune diseases can induce cell stress, resulting in increased cellular concentration of free radicals, which can damage proteins, lipids and nucleic acids (10). Deoxyguanosine has the lowest redox potential of all deoxynucleotides, and is most susceptible to reactions with reactive oxygen species (2). Since telomere tandem repeats are guanosine rich, they present highly susceptible sites for reaction with reactive oxygen species and consequential DNA breaks. Additionally, the shelterin complex attached to the telomeric DNA sequence hampers DNA repair (1). Oxidative stress can also trigger inflammation, which is associated with increased proliferation of immune cells and, consequently, with an increased rate of telomere shortening (9). Inflammation is present in many chronic and autoimmune diseases, such as cardiovascular diseases, rheumatoid arthritis, systemic lupus erythematosus, type 1 and 2 diabetes (9, 11). Telomere dynamics and telomere length studies have shown that longer telomere length and slower telomere erosion rate are associated with better health status, better cognitive function, protection from age-related diseases, healthier lipid profile and lower mortality risk (9).

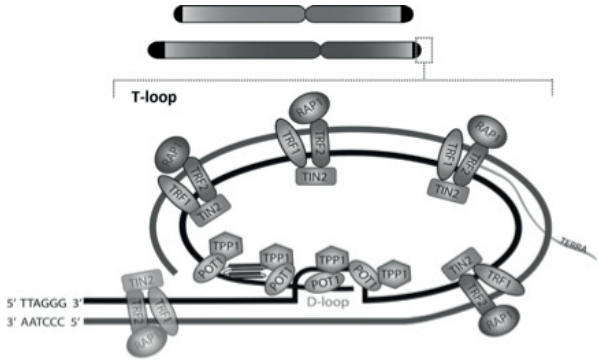

Figure 1. Telomeres and T-loop at the end of telomere. Telomeres are located at the end of chromosomes, their length differs between chromosomes and even between ends of the same chromosome. Proteins of the shelterin complex TRF1, TRF2, RAP1, TIN2, TPP1, POT1 and RNA molecule TERRA are attached to the telomere DNA sequence. The shelterin molecules with telomeric DNA sequence form T-loop, where the 3' end of double-stranded DNA becomes single-stranded, and it is displaced back inside a double-stranded DNA in D-loop area (adopted by reference 1 and 3).

1.1 Telomere Length and Type 1 Diabetes

Type 1 diabetes (T1D) is a chronic autoimmune disease, characterized by the state of hyperglycemia, as a result of an autoimmune destruction of β pancreatic cells (12). Participants with T1D do not produce enough insulin and require exocrine insulin therapy. Disease develops gradually and bursts innately in childhood (13). Self-control of the blood sugar level and appropriate dosages of insulin are crucial to reduce the risk for development of diabetic complications (14).

Increased levels of reactive oxygen species are present during the development, onset and duration of diabetes (10, 15). Only a small number of studies have been published analysing the length of telomeres in T1D. It was reported that T1D subjects have shorter telomere length compared to healthy controls (16); in addition, participants with good glycemic control have longer telomere length than the participants with a poorly-controlled one, but the studies were conducted on a relatively small group of participants (17). The telomere length was also assessed in diabetic participants as a prediction factor for diabetic nephropathy (18) and mortality as a result of diabetic nephropathy (19). Both studies did not show any association between the telomere length and diabetic complications.

Moreover, all published studies have investigated the telomere length in adult population, while the telomere length and telomere dynamics in cohort of juvenile participants with T1D has not been analysed yet. Research on participants with rheumatoid arthritis revealed that participants with an earlier onset of the disease have

shorter telomere length (20), so we aimed to examine this correlation in T1D as well. The aim was also to characterize telomere length and telomere dynamics in juvenile participants and in addition, to evaluate the potential correlation of telomere length with the available clinical parameters that had been determined at the onset of the disease.

2 METHODS

2.1 Participants

Fifty-three participants with diagnosed T1D, 23 boys and 30 girls (1:1.3 male/female ratio), were included into the study. The participants were from 4 to 14 years old, with mean age at the onset of the disease 8.65 ± 2.61 years, and no additional diagnosed autoimmune diseases. All participants were diagnosed and treated at The Department of Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, UMC Ljubljana, Slovenia.

Available data at the first hospitalization of the participants at the onset of the disease for BMI standard deviation score (BMI-SDS) (mean 0.36 ± 1.59), age at the onset of the disease (mean 8.65 ± 2.61 years), serum metabolite of vitamin D 25-hydroxycalcifediol (25-(OH)-D3) concentration (mean 47.00 ± 16.66 nmol/L) and glycated haemoglobin levels (mean 11.04 ± 0.26 %) were collected from the available medical documentation. The research protocol was approved by the Slovene Medical Ethics Committee (Nr: 29/02/13).

2.2 DNA Isolation

Genomic DNA was extracted from whole peripheral blood, collected in blood tubes with EDTA, using a FlexiGene DNA isolation kit (Qiagene, Germany) by the established protocol. Extracted DNA was stored on 4 °C at a concentration of approximately 350 ng/μL for less than 2 years and it was diluted to working DNA stock solution with concentration 5 ng/μL.

2.3 Telomere Length Measurement

We determined ATL with modified Cawthon's method of monochrome multiplex quantitative real time PCR (MMQPCR) (21). The basic principle of this method is the determination of telomere product (T) and amount of reference gen beta-globin (S) in a single well. Triplicated measurements of T and S of the same sample were used to calculate the average T/S ratio. The sample used for normalization of the T/S results across different MMQPCR experiments was the DNA sample of the participant with the lowest level of glycated haemoglobin at the onset of the T1D.

PCR reaction mix was composed by 3 μL/well MeltDoctor™ HRM Master Mix (Applied Biosystems®), 1.00 pmol/well of each telomere primer (telG: ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT; telC: TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAAC), 0.55 pmol/well of each reference gen beta globin primer (hbgu: CGGCGGCGGGCGGCGCGGGCTGGGCGGcttcatccacgttcaccttg; hbgd: GCCCGGCCCGCCGCGCCCGTCCCGCCGgaggagaagtctgccgt) and 0.02 μL/well ROX. Final reaction volume was 6 μL/well and it contained 10 ng of sample DNA.

MMQPCR was performed on 96-well plates on Applied Biosystems 7500 Fast Real Time PCR System with two-part program. We determined Ct value for T factor of T/S analysis in the first run which was immediately followed by the second part of MMQPCR. The Ct value of the second part was used for determination of the amount of the reference gene (S).

The part 1 thermal cycling profile was: 15 min at 95 °C (holding stage), 2 cycles of 15 s at 95 °C and 15 s at 95 °C (cycling stage) and 21 cycles of 15 s at 95 °C, 10 s at 62 °C, 15 s at 71 °C, with signal acquisition (cycling stage), followed by the part 2 thermal profile: 17 cycles of 17 s at 94 °C, 10 s at 62 °C, 15 s at 74°C, 20 s at 84 °C, 15 s at 87 °C with data acquisition (cycling stage), followed holding stage 20 s on 50 °C. For Ct determination, the same threshold cycle levels were used in both, part 1 and part 2, runs. The T/S calculation was performed on the samples where the standard deviation of average Ct-value for T and S factors across the triplicates did not exceeded 10 %.

2.4 Determination of Telomere Length and Statistical Analysis

The Ct data of both experiment runs were used to calculate T/S using ΔΔCt-method. The average T/S of a sample was calculated using data from three parallel experiments, and only results with standard deviation lower than 10 % were included in further analysis. The D'Agostino-Pearson omnibus normality test was performed to assess the data deviation from normal distribution. Consequently, the average T/S values of each sample were correlated against clinical and anthropometric parameters determined at the onset of T1D using Spearman correlation. The differences between upper and lower tertile test groups were assessed with unpaired Welch's t test. The statistical GraphPad Prism software was used for statistical analysis. The p-value below 0.05 was considered to show statistically significant difference or correlation.

3 RESULTS

The ATL was assessed in 53 children with T1D and correlated to clinical and anthropometric parameters determined at the time of the disease onset. Average standard deviation of measured T/S values was 5.25 %.

ATL showed weak negative correlation with BMI-SDS values (0.241; p=0.082). This tendency was confirmed with Welch's test of lower and higher ATL tertiles where

the difference between average BMI-SDS was statistically significant (0.455 ± 0.438, -0.630 ± 0.295; p=0.049). The correlation between ATL and other investigated parameters was not present. The analysis also revealed negative correlation between 25-(OH)-D3 and BMI-SDS (0.364; p=0.018), where the participants in lower tertile of BMI-SDS had higher values of 25-(OH)-D3 than the participants in higher tertile of BMI-SDS (52.86 ± 4.85 nmol/L; 40.66 ± 3.07 nmol/L; p=0.045). The associations between ATL, the participants' age at the time of the T1D onset and the level of glycated haemoglobin were not statistically significant.

4 DISCUSSION

This was the first study where ATL of juvenile participants with T1D at the onset of the disease was investigated. The correlation between ATL and the age of the participants at the onset of T1D was not present. The results show a tendency for negative correlation between BMI-SDS and ATL and are in agreement with previously reported large case-control study on French obese children (22). One of the possible explanations for this phenomena is increased chronic inflammation, resulting in higher leukocytes proliferation (23) due to increased oxidative stress in diabetic participants (24). Additionally, it was observed that participants with higher BMI-SDS had lower serum level of vitamin D. Furthermore, the decreased bioavailability of vitamin D from cutaneous and dietary sources in obese people could be caused by increased bioaccumulation in adipose tissue (25). Previous studies have reported that 25-(OH)-D3 has immunomodulatory and preventive role in T1D and that participants with T1D have higher prevalence of 25-(OH)-D3 deficiency (12). Association between serum vitamin D levels and ATL has already been reported, but this association was not observed in our study (26). This may be due to the relatively small number of participants involved in our study. Nevertheless, the telomere length was reported as a biomarker of negative effects of oxidative stress and inflammation and as such, it has a potential as a predictive factor for various disease development (9), especially due to the fact that recent studies revealed an indirect involvement of shelterin complex proteins with the regulation of metabolism (27).

It is crucial to increase the number of participants in future studies to investigate the potential involvement of the telomere dynamics in the aetiology of T1D. Nevertheless, the results confirm the findings of the previously reported studies, and indicate that the modified MMQPCR method is adequate for ATL assessment. It would also be reasonable to introduce age matching healthy controls to identify the potential additional correlations between ATL and other parameters, and to elucidate if the factors of telomere dynamics have a role in the development of T1D.

5 CONCLUSION

T1D children/adolescents with a shorter ATL tend to have a higher BMI-SDS. Additionally, lower serum vitamin D levels were determined in T1D subjects with a higher BMI-SDS, while associations between serum vitamin D levels, glycated haemoglobin and ATL were not determined. Additional research with a higher number of participants would be required to clearly establish the role of the telomere dynamics in T1D aetiology.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

The research protocol was approved by the Slovene Medical Ethics Committee (Nr: 29/02/13).

REFERENCES

1. O'Sullivan RJ, Karlseder J. Telomeres: protecting chromosomes against genome instability. Nat Rev Mol Cell Biol 2010; 11: 171–81.
2. Fleming AM, Burrows CJ. G-quadruplex folds of the human telomere sequence alter the site reactivity and reaction pathway of Guanine oxidation compared to duplex DNA. Chem Res Toxicol 2013; 26: 593–607.
3. O'Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere length. Biol Proced Online 2011; 13: 3.
4. Von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002; 27: 339–44.
5. Gomez DE, Armando RG, Farina HG, Menna PL, Cerrudo CS, Ghiringhelli PD. et al. Telomere structure and telomerase in health and disease: review. Int J Oncol 2012; 41: 1561–9.
6. Xi H, Li C, Ren F, Zhang H, Zhang L. Telomere, aging and age-related diseases. Aging Clin Exp Res 2013; 25: 139–46.
7. Hohensinner PJ, Goronzy JJ, Weyand CM. Telomere dysfunction, autoimmunity and aging. Aging Dis 2011; 2: 524–37.
8. Weng N. Telomeres and immune competency. Curr Opin Immunol 2012; 24: 470–5.
9. Shalev I. Early life stress and telomere length: investigating the connection and possible mechanisms: a critical survey of the evidence base, research methodology and basic biology. BioEssays News Rev Mol Cell Dev Biol 2012; 34: 943–52.
10. Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 2011; 50: 567–75.
11. Georgin-Lavialle S, Aouba A, Mouthon L, Londono-Vallejo JA, Lepelletier Y, Gabet AS. et al. The telomere/telomerase system in autoimmune and systemic immune-mediated diseases. Autoimmun Rev 2010; 9: 646–51.
12. Chakhtoura M, Azar ST. The role of vitamin d deficiency in the incidence, progression, and complications of Type 1 diabetes mellitus. Int J Endocrinol 2013: 148673. Epub 2013 Mar 13.

13. Drobnič Radobuljac M, Bratina N, Tomori M, Battelino T. Type 1 diabetes and psychosocial risk factors in adolescence. Zdrav Vest 2012; 81: 664-75.
14. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012; 36(Suppl 1): S67–74.
15. Hovnik T, Dolzan V, Bratina NU, Podkrajsek KT, Battelino T. Genetic polymorphisms in genes encoding antioxidant enzymes are associated with diabetic retinopathy in type 1 diabetes. Diabetes Care 2009; 32: 2258–62.
16. Jeanclos E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH. et al. Shortened telomere length in white blood cells of patients with IDDM. Diabetes 1998; 47: 482–6.
17. Uziel O, Singer JA, Danicek V, Sahar G, Berkov E, Luchansky M. et al. Telomere dynamics in arteries and mononuclear cells of diabetic patients: effect of diabetes and of glycemic control. Exp Gerontol 2007; 42: 971–8.
18. Fyhrquist F, Tiitu A, Saijonmaa O, Forsblom C, Groop P-H, FinnDiane Study Group. Telomere length and progression of diabetic nephropathy in patients with type 1 diabetes. J Intern Med 2010; 267: 278–86.
19. Astrup AS, Tarnow L, Jorsal A, Lajer M, Nzietchueng R, Benetos A. et al. Telomere length predicts all-cause mortality in patients with type 1 diabetes. Diabetologia 2010; 53: 45–8.
20. Colmegna I, Diaz-Borjon A, Fujii H, Schaefer L, Goronzy JJ, Weyand CM. Defective proliferative capacity and accelerated telomeric loss of hematopoietic progenitor cells in rheumatoid arthritis. Arthritis Rheum 2008; 58: 990–1000.
21. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res 2009; 37: e21–21.
22. Buxton JL, Walters RG, Visvikis-Siest S, Meyre D, Froguel P, Blakemore AIF. Childhood obesity is associated with shorter leukocyte telomere length. J Clin Endocrinol Metab 2011; 96: 1500–5.
23. Aviv A, Valdes AM, Spector TD. Human telomere biology: pitfalls of moving from the laboratory to epidemiology. Int J Epidemiol 2006; 35: 1424–9.
24. Domínguez C, Ruiz E, Gussinye M, Carrascosa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care 1998; 21: 1736–42.
25. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000; 72: 690–3.
26. Liu JJ, Prescott J, Giovannucci E, Hankinson SE, Rosner B, Han J. et al. Plasma vitamin D biomarkers and leukocyte telomere length. Am J Epidemiol 2013; 177: 1411-7.
27. Martínez P, Gómez-López G, García F, Mercken E, Mitchell S, Flores JM. et al. RAP1 protects from obesity through its extratelomeric role regulating gene expression. Cell Rep 2013; 3: 2059–74.

SUPPORT GROUP FOR PARENTS COPING WITH CHILDREN WITH TYPE 1 DIABETES

SKUPINA ZA STARŠE KOT PODPORA DRUŽINAM PRI SOOČANJU Z OTROKOVO SLADKORNO BOLEZNIJO TIPA 1

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ABSTRACT

Objectives. Type 1 diabetes is one of the most common chronic diseases in childhood. Active parental involvement, parental support in the diabetes management and family functioning are associated with optimal diabetes management and glycemic control. The purpose of this study was to assess parental satisfaction with participation in the group and their perceptions of the impact of the intervention on living and coping with childrens T1D.

Methods. A sample of 34 parents of children with T1D participated in this trend study. The participants' experience and satisfaction with support group was measured by a self- evaluation questionnaire, designed for the purpose of the present study.

Results. Quantitative data show that parents were overall satisfied with almost all measured items of the evaluation questionnaire (wellbeing in the group, feeling secure, experiencing new things, being able to talk and feeling being heard) during the 4-year period. However, parents from the second and third season, on average, found that the support group has better fulfilled their expectations than the parents from the first season ($p = 0,010$). The qualitative analysis of the participants' responses to the open-ended questions was underpinned by four themes: support when confronting the diagnosis, transformation of the family dynamics, me as a parent, exchange of experience and good practice and facing the world outside the family.

Discussion. The presented parent support group showed to be a promising supportive, therapeutic and psychoeducative space where parents could strengthen their role in the upbringing of their child with T1D.

Keywords:
mothers, fathers, emotional regulation, family functioning, relational family model

IZVLEČEK

Izhodišče. *Sladkorna bolezen tipa 1 je ena izmed pogostejših kroničnih bolezni v otroštvu. Optimalno vodenje in presnovna urejenost otrokove sladkorne bolezni sta povezana z aktivno vključenostjo staršev/skrbnikov, podporo otroku pri nadzoru nad boleznijo in funkcionalnostjo družine. Raziskava predstavlja oceno zadovoljstva staršev s programom skupine za starše in njihovo doživljanje vpliva omenjenega programa na življenje otroka in spoprijemanje z njegovo SBT1.*

Metode. *V raziskavi je sodelovalo 34 staršev otrok s SBT1. Udeleženci so izpolnili evalvacijski vprašalnik o izkušnji in zadovoljstvu s skupino za starše, ki je bil sestavljen za namen te raziskave.*

Rezultati. *Kvantitativni podatki so pokazali, da so bili starši v splošnem zadovoljni pri skoraj vseh merjenih postavkah (počutje v skupini, občutek varnosti, odkrivanje novih stvari, možnost pogovora, občutek slišanosti) v štirih sezonah skupine za starše, razen pri postavki izpolnitev pričakovanj. Udeleženci iz druge in tretje sezone so poročali, da je skupina izpolnila njihova pričakovanja v večji meri, kot pa so o svojih pričakovanjih poročali udeleženci iz prve sezone ($p = 0,010$). Kvalitativna analiza odprtih vprašanj je pokazala štiri teme: opora skupine pri soočanju z diagnozo SBT1, preoblikovanje družinske dinamike, vzgoja otroka s SBT1, izmenjava konkretnih izkušenj in dobrih praks ter soočanje z okoljem. Tema »opora skupine pri soočanju z diagnozo SBT1« je prevladujoča v vseh štirih sezonah.*

Razprava. *Skupina za starše se kaže kot pomemben terapevtski, podporni in psihoedukativni dejavnik; v njej starši ob voditeljih in drugih udeležencih krepijo svojo pomembno vlogo, ki jo imajo v življenju otroka s SBT1.*

Ključne besede:
matere, očetje, regulacija čustev, funkcionalnost družine, relacijski družinski model

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1 INTRODUCTION

A diagnosis of chronic illness, such as type 1 diabetes (T1D), in a child, has a substantial impact not only on the child's life but on the entire family (1). Parents have the key role in coping with the new way of life not only as caregivers but also as regulators of the child, and their own emotions influenced by chronic illness and the loss of a healthy child. Relevant literature shows the importance of parental role and experience in coping with T1D (2-4).

T1D is a chronic illness caused by autoimmune destruction process of insulin producing pancreatic ß cells, and is characterized by persistent hyperglycemia (5, 6). Treatment regimen includes frequent blood glucose monitoring, appropriate adjustments of insulin doses that match carbohydrate intake, exercise and stress. Daily self-management regimen is crucial to maintain optimal health, to avoid the most frequent acute complications such as high- (hyperglycemia) or low blood sugar (hypoglycemia) and, in the long run, also reduces the risk of long-term complications (5, 7, 8). The entire family has an important role in daily routine of careful self-management (9). Optimal metabolic control is associated with greater family cohesion (10, 11) and lower family conflicts (2-4, 12). Research shows that parents hold a high level of responsibility, are the main source of the child's support and an important link between the child and the health care team in diabetes management (1, 13). Continuous parent involvement that results in frequent blood glucose measurements is associated with better quality of life and metabolic control in children, but also with higher anxiety and stress in parents (14-17), especially in parents of younger children (18, 19) and mothers of children and adolescents (15). Mothers are more concerned and report greater fear of hypoglycemia (16, 20), parental stress, feelings of depression and diabetes burnout than fathers (15, 17, 21). These findings support the need for parent-based interventions that reduce parent distress while improving coping and supportive involvement (22-24). Studies show that support and psychoeducation groups for parents of children with T1D may decrease the influence of stressful treatment management and improve the quality of life in the sense of emphasizing the provision of information (knowledge about diabetes and general principles for management), or teaching specific coping skills to accomplish increased competence in medical management.

Relational Family Therapy (RFT) is a psycho-biological model based on the assumption that various patterns (of early relationships with parents, physical, emotional and behavioral sensations) are restoring throughout the life cycle on the systemic, interpersonal and intrapsychic level (25). Through interpersonal and systemic matrix of family relationships, members learn and develop skills of functional communication and emotion regulation. Parents of children with T1D find themselves in an entirely new role, and must regulate the child's physical (medication, food, rest) and psychological states (level of stress, emotions), as well as cope with the world outside their families (kindergarten, school, friends, co-workers, etc.), which may lead to the feelings of unacceptability, misunderstandings, guilt, anger and sadness (26). The Relational Family Model traces the mechanism of affect regulation, which plays an important role in the balancing of parental intrapsychic level of experiences as well as experiences in the parent-child relationship. The process of affect regulation may help a parent to identify and regulate her child's distress, influenced by the chronic illness, transform it into a form that is manageable and acceptable for a child, and explore new meanings of the illness (27, 28). The impact of T1D on the entire family directs the need for parent-centered interventions to reduce distress and improve mental health, psychological adjustment and coping.

The present study reports the experiences of the parents of children with T1D enrolled in the parent support group. The purpose of this study was to assess parental satisfaction with participation in the group and their perceptions of the impact of the intervention on living and coping with their children's T1D.

2 METHODS

2.1 Parent Support Group

The parent support group was designed to provide psychosocial support for parents of children with T1D. The program started in 2010, in collaboration with The Department of Endocrinology Diabetes and Metabolic Diseases at University Children's Hospital, Ljubljana, Association for children with metabolic disorders and Franciscan Family Institute. The support group program is based on the relational family paradigm (27), and takes into account the specific dynamics of the family systems with children with chronic illnesses (29, 30). The primary aim of the program is to create a space for awareness, understanding and development of more flexible and adaptive coping strategies for regulating stressful emotions influenced by child's T1D, and to empower parents to be sensitive to their own and children's needs (30-32). The key objectives of the program are to help parents reflect their role as parents, to enhance 'attunement' to children's needs, connection and deeper understanding between a parent and child (31), as well as to improve their own coping and ability for effective and functional living with the child's diabetes. The content of the program was accentuated to parents' challenges in living with the child's T1D from diagnosis onwards, experiences of responsibility and daily stress related to diabetes management, coping with emotions and worries, their view of child's experience of T1D and challenges in adolescents with T1D (e. g. rebellion, setting limits), as well as family adjustment, communication, relationships and support. The therapists used group and psychotherapeutic intervention techniques, such as problem-solving skills, feedback, modeling, the validation of feelings with further questions in collaboration with all the other participants in search of alternative solutions

to a concrete situation. Nine monthly sessions, from September to May, of 2 hour duration, were facilitated by a therapeutic pair of trained marriage and family therapists. The setting of the group was of a closed type for the first three seasons and of an open type for the final season.

2.2 Participants

Forty-seven parents (32 mothers, 15 fathers) of 33 children with T1D participated in the support group on at least one of the four seasons from January 2010 to May 2013. Participating parent's average age was 42.4 years (SD = 6.9), their child's average age was 8.9 years (SD = 5.04). The average duration of child's T1D at the parents' inclusion in the support group was 1.6 years (0 years–10 years; SD = 2.59). The drop-out rate during the first three seasons was four of 21 families. The reasons stated were the change of working times or birth of a new child. Nine families attended the group for two seasons, and one family for three seasons.

The self-evaluation questionnaires were returned by four out of six participants in the first season (2009/10), 11 of 15 participants in the second season (2010/11), 10 of the 12 participants in the third season (2011/12) and by nine of 22 participants in the final (open group type) season (2012/13).

2.3 Self-Evaluation Questionnaire

The self-evaluation questionnaire was designed by the authors for the purpose of the present study, and consisted of two parts. The first part of the questionnaire was related to the parents' well-being in the group. Parents answered to the questions such as 'I felt safe in the group' and rated each of the six items (feelings, expectations, safety, new knowledge, an opportunity to speak and the feeling to be heard) on a five-point Likert scale from 1 - not at all true to 5 - absolutely true. In the fourth season (2012/13), the participants were also asked to quantitatively evaluate the organizational aspect of the group (topics of the meetings, the organization of meetings and the team) on the scale from 1 - very poor to 5 - very good. The second part of the questionnaire consisted of open-ended questions, regarding: the topics that were of the most interest to the participant; the effect or changes that the group might have brought to the participant, to his/her partner or child; the comments on the group organization and structure; the aspects of the group that the participants liked, or the aspects they would change in the program in general.

2.4 Participation in the Parent Support Group and Analyses of the Data

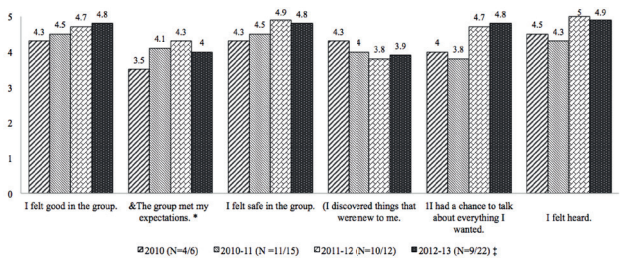
The parents were informed and invited to the support group by the Association for children with metabolic disorders and at the regular outpatient visits at The Department of Endocrinology Diabetes and Metabolic Diseases, University Children's Hospital, Ljubljana. The participation in the group was voluntary. At the first meeting, the parents signed the informed consent of participation in the group and completed the demographic questionnaire. At the final meeting of each group season, the participants were invited to complete the self-evaluation questionnaire. All information about the members was used only for the purpose of the group and present research. The study protocol was approved by the National Medical Ethics Committee (Approval No. 122/04/10).

The quantitative data was analyzed using SPSS software version 19. The Brown-Forsythe test was conducted to compare the differences between the average scores of the first three seasons. A Mann-Whitney U test was used to analyse differences between the third season of the closed group (2011/12) and the open group season (2012/13). The differences were considered statistically significant at the $p < 0,05$. The qualitative data was analysed using content analysis (30). All of the participants' answers were grouped into themes by way of a cyclical process of reflection, observation, and analysis by the consensus of two researchers. Illustrative examples for each theme were then used in the paper.

3 RESULTS

3.1 Group Attendance

The mothers were more than twice as frequently present as the fathers (154 versus 74). The fathers attended the group more frequent than the mothers only in seven of 33 families. More parents visited the open group than the closed group - the open group was attended by 22 parents (2012/13), and the closed group, in the first season (2010), was attended by 9 parents, 14 parents in the second (2010/11), and 16 parents in the third season (2011/12). However, a smaller core of parents was formed within the open group (7 parents), which attended the group throughout the year.



Note:‡the group was of a closed type in the seasons 2010, 2010-11, 2011-12 and of an open type in 2012-13, * the difference is statistically significant.

Figure 1. Parent support group questionnaire mean scores in parents of children with T1D by seasons (1 – not at all true, 5 – true to a great extent).

3.2 Questions on Wellbeing in the Group

Parents in the group were overall satisfied with regards to all six items of the questionnaire (Figure 1). The results show a statistically significant difference with regards to the item 'expectations' ($F(2, 22) = 5{,}757$, $p = 0{,}010$), namely: parents from the second and third season, on average, found that the support group has better fulfilled their expectations than the parents from the first season. Regarding other items, there were no statistically significant differences on: wellbeing in the group ($F(2, 22) = 0{,}761$, $p = 0{,}479$), feeling secure (Welch $F(2, 6{,}722) = 2{,}743$, $p = 0{,}135$), experiencing new things (Welch $F(2, 7{,}090) = 0{,}331$, $p = 0{,}729$), being able to talk ($F(2, 22) = 2{,}158$, $p = 0{,}139$) and the feeling of being heard (Welch $F(2, 6{,}761) = 2{,}166$, $p = 0{,}188$).

There were also no statistically significant differences between the last season of the closed group (2011/12) and the season of the open group (2012/13) on any of the items: wellbeing in the group (M-W = 48,50, $p > 0{,}05$), expectations (M-W = 10,50, $p > 0{,}05$), feeling secure (M-W = 39,50, $p > 0{,}05$), experiencing new things (M-W = 46,00, $p > 0{,}05$), being able to talk (M-W = 48,50, $p > 0{,}05$) and the feeling of being heard (M-W = 44,50, $p > 0{,}05$).

3.3 Questions on Organization and Subjects

The parents in the season 2012/13 (N = 9) also completed the questionnaires about the organisation and subjects discussed in the group. Their average score pertaining to the item on subjects discussed in the group (relevance, importance) was 4.4, their average score pertaining to the item regarding organisation (time, location, informing) was 4.7, and pertaining to the item regarding group leading (attitude of group leaders, moderating, giving feedback) was 4.7.

3.4 Open-Ended Questions

The participants' responses to the open-ended questions are grouped according to similar topics, and illustrative disclosures are presented in Table 1.

Table 1. Disclosures of the participants of the parent support group, according to the most relevant emerging themes.

Disclosures of the participants of the parent support group, according to the most relevant emerging themes.	One thing is what you read in the literature and what doctors and nurses tell you. And another thing is what other parents tell you ... The parents who, by themselves, calculate, weight, inject and question themselves if they are doing it the right way. And who answer their children 'why' and 'how' to go through the disease that will stay with them forever. It is much easier to share our sorrow and joy of our 'sugarplums' (child with T1D) growing up in a group of parents with the same stories. I would like to welcome 'the new family member' (the disease), so that I could face the difficulties and the struggle of living with the disease.
Transformation of the family dynamic	Diabetes is a good teacher. It forces the family into better relationships, so that the family can survive as a whole. You devote to your child more fully and in a different way. It takes a lot of discipline, determination and persistence. When you accept diabetes into your life, it can give you a lot of positive things. But I definitely don't neglect all of distress that accompanies that. I could tell my husband what I experience about the disease and I could also hear what my husband experiences.
Me as a parent	... (The most valuable thing is) to meet other people with the same problems regarding diabetes management, and to see that you are not alone with the problems and that you can get along with the disease. Oh, yes, I would say a lot (changed in him as a parent). I am more tolerant, better educated and prepared for problems that may emerge in the future. And what I think the best outcome of the group is: despite of the reality of living with my son's T1D and a heap of limitations and regulations because of this, the life and coping with daily things is simpler and better – less stressful and tiring. ... in the situation when 'you lose control,' I can calm down much easier and count to 10 before I respond.
Exchange of experience and good practice	Exchange of experiences, how to respond in different everyday situations (was very important). (A great value in) ... expert help of the group and qualified group leaders. I have more information about everything - upbringing and living with my son's diabetes. One has so much faith in those words, you just absorb them. What she (the doctor) says is as if you hear this for the first time, even though you have heard this often.
Facing the world outside the family	This group means a lot to me because I feel I am accepted and understood. I miss that in my everyday life, with co-workers, friends ...

Support when confronting the diagnosis

One of the most important themes that emerged was the feeling of emotional support and being understood by the other parents that are in a similar situation.

Transformation of the family dynamics

T1D requires adjustments of a family by re-shaping the family member roles and their dynamics (i.e. time that parents spend with the child with T1D, other children and with each other; time for everyday activities; family roles and burden of responsibility of every family member, etc.), and also to re-establish adequate relationships to the world outside the family (i.e. kindergarten, school, friends and relatives, etc.). In most of the families, the mothers took over the majority of the burden of T1D. The fathers, on the other hand, distanced themselves from emotional burden and focused on more technical aspects of family functioning (finances, logistics, etc.).

Me as a parent

The participants serve each other as models of emotion regulation. In this way, they got a better insight into more or less functional strategies of coping with distress. For most, this was a very valuable experience. They experienced the group as a space where they could evaluate their part in disease management, their experience of different emotions, and as a space where they got enough emotional support, so that they could respond to their children's emotional problems and challenges of growing up with T1D.

Exchange of experience and good practice

The participants pointed out the need for practical solutions to the problems of living with T1D. Some of them found value especially in expert information. Furthermore, the visit of the paediatric diabetologist turned out to be especially appreciated and valued.

Facing the world outside the family

The participants experienced the group environment supporting and understanding, as opposed to their communities (friends, family, work).

4 DISCUSSION

The article presents a quantitative assessment of the participants' satisfaction with the support group for parents of children with T1D, and a qualitative exploration of the perceived impact of the group to their functioning.

In all of the seasons, the parents experienced the support group as a safe space where they could connect, talk and also be heard about their challenges and feelings. According to the Relational Family Model, a sense of security is essential for a functional affect regulation (24, 25). For the parents of children with T1D, the biggest challenge is to accept the disease and transform their relationship to the children's diabetes. The ability of a family's coping with difficulties of a child's T1D depends on the mental health of the parents (14-17, 20, 31).

When considering a sense of security, it is important if the group is open or closed. In our instance, in the first three seasons, the group was closed so that only the members from the first meeting could attend the meetings. The only significant difference between these seasons was with regards to the item expectations. The lowest score was in the first season, which can be explained by the fact that the program of the group developed through the seasons so that the needs of the parents could be better met. When comparing the last season of the closed group (2011/12) with the season of the open group (2012/13), there was no statistically significant differences on any item. That indicates that, also in the open group, when the parents could join the group at any meeting, the parents felt that they can express themselves and be heard. Possibly, this means that the closeness of the group is not the most important factor for the parents' feelings of security. The detailed analysis of attendance of the season 2012/13 revealed an establishment of a smaller group of parents who regularly attended the meetings, possibly contributing to a stronger sense of security among new members. Moreover, the experience of being a parent of a child with T1D could contribute to a stronger and faster development of the feelings of empathy and understanding among the members. The f finding was supported also by their reflections.

The participants also evaluated the organisation of the open group in the last season. The results of the questionnaires indicated that parents found the open group setting with monthly two hour meetings suitable.

The mothers visited the support group twice as frequently as the fathers. The reason could be a greater engagement of the mothers in the responsibility and care for a child with T1D, a finding in accordance with other research (3, 15, 16). In the study by Streisand et al., 79 % of the mothers took care for injecting the insulin and, in 70 % of the cases, they took care for blood glucose measurements (15). The fathers' generally lower involvement in the process of disease management is also connected with worse treatment outcomes in adolescents with T1D (3, 16).

The topics the participants pointed out in the evaluation questionnaires are in accordance with the themes other researchers found to be important for the parents of children with T1D (27). The participants pointed out that emotional support of other parents was the most

important benefit of the group. This finding is in line with their feelings of social isolation, loss of the healthy child and, consequently, the way of life they used to know. When their child is diagnosed with T1D, the family life changes dramatically. Because of the constant threats of hypoglycaemia and a fear of complications, later in life, the parents are overwhelmed with emotions of sadness, fear, anger, guilt, which often isolates them from a wider social network (17, 20, 21). They are faced with a completely new challenge of regulating their children's physical (food, rest, blood sugar) and psychical states (stress, emotions). Moreover, they must communicate their new circumstances and needs to a wider community (relatives, friends, school or kindergarten, co-workers, etc.), which can trigger new feelings of alienation, misunderstanding, guilt, anger and overall stress (20).

Parents have also noticed that the group helped to improve their family dynamics. T1D influences a family profoundly: the roles in the family change, the hierarchy changes, also communication, interpersonal relations and finances (13, 27). The child and also other family members must learn to regulate the effects through their interpersonal relationships. Good family functioning and coping with challenges of T1D leads to a better glycemic control (10, 11). On the other hand, conflicts, unclear boundaries between family systems, unclear or rigid rules and undefined roles in the family lead to worse diabetes management and glycemic control (2-4, 12). Relational family model asserts that dysfunctional family system achieves its homeostasis by transmitting tension to the child who becomes a carrier of unresolved family conflicts, and is therefore unable to functionally express and regulate emotions (23, 32). This is especially crucial in the families with children with T1D. When evaluating the group, one of the mothers pointed out that she could express to her husband what she felt and also that she could hear what he was experiencing. In the functional regulation of emotions in the family, it is crucial for the parents to be heard and understood among themselves. Their interpsychic and interpersonal emotion regulation, furthermore, enables them to feel the child's distress and help to regulate it and transform it into a form the child can manage (24, 25).

The parents felt more competent in their upbringing after attending the group, as a consequence of gaining new knowledge and exchanging good practice in the management of diabetes, which they got from other parents as well as from the group leaders and the diabetologist. The perceived change helped them to adapt to new circumstances of their family, reduced the anxiety and stress in the family, which are all important themes in the families with T1D (27). Another important aspect that the participants perceived as helpful in the family's adaptation to the disease was open communication between the diabetic team and the family members. Communication about the possible disease complications and the accompanying emotions is very important for the family in order to be able to accept the new situation of living with the T1D, to try to find the meaning in the disease and to start to live anew (33). In other words, the parents, in the management of their children's T1D, do not become just medical experts and dietetics for T1D, but remain the most important regulators of all difficult emotional states that T1D awakes in the child and other family members.

However, a few limitations of the study should be considered. There were no standardised measures used in the study which would more accurately indicate the process of the group and specific differences and changes in the parents' experiencing pre- and post-intervention. Additional data about children's psychosocial and diabetes characteristics were lacking, which could show a potential pathway to the parental coping with the child and T1D, and thereby limiting the analysis of the results.

5 CONCLUSION

The research in the field of psychological support for parents with chronically ill children is scarce. T1D is a big challenge, not only for the child, but also for other family members facing this new physical and emotional state. The parent support group helps the parents to express their concerns, to give support to one another and, especially, to recognise, accept and regulate their children's physical and psychological changes and needs. The presented parent support group showed to be a promising supportive, therapeutic and psychoeducative space, where parents could strengthen their role in the upbringing of their child with T1D. Effective psychosocial support to families is a part of integrative healthcare in children and adolescents with T1D.

For the future work of the support group, it would be important to employ a quantitative analysis of the changes in the experience of the parents, using standardised measures, and to follow the process and the dynamics of the group.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

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REFERENCES

1. Snoek F, Skinner C. Psychology in diabetes care. 2nd ed. West Sussex: John Wiley & Sons Ltd, 2005.
2. Anderson B, Vangsness L, Connell A, Butler D, Goebel-Fabbri A, Laffel LMB. Family conflict, adherence, and glycaemic control in youth with short duration Type 1 diabetes. Diabet Med 2002; 19: 635-42.
3. Shorer M, David R, Schoenberg-Taz M, Levavi-Lavi I, Phillip M, Meyerovitch J. Role of parenting style in achieving metabolic control in adolescents with type 1 diabetes. Diabetes Care 2011; 34: 1735-7.
4. Wiebe DJ, Berg CA, Korbel C, Palmer DL, Beveridge RM, Upchurch R. et al. Children's appraisals of maternal involvement in coping with diabetes: enhancing our understanding of adherence, metabolic control, and quality of life across adolescence. J Pediatr Psychol 2005; 30: 167-78.
5. Association AD. Standards of medical care in diabetes 2013. Diabetes Care 2013; 36(Suppl 1): 11-66.
6. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. Lancet 2009; 373: 2027-33.
7. Bratina N, editor. Sladkorčki: vse, kar ste želeli vedeti o sladkorni bolezni. Ljubljana: Društvo za pomoč otrokom s presnovnimi motnjami, 2012.
8. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329: 977-86.
9. Delamater AM. Psychological care of children and adolescents with diabetes. Pediatr Diabetes 2009; 10: 175-84.
10. Cohen D, Lumley M, Naar-King S, Partridge T, Cakan N. Child behavior problems and family functioning as predictors of adherence and glycemic control in economically disadvantaged children with type 1 diabetes: a prospective study. J Pediatr Psychol 2004; 29: 171-84.
11. Davis C, Delamater A, Shaw K, La Greca A, Eidson M, Perez-Rodriguez J. et al. Parenting styles, regimen adherence, and glycemic control in 4- to 10-year-old children with diabetes. J Pediatr Psychol 2001; 26: 123-9.
12. Tsamparli A, Kounenou K. The Greek family system when a child has diabetes mellitus type 1. Acata Pediatr 2004; 93: 1646-53.
13. Drotar D. Psychological interventions in childhood chronic illness. Washington DC: American Psychological Association, 2006.
14. Lewin AB, Storch EA, Silverstein JH, Baumeister AL, Strawser MS, Geffken GR. Validation of the pediatric inventory for parents in mothers of children with type 1 diabetes: an examination of parenting stress, anxiety, and childhood psychopathology. Fam Syst Health 2005; 23: 56-65.
15. Streisand R, Swift E, Wickmark T, Chen R, Holmes CS. Pediatric parenting stress among parents of children with type 1 diabetes: the role of self-efficacy, responsibility, and fear. J Pediatr Psychol 2005; 30: 513-21.
16. Haugstvedt A, Wentzel-Larsen T, Graue M, Sovik O, Rokne B. Fear of hypoglycaemia in mothers and fathers of children with Type 1 diabetes is associated with poor glycaemic control and parental emotional distress: a population-based study. Diabet Med 2010; 27: 72-8.
17. Stallwood L. Influence of caregiver stress and coping on glycemic control of young children with diabetes. J Pediatr Health Care 2005; 19: 293-300.
18. Monaghan MC, Hilliard ME, Cogen FR, Streisand R. Nighttime caregiving behaviors among parents of young children with type 1 diabetes: associations with illness characteristics and parent functioning. Fam Syst Health 2009; 27: 28-38.
19. Patton S, Dolan L, Henry R, Powers S. Fear of hypoglycemia in parents of young children with type 1 diabetes mellitus. J Clin Psychol Med Settings 2008; 15: 252-9.
20. Landolt M, Vollrath M, Laimbacher J, Gnehm H, Sennhauser F. Prospective study of posttraumatic stress disorder in parents of children with newly diagnosed type 1 diabetes. J Am Acad Child Adolesc Psychiatry 2005; 44: 682-9.
21. 21. Streisand R, Mackey ER, Elliot BM, Mednick L, Slaughter IM, Turek J. et al. Parental anxiety and depression associated with caring for a child newly diagnosed with type 1 diabetes: opportunities for education and counseling. Patient Educ Couns 2008; 73: 333-8.
22. Schore A. Affect regulation and the repair of the self. New York: Norton, 2003.
23. Atkin K, Ahmad WI. Family care-giving and chronic illness: how parents cope with a child with a sickle cell disorder or thalassaemia. Health Soc Care Community 2000; 8: 57-69.
24. Gostečnik C. Inovativna relacijska družinska terapija: inovativni psiho-biološki model. Ljubljana: Brat Frančišek, Teološka fakulteta in Frančiškanski družinski inštitut, 2011.
25. Gostečnik C. Relacijska paradigma in travma. Ljubljana: Brat Frančišek, Frančiškanski družinski inštitut; 2008.
26. Couch R, Jetha M, Dryden DM, Hooten N, Liang Y, Durec T. et al. Diabetes education for children with type 1 diabetes mellitus and their families. Evi Rep Technol Assess 2008; 166: 1-144.
27. Whittemore R, Jaser S, Chao A, Jang M, Grey M. Psychological experience of parents of children with type 1 diabetes: a systematic mixed-studies review. Diabetes Educ 2012; 38: 562-79.
28. Hoey H. Psychosocial factors are associated with metabolic control in adolescents: research from the Hvidoere Study Group on Childhood Diabetes. Pediatr Diabetes 2009; 10(Suppl 13): 9-14.
29. Siegel DJ, Bryson TP. The whole-brain child: 12 revolutionary strategies to nurture your child's developing mind. New York: Delacorte Press; 2011.
30. Snape D, Spencer L. The foundations of qualitative research. In: Ritchie J, Lewis J, editors.Qualitative research practice: a guide for social science students and researchers. London: Sage; 2003.
31. Jaser SS, Whittemore R, Ambrosino JM, Lindemann E, Grey M. Mediators of depressive symptoms in children with type 1 diabetes and their mothers. J Pediatr Psychol 2008; 33: 509-19.
32. Smyth JM, Arigo D. Recent evidence supports emotion-regulation interventions for improving health in at-risk and clinical populations. Curr Opin Psychiatry 2009; 22: 205-10.
33. Gostečnik C. Sistemske teorije in praksa. Ljubljana: Brat Frančišek in Frančiškanski družinski inštitut; 2010.

NEWBORN SCREENING IN SLOVENIA

PRESEJANJE NOVOROJENCEV V SLOVENIJI

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ABSTRACT

Introduction. Newborn screening in whole Slovenia started in 1979 with screening for phenylketonuria (PKU). Congenital hypothyroidism (CH) was added into the programme in 1981. The aim of this study was to analyse the data of neonatal screening in Slovenia from 1993 to 2012 for PKU, and from 1991 to 2012 for CH.

Methods. Blood samples were collected from the heels of newborns between the third and the fifth day after birth. Fluorometric method was used for screening for PKU, CH screening was done by dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA).

Results. From 1993 to 2012, from 385,831 newborns 57 were identified with PKU. 184 newborns out of 427,396 screened from 1991 to 2012, were confirmed for CH. Incidences of PKU and CH in the periods stated are 1:6769 and 1:2323, respectively.

Conclusions. Successful implementation of newborn screening for PKU and CH has helped in preventing serious disabilities of the affected children. Adding screening for new metabolic diseases in the future would be beneficial.

Keywords:
congenital hypothyroidism, phenylketonuria, mass screening, incidence, Slovenia

IZVLEČEK

***Uvod**. Presejanje novorojencev v Sloveniji se je začelo leta 1979 s presejanjem za fenilketonurijo (PKU). Leta 1981 je bil v program presejanja dodan še kongenitalni hipotireoidizem (CH). Cilj te raziskave je analiza podatkov presejanja novorojencev v Sloveniji v obdobju med letoma 1993 in 2012 za PKU ter med letoma 1991 in 2012 za CH.*

***Metode**. Vzorci krvi so bili odvzeti petim novorojencem med tretjim in petim dnem življenja. Pri presejanju za PKU se uporablja fluorometrična metoda, presejanje za CH pa poteka z metodo DELFIA.*

***Rezultati**. Od leta 1993 do leta 2012 je bil presejalni test za PKU izveden pri 358.831 novorojencih. Pri 57 otrocih je bil PKU potrjen. Pri 427.396 novorojencih med letoma 1991 in 2012 je bil izveden presejalni test za CH. Pri 184 otrocih je bil CH potrjen. V navedenih obdobjih je bila incidenca PKU 1:6769 in incidenca CH 1:2323.*

***Zaključki**. Uspešna implementacija presejanja novorojencev za PKU in CH je imela pomembno vlogo pri preprečevanju resnih zapletov pri obolelih otrocih. Smiselno bi bilo v program presejanja vključiti nove metabolne bolezni.*

***Ključne besede**:
kongenitalnihi potireoidizem, fenilketonurija, presejalni testi, incidenca, Slovenija*

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1 INTRODUCTION

Newborn screening for metabolic diseases is an important public health programme, as an early identification of affected children can help in preventing disabilities and even death (1, 2). It is available in many developed countries and in all neighbouring countries of Slovenia (3-6). Newborn screening in Slovenia started in 1979 with screening for phenylketonuria (PKU) (7). Screening for congenital hypothyroidism (CH) started 2 years later in 1981 (8). PKU is an inborn error in amino acid metabolism, caused by mutations of phenylalanine hydroxylase gene. Phenylalanine hydroxylase converts phenylalanine to tyrosine, decreased activity of the enzyme leads to increased phenylalanine in the blood and the brain, which can have detrimental effects, such as intellectual impairment and other symptoms, like autism, seizures and motor deficits (9). CH, one of the most common preventable causes of mental retardation, is a thyroid hormone deficiency present at birth (10). Screening for PKU and CH in Slovenia helped to improve the outcome of most of the affected children. Screening for both diseases has been shown to be cost saving (11, 12).

2 METHODS

2.1 Organisation

Analyses were done at the University Medical Centre Ljubljana, Department of Nuclear Medicine. Only serum phenylalanine (Phe) was analysed at the University Medical Centre Ljubljana, University Children's Hospital, Unit for Special Laboratory Diagnostics. All children with elevated Phe and TSH values were followed-up at the University Medical Centre Ljubljana, University Children's Hospital, Department of Endocrinology, Diabetes and Metabolic Diseases.

2.2 Specimen Collection

Blood samples from newborns were collected between the third and the fifth day after birth from the heels of newborns. Samples were collected onto the filter paper Whatman 903 and dried. The dried blood samples were sent by mail (from nurseries not located in Ljubljana) or by a courier service (from the nursery in Ljubljana) to the Department of Nuclear Medicine.

2.3 Laboratory Methods

PKU was detected by quantitative determination of Phe in dried blood spot. From January 1979 to June 1992, screening for PKU was performed by the Guthrie method with the Phe cut off value of 0.12 mmol/L (7). Since July 1992, a fluorometric method was used (Neonatal Phenylalanine kit, PerkinElmer). For the fluorometric method, Phe values < 0.12 mmol/L were treated as normal after the responsible analyst approved them. Values of Phe between 0.12 mmol/L and 0.20 mmol/L, including 0.12 mmol/L and 0.20 mmol/L, were considered as increased threshold values. In this case, the analysis was repeated from a new dried blood spot which was acquired from the nursery. Increased values of Phe ≥ 0.20 mmol/L required confirmational analysis of Phe, which was quantified from a new serum blood sample. Serum blood sample was taken after contacting the nursery where the original sample was taken. It was quantified by the use of the ninhydrin and L-leucyl-L-alanine fluorometric test. PKU was confirmed by elevated serum Phe.

CH was detected by measuring the value of thyroid-stimulating hormone (TSH). From August 1981 to April 1989, radioimmunoassay (RIA) was used for this purpose (8). From then on, analysis was done by dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA® Neonatal hTSH kit, PerkinElmer). Values for TSH were given as mU/L. The cut off value for RIA was 20 mU/L. The measured values with the current method were evaluated as follows. Values up to 8 mU/L were considered normal after the approval by the responsible analyst. In the case of TSH, values between 8 and 20 mU/L, including 8 and 20 mU/L, were considered as increased threshold values, analysis was repeated from a new dried blood spot acquired from the nursery. When TSH values were ≥ 20 mU/L, they were considered as increased values and confirmational analysis of TSH was done from a new serum blood sample taken after contacting the nursery where the original sample was taken. Analyses were done by ADVIA Centaur TSH-Ultra assay (Siemens). CH was confirmed by elevated serum TSH.

2.4 Quality Control

The dried blood spot controls were included in every analytical batch to monitor accuracy and precision within the system. The laboratory participated in UK NEQUAS scheme (http://www.ukneqas.org.uk/) for external international quality control for PKU and CH, and in RFB DGKL scheme (http://www.dgkl-rfb.de/) for external international quality control for CH.

3 RESULTS

3.1 Metabolic Phenotypes of PKU Patients

PKU was classified into different metabolic phenotypes, based on the measured Phe value in blood. Normal Phe value was between 0.05 mmol/L and 0.12 mmol/L. In mild hyperphenylalaninaemia, which does not require any dietary treatment to prevent neurological damage, Phe values were between 0.12 mmol/L and 0.60 mmol/L (9, 13). In mild PKU, Phe values were between 0.60 mmol/L and 0.90 mmol/L, in moderate PKU, Phe values were between 0.90 mmol/L and 1.20 mmol/L (some authors

do not use the term moderate PKU and consider mild PKU between 0.60 mmol/L and 1.20 mmol/L). Patients with classic phenylketonuria had Phe values higher 1.20 mmol/L (9, 13).

3.2 Incidence of PKU and CH

Results for PKU screening from 1993 to 2012, and for CH screening from 1991 to 2012, are given in Table 1 and Table 2. The numbers of positive results for both PKU and CH are given for the period between 2000 and 2012, older data was not available. Results of PKU screening from the implementation of screening until April 1993, and of CH screening until July 1991, were already published (7, 8). From 1993 to 2012, 57 cases were diagnosed positive for PKU, which is up to 6 positive cases of PKU per year. Between 1991 and 2012, there were from 3 to 16 positive cases of CH annually, which amounts to a total of 184 patients with CH. That gives average incidences of 1:6769 for PKU and 1:2323 for CH. Incidence of PKU in Europe is between 1:3000 and 1:30000, of CH between 1:1300 and 1:13000 (14).

Table 1. Neonatal screening for PKU in Slovenia from 1993 to 2012 (the number of live births annually was taken from the webpage of Statistical Office of the Republic of Slovenia, www.stat.si). N/A = data not available. / = Incidence can not be calculated as there were no confirmed cases in that year.

Year	No. of newborns	No. of positive results	No. of confirmed cases	No. of classic PKU	No. of moderate PKU	No. of mild PKU	Incidence
1993	19793	N/A	5	4	1	0	1 : 3959
1994	19463	N/A	3	2	0	1	1 : 6488
1995	18980	N/A	3	2	0	1	1 : 6327
1996	18788	N/A	2	2	0	0	1 : 9394
1997	18165	N/A	3	1	1	1	1 : 6055
1998	17856	N/A	5	4	1	0	1 : 3571
1999	17533	N/A	2	2	0	0	1 : 8767
2000	18180	265	3	1	0	2	1 : 6060
2001	17477	167	1	1	0	0	1 : 17477
2002	17501	368	0	0	0	0	/
2003	17321	476	3	1	0	2	1 : 5774
2004	17961	520	6	4	1	1	1 : 2994
2005	18157	556	4	3	0	1	1 : 4539
2006	18932	154	2	1	0	1	1 : 9466
2007	19823	112	1	1	0	0	1 : 19823
2008	21817	112	3	2	0	1	1 : 7272
2009	21856	196	6	5	0	1	1 : 3643
2010	22343	182	4	2	0	2	1 : 5586
2011	21947	154	1	0	0	1	1 : 21947
2012	21938	157	0	0	0	0	/

Table 2. Neonatal screening for CH in Slovenia from 1991 to 2012 (the number of live births annually was taken from the webpage of Statistical Office of the Republic of Slovenia, www.stat.si). N/A = data not available.

Year	No. of newborns	No. of positive results	No. of confirmed cases	Incidence
1991	21583	N/A	9	1 : 2398
1992	19982	N/A	7	1 : 2855
1993	19793	N/A	3	1 : 6598
1994	19463	N/A	8	1 : 2433
1995	18980	N/A	4	1 : 4745
1996	18788	N/A	6	1 : 3131
1997	18165	N/A	4	1 : 4541
1998	17856	N/A	9	1 : 1984
1999	17533	N/A	11	1 : 1594
2000	18180	127	14	1 : 1299
2001	17477	176	7	1 : 2497
2002	17501	201	8	1 : 2188
2003	17321	258	14	1 : 1237
2004	17961	238	10	1 : 1796
2005	18157	214	5	1 : 3631
2006	18932	238	8	1 : 2367
2007	19823	187	16	1 : 1239
2008	21817	185	12	1 : 1818
2009	21856	160	4	1 : 5464
2010	22343	195	8	1 : 2793
2011	21947	128	8	1 : 2743
2012	21938	207	9	1 : 2438

4 DISCUSSION

Worldwide newborn screening started in 1962, in Massachusetts, USA, with the introduction of bacterial inhibition assay for PKU (Guthrie test) (15). Since then, numerous countries have incorporated newborn screening in their public health programmes, and it is, nowadays, an established medical practice in developed countries (3-5). Screening has also expanded to include more diseases, beginning with CH and galactosemia, while many others were added later on (3, 16). More than 10 diseases are screened for in many European countries (17), while in the USA, more than 20 diseases are included in the newborn screening panels (18, 19).

With the technological advances came the introduction of tandem mass spectrometry (MS/MS), which allowed accurate measurements of acylcarnitines, amino acids and other metabolites important in diagnosing metabolic disorders (20-22). MS/MS is being increasingly used for newborn screening, as it provides important advantages over other techniques, such as the ability to screen for several diseases in a single run, short time of analysis, selectivity and sensitivity (23).

In the countries of southeastern Europe, including Slovenia, MS/MS has not yet been implemented into newborn screening programmes (24). In Slovenia, a pilot study is under way (25) and will be the first expanded newborn screening in Slovenia, which will make an important contribution in designing the optimal strategy for screening Slovene newborns for inborn errors of metabolism. It will also significantly contribute to the evaluation of the frequency of occurrence of each inborn error of metabolism in our population, as reports from countries that have already incorporated newborn screening showed that prevalences for some diseases are higher than expected (26-29). Slovene newborn screening is an area that should be expanded, and thus brought closer to other countries in the European Union (17, 18) and other parts of the developed world (3, 16, 30-33).

Early detection of PKU and CH can improve the outcomes of patients (31). One major downside of newborn screening using less sensitive methods is the high number of false positive results. An increase of stress has been shown in parents of infants with false positive screening results (34). With the use of MS/MS, there is a significantly lower number of false positive results (35).

Incidence of classic PKU in Slovenia is 1:10153, while the total incidence of all forms of PKU (classic, moderate and mild PKU) is 1:6769. Incidence of classic PKU in Slovenia between 2001 and 2010, was previously reported to be around 1:10000 (36), which is consistent with here reported data. Incidence of PKU in Europe in 2004, is between 1:3000 and 1:30000 (14). Incidence of CH in Slovenia, which is 1 : 2323, is on the lower end in Europe, where the incidence is from 1:1300 to 1:13000 (14). Our incidence (1:2323) is higher than the incidence reported in 2007 in Slovenia, which was 1:3100 (37).

Guidelines in several European countries recommend starting the treatment for PKU as early as possible for the best outcomes (38). Treatment for PKU is with a Phe restricted diet (39). According to the literature, treatment with levothyroxine for CH should start in newborns within 2 to 3 weeks of age (40). In Slovenia, treatment for both PKU and CH starts immediately after the confirmation of the disease, which is within two weeks after birth. This is in line with the previously stated limits of treatment, and shows that newborn screening has been successfully established for the best outcomes of the affected children.

5 CONCLUSIONS

The newborn screening programme in Slovenia has been successfully implemented in its public health programme, and has been beneficial for a significant number of affected newborns since the start of the programme. Based on positive experiences in screening for PKU and CH, and on the results from the expansion of screened conditions in developed countries, expansion of screened conditions should be the next step towards a better health programme in Slovenia.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Not required.

REFERENCES

1. Van Karnebeek CDM, Stockler S. Treatable inborn errors of metabolism causing intellectual disability: a systematic literature review. Mol Genet Metab 2012; 105: 368–81.
2. Howell R, Terry S, Tait VF, Olney R, Hilton CF, Grosse S. et al. CDC grand rounds: newborn screening and improved outcomes. MMWR Morb Mortal Wkly Rep 2012; 61: 390–3.
3. Therrell BL, Adams J. Newborn screening in North America. J Inherit Metab Dis 2007; 30: 447–65.

4. Burgard P, Cornel M, Filippo FD. Report on the practices of newborn screening for rare disorders implemented in member states of the European Union, candidate, potential candidate and EFTA countries. 2012.
5. Padilla CD, Therrell BL. Newborn screening in the Asia Pacific region. J Inherit Metab Dis 2007; 30: 490–506.
6. Sarnavka V. Rezultati metaboličkog probira u Hrvatskoj. Hrvat Časopis Javno Zdr 2005; 1.
7. Battelino T, Kržišnik C, Pavlin K. Early detection and follow up of children with phenylketonuria in Slovenia. Zdrav Vestn 1994; 63(Suppl 1): s25–8.
8. Kržišnik C, Battelino T, Bratanič N, Hojker S, Pavlin K, Žerjav-Tanšek M. et al. Results of screening for congenital hypothyroidism during the ten-year period (1981-1991) in Slovenia. Zdrav Vestn 1994; 63(Suppl 1): s29–31.
9. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. Lancet 2010; 376: 1417–27.
10. Rastogi M V, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis 2010; 5: 17.
11. Lord J, Thomason MJ, Littlejohns P, Chalmers RA, Bain MD, Addison GM. et al. Secondary analysis of economic data: a review of cost-benefit studies of neonatal screening for phenylketonuria. J Epidemiol Community Health 1999; 53: 179–86.
12. Geelhoed EA, Lewis B, Hounsome D, O'Leary P. Economic evaluation of neonatal screening for phenylketonuria and congenital hypothyroidism. J Paediatr Child Health 2005; 41: 575–9.
13. Guldberg P, Rey F, Zschocke J, Romano V, François B, Michiels L. et al. A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. Am J Hum Genet 1998; 63: 71–9.
14. Loeber JG. Neonatal screening in Europe: the situation in 2004. J Inherit Metab Dis 2007; 30: 430–8.
15. MacCready RA, Hussey MG. Newborn phenylketonuria detection program in Massachusetts. Am J Public Health Nations Health 1964; 54: 2075–81.
16. Harms E, Olgemöller B. Neonatal screening for metabolic and endocrine disorders. Dtsch Arztebl Int 2011; 108: 11–21.
17. Loeber JG, Burgard P, Cornel MC, Rigter T, Weinreich SS, Rupp K. et al. Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 1. From blood spot to screening result. J Inherit Metab Dis 2012; 35: 603-11.
18. Rinaldo P, Zafari S, Tortorelli S, Matern. Making the case for objective performance metrics in newborn screening by tandem mass spectrometry. Ment Retard Dev Disabil Res Rev 2006; 12: 255-61.
19. De Jesús VR, Mei JV, Bell CJ, Hannon WH. Improving and assuring newborn screening laboratory quality worldwide: 30-year experience at the Centers for Disease Control and Prevention. Semin Perinatol 2010; 34: 125-33.
20. Levy HL. Newborn screening by tandem mass spectrometry: a new era. Clin Chem 1998; 44: 2401–2.
21. Sweetman L. Newborn screening by tandem mass spectrometry: gaining experience. Clin Chem 2001; 47: 1937–8.
22. Sweetman L. Newborn screening by tandem mass spectrometry (MS-MS). Clin Chem 1996; 42: 345–6.
23. Chace DH. Mass spectrometry in newborn and metabolic screening: historical perspective and future directions. J Mass Spectrom 2009; 44: 163–70.
24. Groselj U, Tansek MZ, Smon A, Angelkova N, Anton D, Baric I. et al. Newborn screening in southeastern Europe. Mol Genet Metab 2014; 113: 42-5.
25. Smon A, Murko S, Repic Lampret B, Battelino T. Pilot research on expanding Slovenian newborn screening programme for inherited metabolic disorders detectable by tandem mass spectrometry. Chem Listy 2014; 108: s182-6.
26. Derks TG, Boer TS, van Assen A, Bos T, Ruiter J, Waterham MR. et al. Neonatal screening for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in The Netherlands: the importance of enzyme analysis to ascertain true MCAD deficiency. J Inherit Metab Dis 2008; 31: 88-96.
27. Lim JS, Tan ES, John CM, Poh S, Yeo SJ, Ang JSM. et al. Inborn Error of Metabolism (IEM) screening in Singapore by electrospray ionization-tandem mass spectrometry (ESI/MS/MS): an 8 year journey from pilot to current program. Mol Genet Metab 2014; 113: 53-61.
28. Wilcken B, Wiley V, Hammond J, Carpenter K. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med 2003; 348: 2304-12
29. Schulze A, Lindner M, Kohlmüller D, Olgemöller K, Mayatepek E, Hoffmann GC. Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 2003; 116: 1399-406.
30. Tarini BA, Christakis DA, Welch HG. State newborn screening in the tandem mass spectrometry era: more tests, more false-positive results. Pediatrics 2006; 118: 448–56.
31. Wilcken B, Haas M, Joy P, Wiley V, Bowling F, Carpenter K. et al. Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. Pediatrics 2009; 124: 241–8.
32. Kasper DC, Ratschmann R, Metz TF, Mechtler TP, Möslinger D, Konstantopoulou V. et al. The national Austrian newborn screening program - eight years experience with mass spectrometry: past, present, and future goals. Wien Klin Wochenschr 2010; 122: 607–13.
33. Pollitt RJ. Introducing new screens: why are we all doing different things? J Inherit Metab Dis 2007; 30: 423–9.
34. Gurian EA, Kinnamon DD, Henry JJ, Waisbren SE. Expanded newborn screening for biochemical disorders: the effect of a false-positive result. Pediatrics 2006; 117: 1915–21.
35. Schulze A, Lindner M, Kohlmüller D, Olgemöller K, Mayatepek E, Hoffmann GF. Expanded newborn screening for inborn errors of metabolism by electrospray ionization - tandem mass spectrometry: results, outcome, and implications. Pediatrics 2003; 111: 1399–406.
36. Grošelj U, Tanšek MŽ, Podkrajšek KT, Battelino T. Genetske in klinične značilnosti bolnikov s fenilketonurijo v Sloveniji. Zdrav Vestn 2013; 82: 767–77.
37. Avbelj M, Tahirovic H, Debeljak M, Kusekova M, Toromanovic A, Krzisnik C. et al. High prevalence of thyroid peroxidase gene mutations in patients with thyroid dyshormonogenesis. Eur J Endocrinol 2007; 156: 511–9.
38. Blau N, Bélanger-Quintana A, Demirkol M, Feillet F, Giovannini M, MacDonald A. et al. Management of phenylketonuria in Europe: survey results from 19 countries. Mol Genet Metab 2010; 99: 109–15.
39. Tanšek MŽ. Fenilketonurija - zgodba o uspešnem zdravljenju presnovne bolezni. Slov Pediatr 2003; 10: 225–35.
40. Balhara B, Misra M, Levitsky LL. Clinical monitoring guidelines for congenital hypothyroidism: laboratory outcome data in the first year of life. J Pediatr 2011; 158: 532-7.

COMPARISON OF A WEB-BASED DIETARY ASSESSMENT TOOL WITH SOFTWARE FOR THE EVALUATION OF DIETARY RECORDS

PRIMERJAVA SPLETNE APLIKACIJE IN RAČUNALNIŠKEGA PROGRAMA ZA OVREDNOTENJE PREHRANSKIH DNEVNIKOV

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ABSTRACT

Background. Dietary assessment in clinical practice is performed by means of computer support, either in the form of a web-based tool or software. The aim of the paper is to present the results of the comparison of a Slovenian web-based tool with German software for the evaluation of four-day weighted paper-and-pencil-based dietary records (paper-DRs) in pregnant women.

Methods. A volunteer group of pregnant women (n=63) completed paper-DRs. These records were entered by an experienced research dietitian into a web-based application (Open Platform for Clinical Nutrition, OPEN, http://opkp.si/en, Ljubljana, Slovenia) and software application (Prodi 5.7 Expert plus, Nutri-Science, Stuttgart, Germany, 2011). The results for calculated energy intake, as well as 45 macro- and micronutrient intakes, were statistically compared by using the non-parametric Spearman's rank correlation coefficient. The cut-off for Spearman's rho was set at >0.600.

Results. 12 nutritional parameters (energy, carbohydrates, fat, protein, water, potassium, calcium, phosphorus, dietary fiber, vitamin C, folic acid, and stearic acid) were in high correlation (>0.800), 18 in moderate (0.600-0.799), 11 in weak correlation (0.400-0.599), while 5 (arachidonic acid, niacin, alpha-linolenic acid, fluoride, total sugars) did not show any statistical correlation.

Conclusion. Comparison of the results of the evaluation of dietary records using a web-based dietary assessment tool with those using software shows that there is a high correlation for energy and macronutrient content.

Keywords:
web-based dietary assessment tools, dietary records, comparison, pregnant women

IZVLEČEK

***Izhodišča**. V klinični praksi za ovrednotenje prehranskih dnevnikov običajno uporabljamo računalniško podporo, bodisi v obliki računalniškega programa ali spletne aplikacije. Namen članka je predstaviti rezultate primerjave nemškega računalniškega programa in slovenske spletne aplikacije za ovrednotenje prehranskega vnosa na osnovi metode štiridnevnega papirnega tehtanega prehranskega dnevnika (papirni PD), ki so ga vodile nosečnice.*

***Metode**. Skupina nosečnic prostovoljk (n=63) je vodila papirni PD. Izkušeni klinični dietetik je vnesel dnevnike v spletno aplikacijo (Odprta platforma za klinično prehrano, OPKP, http://opkp.si, Ljubljana, Slovenija) in računalniški program (Prodi 5.7 Exper Plus, Nutri-Science, Stuttgart, Germany, 2011). Rezultate za izračunani energijski vnos ter vnos 45 makro- in mikrohranil s pomočjo aplikacije in programa smo statistično primerjali z neparametričnim Spearmanovim koeficientom (>0,600).*

***Rezultati**. Visoko korelacijo (>0,800) med metodama smo ugotovili za 12 hranil (energija, ogljikovi hidrati, skupne maščobe, beljakovine, voda, kalij, kalcij, fosfor, skupna prehranska vlaknina, vitamin C, folna kislina in stearinska kislina), zmerno (0,600-0,799) za 18 hranil, šibko (0,400-0,599) za 11 hranil, medtem ko za 5 hranil ni bilo korelacije (arahidonska kislina, niacin, alfa-linolenska kislina, fluor, skupni sladkorji).*

***Zaključki**. Rezultati ovrednotenja prehranskih dnevnikov s spletno aplikacijo in računalniškim programom so v visoki korelaciji za energijsko vrednost in vsebnost makrohranil.*

Ključne besede:
spletne aplikacije za ovrednotenje prehranskih dnevnikov, prehranski dnevniki, primerjava, nosečnice

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1 INTRODUCTION

Dietary records are an important tool for estimating food and nutrient intakes in different groups of the population. In addition to dietary records, there are other methods of dietary assessment (food frequency questionnaires, 24-hour recall method), but paper dietary recording (paper-DR) has proven to be the best and most accurate way of evaluating food and nutrient intake (1-5). In the dietary record approach, each respondent must describe the foods and amounts consumed, including the name of the food (brand name, if possible), preparation methods, recipes for food mixtures and portion sizes consumed over a certain period of time (6-7). The amounts consumed can be measured either by using a scale or estimated by household measures or models, pictures or without visual aids (8). Ideally, the recording is done at the time of eating in order to avoid reliance on memory. A recording period of more than seven consecutive days is usually unsatisfactory, because of respondent fatigue or non-compliance (8). The duration most often used in the literature is three or four days of dietary recording (two or three weekdays and one weekend day), which has previously given acceptable and reliable data, and caused relatively low dropout (9-10).

There is an increase in computer support tools (such as as software, web-based applications or mobile applications) available for both the general population and nutritional experts, which have received an increasing attention for large-scale population nutrition research (7, 11-17). The goal of computer support for the general population is to facilitate and simplify recording, as well as to be able to access the results quickly. Computer support tools allow end-users to enter food intake and receive feedback relating to energy and nutrient intake. The collected dietary data can be processed and calculated in place, or exported by a research dietitian for data analysis. Despite the availability of novel tools, the usual method of self-monitoring continues to be the paper-DR, which is time consuming, tedious and inconvenient for study volunteers, as well as for the research dietitians (11, 18).

We aimed to compare matching results of four-day paper-DRs kept by 63 pregnant women (hereinafter referred as volunteers), entered by an experienced research dietitian into the web-based application Open Platform for Clinical Nutrition (OPEN; hereinafter referred as web-DR) and the software Prodi 5.7 Expert plus, Nutri-Science, Stuttgart, Germany, 2011 (Prodi; hereinafter referred as SW-DR). Our objective was to examine whether web-DR and SW-DR yield similar results of energy and nutritional intake estimates for 45 macro- and micronutrients.

2 METHODS

2.1 Study Design

This pilot study is a part of the Slovenian research project entitled 'The role of human milk in development of a breast fed child's intestinal microbiota' or 'My-Milk,' in short, which has been described elsewhere (available at: www.moje-mleko.si/en) (19). Briefly, the 'My-Milk' study aims to elucidate the role of microbiota and the fatty acid composition of mother's milk in the development of intestinal microbiota and the overall health status of a newborn infant.

Within this pilot study, we aimed to determine whether web-DR is equivalent to SW-DR, which would substantially reduce logistical and cost burdens in clinical practice, since the web-DR could be recorded directly by the volunteer/user/patient and only checked by a dietitian. Volunteers were included in the study if they were healthy and willing to participate by keeping a paper-DR at home throughout four consecutive days, including one weekend day (from Sunday to Wednesday), because of the protocol of 'My-Milk' study.

They were recruited from January until May 2011, at the Gynecological Clinic, University Medical Centre Ljubljana, while attending the 'School for Parents.' The volunteers came mainly from Ljubljana (the capital of Slovenia) and its surrounding areas. The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Ljubljana, Slovenia (No. 32/07/2010), and is registered at ClinicalTrials.gov (NCT01548313).

The volunteers received 15 minutes of oral instruction from a research dietitian, as well as written instructions on how to keep a paper-DR. We provided them with a kitchen scale, with 1 g resolution (CTC, Clatronic® International GmbH), and asked them not to make any dietary changes during the trial. We recorded the basic anthropometrical measurements (age, week of pregnancy, body height and pre-pregnancy body mass for each volunteer) and basic socio-demographic data (level of education and employment status). Body mass was measured with a certified medical scale to the nearest 0.1 kg and body height to the nearest 0.5 cm (Seca digital scale 769, Germany). The volunteers' data were coded and all information was kept confidential.

2.2 Study Population

By the end of May 2011, 65 volunteers had been approached for study recruitment; two of them withdrew from the study because of lack of interest. In total, 63 volunteers completed the paper-DR. Their average age was 30.4 (±4.0) years, they were in the 30.7th (±4) week of pregnancy and had a pre-pregnancy body mass index of 25.3 (±3.6) kg/m2. The majority of the volunteers were better educated (postgraduate: 13 (21%); tertiary: 42

(67%); secondary: 8 (13%), primary: 0 (0%)), and all were employed.

2.3 Methodology of the Comparative Study

We asked the volunteers to record the intake of all foods, drinks and food supplements consumed over four consecutive days; from Sunday to Wednesday.

The sum values of recording for all four days together for web-DR (n=63) and SW-DR (n=63) were compared for energy and 45 macro- and micronutrients (hereinafter referred to as 46 parameters) (Table 1).

We selected the list of observed nutrients on the basis of the previous study comparing nutrient intake of Slovenian adolescents (20) and, additionally, on the basis of nutrients that are of special interest in the 'My-Milk' study (i.e., fatty acids: linoleic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) (19). For dishes specified in the diaries, we used Slovenian traditional recipes and frequently used recipes to identify the ingredients.

2.3.1 Paper-DR

The paper-DR had five pages, including one page of instructions with an example of one daily dietary record. Detailed information regarding the: a) time of consumption, b) quantities in grams/milliliters or, exceptionally, also in household measures (such as cup, tablespoon, teaspoon, cup of coffee, slice of bread, etc.), c) foods with brand names when appropriate, and the type of preparation were requested. The paper-DRs were checked when received by the experienced research dietitian.

The research dietitian entered the paper-DR into the web-DR and SW-DR and checked the entries twice.

2.3.2 Web-DR (OPEN)

OPEN is the first Slovenian web-based tool for assessment of dietary intake, as well as for diet planning, and it has been described in more detail elsewhere (21-22). Briefly, it consists of data from Slovenian (23-24), European (25) and, to a limited extent, also American (26) food composition tables. To support its use in different countries and languages, OPEN allows translation of the user interface into any language, as well as the use of any food composition dataset that complies with Food data structure and format standard (BS EN 16104:2012). To calculate food composition data for traditional and frequently consumed Slovenian dishes, OPEN applied a recipe-calculation procedure, originally recommended by INFOODS (27) and recognized by EuroFIR (28). In order to prove the efficiency and correctness of the recipe-calculation procedure applied within OPEN, the energy and nutrient contents of composite samples of daily meals (each sampled four times) were compared by using both analytical and calculation techniques (29, 30). The data included for each food item from paper-DRs were: the amount consumed, the date and time of consumption. After a meal had been entered by the research dietitian, OPEN stored the information.

2.3.3 SW-DR (Prodi)

Prodi is German software for nutritional counseling and nutritional therapy available in German and English language. It supports meal planning and calculation, as well as documentation of the consultancy. Foods and their ingredients are readily available, calculated and compared.

In this pilot study, we used Prodi 5.7 Expert plus Nutri-Science, Stuttgart, Germany, 2011, which contains the database of approximately 14,800 foods from the Bundeslebensmittelschlüssel 3.01 (BLS 3.01) database, Fachmann-Kraut-Nährwerttabellen (FKN, Stuttgart, 2005) database, and industrial products and dietetic foods.

2.4 Statistical Analysis

We applied the Shapiro-Wilk normality test to determine whether or not the dataset was modeled with a normal distribution. The dataset of observed parameters did not have a normal distribution, so non-parametric Spearman's rho coefficients were used to measure the correlation of results of nutrient intake calculated by OPEN and Prodi. We defined acceptable correlation as being 0.600 or more. Statistical analyses were performed using the statistical software SPSS ver. 21 (SPSS Inc, Chicago, IL, 2012).

3 RESULTS

Our data show that there was no systematic error in entering. For all 126 DRs (63 in web-DR and 63 in SW-DR), we first calculated the average, SD and median values. We then calculated Spearman's rho correlation coefficients for 46 parameters to check the correlation between web-DR and SW-DR.

In the Table 1 the average and median daily nutrition content for 46 nutritional parameters from paper-DRs (n=63) entered into web-DR and SW-DR. Figure 1 shows the Spearman's rho correlation coefficients for 46 nutritional parameters. The Spearman's correlation coefficient for parameters ranged from -0.05 for total sugars to 0.95 for water.

Table 1. Averages and medians of daily nutrient content for all parameters calculated from 63 four-day paper-based dietary records (paper-DR) entered into web-based dietary records (web-DR) and software-based dietary records (SW-DR).

	Average(SD)		Median	
	web-DR	SW-DR	web-DR	SW-DR
Energy [kcal]	2017.21(386.53)	1994.89(363.02)	2094.13	2025.50
[kJ]	8350.63(1618.03)	8350.63(1519.58)	8766.00	8478.75
Carbohydrates [g]	263.14(56.91)	243.59(49.47)	270.79	241.75
Total sugar [g]	114.77(32.45)	0.61(2.70)	111.95	0.00
Starch [g]	78.35(30.79)	116.00(30.35)	79.76	117.50
Dietary fiber [g]	23.64(8.07)	28.15(9.39)	22.63	27.80
Fats [g]	71.50(18.30)	74.20(19.23)	68.97	74.25
SFA* [g]	25.38(6.94)	25.72(8.43)	24.92	25.25
Myristic acid [g]	2.92(1.11)	3.30(1.09)	2.80	3.33
Palmitic acid [g]	12.62(3.40)	13.14(3.67)	12.28	13.10
Stearic acid [g]	5.65(1.69)	6.05(2.09)	5.62	5.80
MUFA** [g]	18.72(5.36)	22.99(7.88)	18.05	21.75
Oleic acid [g]	13.35(4.89)	21.75(6.88)	12.57	21.13
PUFA*** [g]	11.26(3.24)	12.84(5.90)	10.45	11.40
Linoleic acid [g]	10.72(3.37)	11.40(5.53)	10.09	9.15
Alpha-Linolenic acid [g]	1.48(0.63)	1.06(0.34)	1.27	1.00
Arachidonic acid [g]	0.10(0.06)	0.17(0.16)	0.08	0.10
Eicosapentaenoic acid [g]	0.04(0.07)	0.05(0.08)	0.01	0.00
Docosahexaenoic acid [g]	0.13(0.26)	0.17(0.16)	0.03	0.10
Cholesterol [mg]	253.41(102.34)	254.68(103.62)	230.08	231.50
Proteins [g]	78.64(16.90)	79.02(17.95)	78.30	79.10
Water [g]	2928.89(1355.09)	2758.93(872.28)	2707.06	2808.50
Alcohol [g]	0.57(1.09)	0.64(1.17)	0.06	0.15
Vitamins:				
Biotin [μg]	35.97(11.15)	52.08(20.42)	34.76	49.75
Folic acid [μg]	388.64(106.78)	298.59(109.33)	377.37	277.25
Niacin [μg]	30698.05(8505.74)	27343.77(7964.66)	30861.95	26568.50
Pantothenic acid [mg]	6.13(2.04)	5.94(2.09)	5.68	5.40
Vitamin A [mg]	0.76(1.34)	0.41(0.20)	0.52	0.36
Riboflavin [mg]	1.91(0.48)	1.65(0.49)	1.91	1.62
Thiamine [mg]	1.47(0.38)	1.36(0.53)	1.42	1.22
Vitamin B_{12} [μg]	3.90(1.26)	5.23(2.08)	3.87	4.83
Vitamin B_6 [mg]	1.89(0.48)	1.81(0.48)	1.86	1.82
Vitamin C [mg]	173.69(93.75)	189.07(74.99)	156.09	171.25
Vitamin D [μg]	2.58(2.64)	2.50(3.07)	1.73	1.75
Vitamin E [mg]	11.96(4.18)	15.10(6.01)	11.00	14.68
Minerals:				
Calcium [mg]	1106.75(508.97)	1084.81(320.02)	1042.99	1088.25
Magnesium [mg]	571.99(1100.57)	386.16(102.35)	363.15	376.50
Phosphorus [mg]	1349.86(305.69)	1475.62(352.82)	1334.92	1437.00

	Average(SD)		Median	
	web-DR	SW-DR	web-DR	SW-DR
Potassium [mg]	3349.91(783.74)	3557.52(922.98)	3294.96	3418.00
Sodium [mg]	3130.55(2416.03)	2343.81(872.59)	2474.43	2178.25
Chloride [mg]	4934.61(3759.24)	3951.81(1386.93)	4074.61	3595.75
Trace elements:				
Iron [mg]	14.06(3.79)	14.28(3.71)	14.07	13.98
Copper [µg]	1684.52(440.59)	2472.60(595.37)	1721.94	2546.25
Fluoride [µg]	297.19(255.45)	906.85(341.91)	241.35	857.50
Iodine [µg]	132.65(117.95)	171.22(62.78)	98.48	161.00
Manganese [µg]	5194.11(3363.54)	4738.35(1730.32)	4348.27	4438.50
Zinc [mg]	9.64(2.34)	12.31(2.88)	9.96	12.25

* Sum of saturated fatty acids
** Sum of monounsaturated fatty acids
*** Sum of polyunsaturated fatty acids

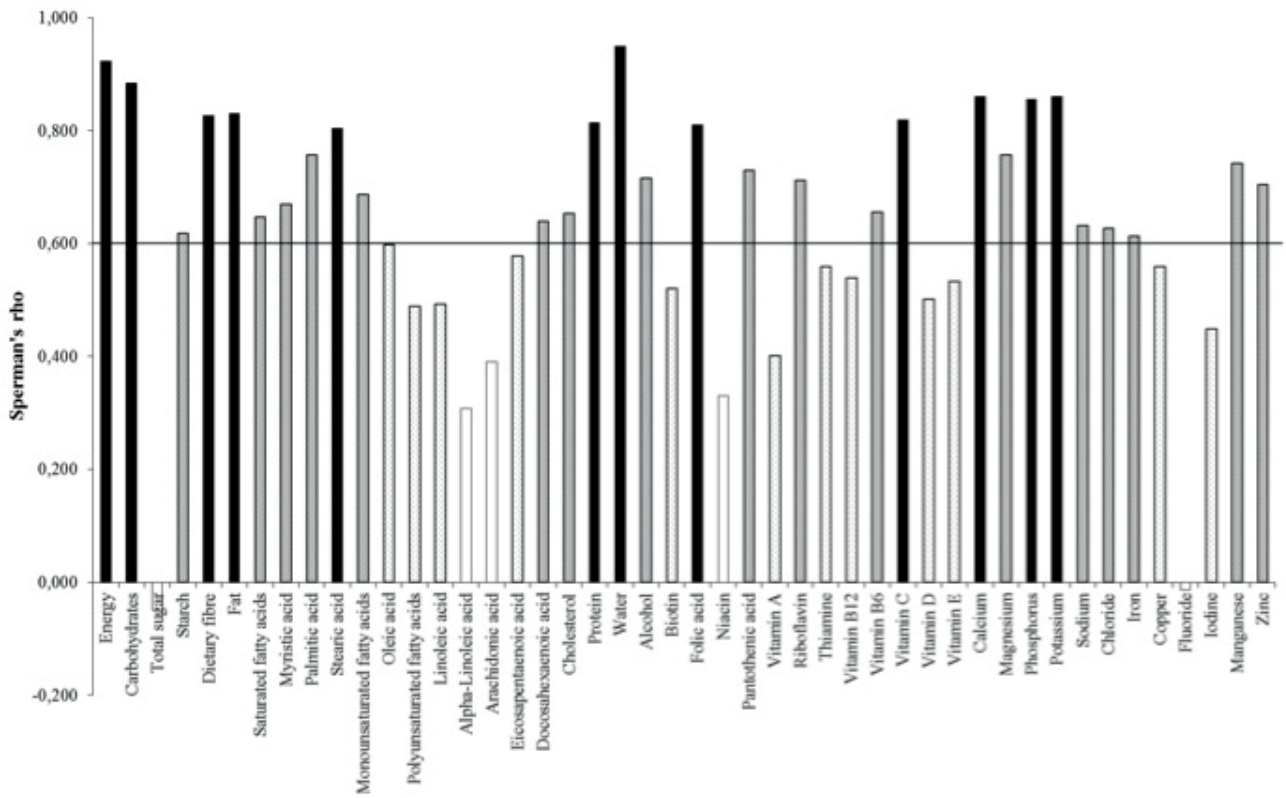

Figure 1. Spearman's rho correlation coefficients for 46 parameters calculated from 63 four-day dietary records recorded by 63 volunteers and entered into web- and software-based dietary records by a dietitian (cut-off for Spearman's rho as strongly positive correlation was set at >0.800 (black columns), as medium significant at 0.600-0.799 (grey columns), as weak at 0.400-0.599 (spotted columns) and no correlation (white columns)). Spearman's rho coefficients for all studied correlations are significant at the 0.05 level.

4 DISCUSSION

To the best of our knowledge, this is the first study to compare two self-administered methods (web-DR versus SW-DR) completed by the same persons and entered into both applications by the same research dietitian. We have already compared the assessment of dietary intake using paper-DR as the gold standard (1) versus the novel web-DR completed by the same volunteers (31). There was no difference between total matching of paper-DR versus web-DR. The next step was to compare the two most frequently used dietary record softwares in clinical practice in Slovenia; OPKP (Web-DR) and Prodi (SW-DR).

Average ranges (median) are not different between the two methods (Table 1). The basic parameters in nutrition assessment (i.e., energy, carbohydrates, proteins, fat and water) were highly correlated between the methods (>0.800) (Figure 1). As expected, some of the parameters, such as arachidonic acid, niacin, alpha-linolenic acid, fluoride and total sugars, did not correlate. In our opinion, the reason for the discrepancy with arachidonic acid, niacin and alpha-linolenic acid was mainly a lack of compositional data for branded food items. Namely, foods rich in these nutrients are meat and meat products, fish and fish products, eggs and egg products, nuts and nut products, and grain-based products. In OPEN, the Slovenian food composition data for meat, fish and their products were used. Since meat of Slovenian origin accounts for the largest share of meat consumed in this country, a comparison of compositional data on Slovenian meat with data from the literature was made, showing a wide variation, particularly for the total fat content, fatty acid composition and cholesterol content (24).

In the case of fluoride and total sugars, the differences were due to different food composition databases (mainly there is no data for total sugar in Prodi). The lack of correlation could also be due to human error but this is less likely, because all paper-DRs were entered into the web-DR and SW-DR by the same research dietitian and they were checked twice.

Values for total saturated, monounsaturated, and polyunsaturated fatty acids may include individual fatty acids not reported; therefore, the sum of their values may exceed the sum of the individual fatty acids. In rare cases, the sum of the individual fatty acids may exceed the sum of the values given for the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). These differences are generally caused by rounding and should be relatively small.

Various instruments are used nowadays to assess nutrient intake and food consumption, each with advantages and disadvantages (16). However, it makes more sense to use a DR that is supported by devices that are integrated into the daily lives of people (computer, tablet, smartphone, etc.), as has already been described in the literature (14, 18, 32, 33).

Our study also had some other limitations. Firstly, not participants themselves, but the research dietitian entered the paper-DR into the web-DR and SW-DR. It would be interesting to analyze the matching of dietary assessment with both methods, conducted by the same volunteers.

Secondly, when the exact food was not available in the OPEN food composition database, the closest substitute was used (the research dietetic sometimes selected a different substitute in OPKP to that chosen in Prodi).

Thirdly, some technical limitations with web-DR were observed/reported, such as a slower internet connection speed, which can decrease the user-friendliness of OPEN; users, consequently, had to wait longer than expected for the food list to appear on the screen. In the Probst and Tapsell study (34), it was reported that spelling errors and errors in the identification of specific foods can also cause problems, especially with self-administered web-based dietary records, but not in SW-DR. There was the same problem in our case, especially in relation to some local traditional foods that have different names for the same items across Slovenia (e.g., lard), or some newly adopted international words (e.g., pizza, ketchup).

5 CONCLUSION

Our study shows that web-DR (OPEN) provides dietary intake data information of equal or superior quality to that of SW-DR (Prodi), mainly because it is based on Slovenian food composition data, which are integrated in OPEN. The use of advanced technology in DR recording has shown and continues to show great promise. We have shown that either one of the nutritional dietary record softwares can be used in clinical practice.

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

The study protocol was approved by the Slovene National Medical Ethics Committee (No. 32/07/2010).

REFERENCES

1. Rosalind SG. Validity in dietary assessment methods. In: Rosalind SG, editor. Principles of nutritional assessment. 2nd ed. New York: Oxford University Press, 2005: 149–58.
2. Ferro-Luzzi A. Keynote paper: individual food intake survey methods. FAO Corp Doc Repos. Available September 26, 2014 from: http://www.fao.org/docrep/005/Y4249E/y4249e0a.htm#bm10.
3. Chinnock A. Validation of an estimated food record. Public Health Nutr 2007; 9: 934–41.
4. Kobe H, Kržišnik C, Fidler Mis N. Under- and over-reporting of energy intake in Slovenian adolescents. J Nutr Educ Behav 2012; 6: 574–84.
5. Day N, McKeown N, Wong M, Welch A, Bingham S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. Int J Epidemiol 2001; 30: 309–17.
6. Burke LE, Sereika S, Choo J, Warziski M, Music E, Styn M. et al. Ancillary study to the PREFER trial: a descriptive study of participants' patterns of self-monitoring-rationale, design and preliminary experiences. Contemp Clin Trials 2006; 27: 23–33.
7. Thompson FE, Subar AF. Dietary assessment methodology. In: Coulston AM, Boushey CJ, Ferruzzi MG, editors. Nutrition in the prevention and treatment of disease. 3rd ed. Oxford: Elsevier, 2013: 5–46.
8. Thompson FE, Byers T. Dietary assessment resource manual. J Nutr 1994; 124: 245–317.
9. Jackson B, Dujovne CA, DeCoursey S, Beyer P, Brown EF, Hassanein K. Methods to assess relative reliability of diet records: minimum records for monitoring lipid and caloric intake. J Am Diet Assoc 1986; 86: 1531–5.
10. Gersovitz M, Madden JP, Smiciklas-Wright H. Validity of the 24-hr dietary recall and seven-day record for group comparisons. J Am Diet Assoc 1978; 73: 48–55.
11. Illner AK, Freisling H, Boeing H, Huybrechts I, Crispim SP, Slimani N. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. Int J Epidemiol 2012; 41: 1187–203.
12. Lieffers JRL, Hanning RM. Dietary assessment and self-monitoring with nutrition applications for mobile devices. Can J Diet Pract Res 2012; 73: 253–60.
13. Baranowski T, Islam N, Baranowski J, Cullen KW, Myres D, Marsh T. et al. The food intake recording software system is valid among fourth-grade children. J Am Diet Assoc 2002; 102: 380–5.
14. Carine V A, Marc C, Denise H, Lea M. Feasibility of the young children's nutrition assessment on the web. J Am Diet Assoc 2009; 109: 1896–902.
15. EFSA. General principles for the collection of national food consumption data in the view of a pan-European dietary survey 1. EFSA J 2009; 7: 1–51.
16. Burke L, Warziski M, Starrett T, Choo J, Music E, Sereika S. et al. Self-monitoring dietary intake: current and future practices. J Ren Nutr 2005; 15: 281–90.
17. Ming Yan Chung L, Wai Yee Chung J, Kwok Shing Wong T. Usability test of an interactive dietary recording. Int Electron J Heal Educ 2009; 12: 123–34.
18. Beasley JM, Riley WT, Davis A, Singh J. Evaluation of a PDA-based dietary assessment and intervention program: a randomized controlled trial. J Am Coll Nutr 2008; 27: 280–6.
19. Bratanič B, Fidler Mis N, Benedik E, Rogelj I. A short presentation of the research project "The role of human milk in development of breastfed child's intestinal microbiota."In: Rogelj I, Fidler Mis N, Bogovič Matijašić B, editors. Moje-mleko: zbornik prispevkov: mednarodni znanstveni simpozij Vloga humanega mleka v razvoju črevesne mikrobiote dojenčka. Ljubljana: Biotehniška fakulteta, 2011: 30–4.
20. Fidler Mis N, Kobe H, Štimec M. Dietary intake of macro- and micronutrients in slovenian adolescents: comparison with reference values. Ann Nutr Metab 2012; 61: 305–13.
21. Computer web-based software: the Open Platform for Clinical Nutrition (OPEN). Available September 26, 2014 from: http://www.opkp.si/sl_SI/fooddiary/diary.
22. Koroušić Seljak B. Computer-based dietary menu planning. J Food Comp Anal 2009; 22: 414–20.
23. Golob T, Stibilj V, Žlender B, Kropf U, Korošec M, Polak T. et al. Slovenske prehranske tabele: meso in mesni izdelki. Ljubljana: Biotehniška fakulteta, 2006.
24. Korošec M, Golob T, Bertoncelj J, Stibilj V, Koroušić Seljak B. The Slovenian food composition database. Food Chem 2013; 140: 495–9.
25. Souci S, Fachmann W, Kraut H. Food composition and nutrition tables. Stuttgart: MeDRharm, 2008.
26. USDA. National nutrient database for standard reference. Release 17, 2011.
27. Greenfield H, Southgate DAT. Food composition data – production, management and use. 2nd ed. Rome: Food and Agriculture Organization of the United Nations, 2003.
28. Reinivuo H, Laitinen K. Proposal for the harmonization of recipe calculation procedures. WP2.2. Composite Foods, Finland: National Public Health Institute, 2007.
29. Koroušić Seljak B, Pokorn D, Stibilj V, Pograjc L. Evaluation of a web application for dietary menu planning. 2nd International EuroFIR Congress. 26th-27th Sept. 2007. Granada: EuroFIR, 2007: 119.
30. Koroušić Seljak B, Stibilj V, Pograjc L, Fidler Mis N, Benedik E. Food composition databases for effective quality nutritional care. Food Chem 2013; 140: 553–61.
31. Benedik E, Koroušić Seljak B, Simčič M, Rogelj I, Bratanič B, Ding EL. et al. Comparison of paper- and web-based dietary records: a pilot study. Ann Nutr Metab 2014; 64: 156–66.
32. Acharya SD, Elci OU, Sereika SM, Styn MA, Burke LE. Using a personal digital assistant for self-monitoring influences diet quality in comparison to a standard paper record among overweight/obese adults. J Am Diet Assoc 2011; 111: 583–8.
33. Yon BA, Johnson RK, Harvey-Berino J, Gold BC, Howard AB. Personal digital assistants are comparable to traditional diaries for dietary self-monitoring during a weight loss program. J Behav Med 2007; 30: 165–75.
34. Probst Y, Tapsell LC. Overview of computerized dietary assessment programs for research and practice in nutrition education overview of computerized dietary assessment programs for research. J Nutr Educ Behav 2005; 37: 20–6.

CYTOGENETIC AND MOLECULAR GENETIC CHARACTERIZATION OF CHILDREN WITH SHORT STATURE

CITOGENETSKA IN MOLEKULARNO GENETSKA OPREDELITEV NIZKE RASTI PRI OTROCIH

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ABSTRACT

Background. The deficiency of *SHOX* gene (short stature homeobox-containing gene) has been recognized as the most frequent monogenetic cause of short stature. *SHOX* gene has been associated with short stature in Turner syndrome and Leri Weill dyschondrosteosis as well with non-syndromic idiopathic short stature. The aim of this study was to determine the frequency of *SHOX* deletions and mutations in a cohort of Slovenian children with short stature, and to delineate indications for routine *SHOX* gene mutation screening.

Methods and results. 40 selected subjects with idiopathic short stature were screened for entire *SHOX* gene deletion and for mutations in the *SHOX* gene coding region (exon 2 to 6), together with sequences flanking the exon-intron boundaries. FISH analysis on metaphase and interphase spreads revealed no entire gene deletion. Additionally, no pathogenic point mutations or smaller deletion/duplications were identified in this study group.

Conclusions. *SHOX* gene deletions and point mutations are not a common cause of idiopathic short stature in a cohort of Slovenian children with short stature. Therefore, the frequency of *SHOX* mutations must be much lower as expected based on the reported data.

Keywords:
SHOX gene, idiopathic short stature, FISH analysis, DNA sequnecing

IZVLEČEK

Izhodišča. *Razlike v številu aktivnih kopij gena SHOX (short stature homeoboxcontaining gen) so najpomembnejši monogenetski vzrok nizke rasti. Vpliv gena SHOX je opredeljen pri razvoju nizke rasti v sklopu Turnerjevega sindroma ali Leri-Weillove dishondrosteoze ter je lahko vzrok nesindromske idiopatske nizke rasti. Namen raziskave je določiti frekvenco delecij in mutacij gena SHOX v skupini slovenskih otrok z nizko rastjo ter opredeliti smernice za presejalno testiranje mutacij v genu SHOX.*

Metode in rezultati. *Izbranih 40 preiskovancev z nizko rastjo smo genetsko testirali za delecijo celotnega gena SHOX ter pojavnost mutacij v celotnem kodirajočem področju gena (eksoni 2 do 6) skupaj z mejami med eksoni in introni. Z analizo FISH na metafaznih in interfaznih kromosomih pri nobenem izmed preiskovancev nismo odkrili delecije. Prav tako pri nobenem izmed 40 preiskovancev nismo odkrili točkovne mutacije ali manjše delecije/duplikcije.*

Zaključki. *Delecije in točkovne mutacije gena SHOX v izbrani skupini slovenskih otrok z nizko rastjo niso pogost razlog za idiopatsko nizko rast. Zato predvidevamo, da je frekvenca mutacij gena SHOX nižja, kot bi pričakovali glede na podatke iz literature.*

Ključne besede:
gen SHOX, idiopatska nizka rast, analiza FISH, sekveniranje DNK

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1 INTRODUCTION

Short stature is a frequent childhood developmental condition with an incidence of 3 in 100 (1, 2). With the majority of these individuals, the underlying cause remains unknown and the condition is referred to as idiopathic short stature (ISS). It has been long known that human height follows a polygenic mode of inheritance. To date, there is about 50 genes and regions of the genome associated with height (3, 4). Among them, the deficiency of short stature homeobox-containing gene (*SHOX* gene) has been found as the most frequent monogenetic cause of short stature (5, 6).

Numerous studies in the last decade have indicated that syndromic short stature and idiopathic growth retardation are associated with *SHOX* deficiency. Nullzygosity of *SHOX* results in Langer mesomelic dysplasia (LMD), while haploinsufficiency of *SHOX* leads to Leri-Weill dyschondrosteosis (LWD) and short stature observed in Turner syndrome (TS). Heterozygous *SHOX* mutations or *SHOX* deletions were detected in 2-15% of individuals with idiopathic short stature (ISS) (6-8). The phenotypic outcome of *SHOX* deficiency is extremely variable: from most severe LMD, milder LWS, to isolated *SHOX*-related short stature at the mildest end of the spectrum. On the contrary, the over-expression of the gene is associated with tall stature in Klinefelter syndrome (Figure 1). It is important to emphasize that short stature can also be non-pathological in the case of familial short stature and constitutional delay (9).

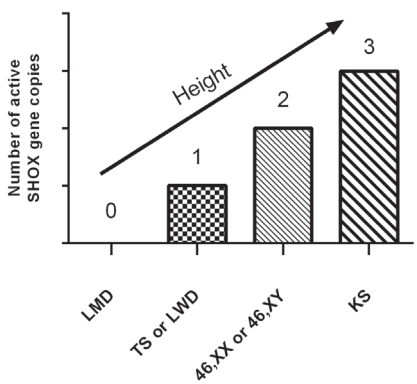

Figure 1. Number of active copies of SHOX gene associated with human height. LMD, Langer mesomelic dysplasia; TS, Turner syndrome; LWD, Leri-Weill dyschondrosteosis; KS, Klinefelter syndrome.

The evolutionary and biological basis of human height is not fully understood, especially its links to disease (10). The aim of this study was to determine the frequency of *SHOX* deletions and mutations in a cohort of Slovenian children with ISS, and to delineate indications for routine *SHOX* gene mutation screening.

1.1 *SHOX* Gene

The *SHOX* gene resides in pseudoautosomal region (PAR1) on the short arm of Xp and Yp, and has an important role in mediating linear growth. This telomeric PAR1 region spans over 2.7 Mb, and contains, so far, 29 known genes which escape X inactivation leading to the expression of *SHOX* from both sex chromosomes. The inheritance of pseudoautosomal region therefore mimics an autosomal dominant mode of inheritance (1, 11). The *SHOX* gene is composed of 6 exons: the last one has two alternatively spliced forms (exon 6a and exon 6b) encoding two different isoformes; *SHOX*a and its shortened version of *SHOX*b (11). Both isoformes function as transcriptional activators binding on specific regulatory region on DNA (7).

To date, there is about 250 *SHOX* gene mutations listed in Human Genome Mutation Database (HGMD Professional 2013.3), and an additional 1000 unique gene variants in *SHOX* database at www.shox.uni-hd.de. Among them, gross deletions and nucleotide substitutions are the most frequent ones. At the University Children's Hospital Ljubljana, we have established a method using both cytogenetic and molecular genetic technique for the assessment of *SHOX* deficiency in Slovenian children with short stature.

2 PATIENTS AND METHODS

From 2011 to 2014, we have tested 107 short stature children (92 females, 15 males, mean age 10.5±4.4). They were all referred for cytogenetic testing to the Cytogenetic laboratory at the Unit for Special Laboratory Diagnostics at the University Children's Hospital, University Medical Centre Ljubljana. After standard cytogenetic analyses, 16 girls were diagnosed with Turner syndrome or Turner syndrome variant. The subjects found to harbour structural chromosomal rearrangement were also excluded from further molecular analysis. The remaining patients were clinically examined at The Department of Endocrinology, Diabetes and Metabolism, University Children's Hospital, University Medical Centre, Ljubljana. Idiopathic short stature (ISS) was defined as the height below the 5th percentile for chronological age and sex and the absence of specific causative disorder. After thorough diagnostic work-up, overall 40 selected individuals (34 females, 6 males, mean age 9.1±4.1) were included in the further molecular investigation of short stature. All cytogenetics and molecular-genetics studies were undertaken with fully informed consent. The study followed the principles of the Declaration of Helsinki.

2.1 Cytogenetic Investigation and FISH Analysis

All patients were previously screened for chromosomal aneuploidy and/or structural rearrangement using standard cytogenetic analysis on stimulated lymphocytes. Only children with normal karyotype in 30 metaphases studied by GTG banding at the 500 band level were enrolled in subsequent fluorescence in situ hybridisation (FISH). FISH analysis was performed on metaphase and interphase chromosome spreads using a probe specific for the *SHOX* gene. We have selected BlueFish probe RP13-391G2 (BlueGnome) hybridizing to cytoband Xp22.33 starting from 562252 to 630112. For internal control we used chromosome X centromere probe (DXZ3 locus) and/or chromosome Y probe (DYZ1 locus) in parallel hybridisation.

2.2 DNA Sequencing Analysis

DNA was extracted directly from fixed cytogenetic cell suspensions with fast and simple isolation protocol using dedicated QiAmp DNA mini isolation kit, together with automated isolation system Qiacube (Qiagen, Hilden, Germany) (13). The amount of isolated DNA with concentrations between 10–15 ng/µl was suitable for PCR amplification of all *SHOX* exons. When available, genomic DNA was isolated from peripheral blood with the FlexiGene DNA Kit 250 (Qiagen, Hilden, Germany). *SHOX* gene coding region was PCR amplified using in-house designed sets of primers (sequences available upon request). Amplicons were sequenced using BigDye Terminator v.3.1 Cycle Sequencing Kit and 3500 Genetic Analyzer capillary electrophoresis system (Life Technologies, Foster City, CA, USA).

3 RESULTS

40 patients were diagnosed with ISS (no evidence of organic disease, normal wrist X-rays as indicator of bone age and presence of Madelung deformity, normal endocrine screen and normal growth hormone secretion assessed by growth hormone levels after provocative testing with Arginine or L-dopa). Short stature with disproportions of bodily parts (possible skeletal dysplasia) was present in 2 subjects, and 7 subjects had mild dysmorphic features that were not assigned to a known syndrome. None of the participants presented with the Madelung deformity, which was assessed clinically and with radiological imaging. Clinical characteristics of the selected participants are summarized in Table 1. FISH analysis on metaphase and interphase spreads revealed no entire gene deletion. Additionally, no pathogenic point mutations or smaller deletion/duplications were identified in any of the 40 participants.

Table 1. Clinical features of children with idiopathic short stature screened for SHOX gene mutations.

N	40
Age (years)	9.1±4.1
Gender	34 female (85.0 %), 6 male (15.0 %)
Height SDS	-1.95±0.46
Bone age SDS	-0.93±1.02
Target height SDS	-0.20±0.65
Possible skeletal dysplasia	2 (5.0 %)
Dysmorphic features	7 (17.5 %)

N: number of patients; SDS: standard deviation score

4 DISCUSSION

The clinical phenotypes of *SHOX* haploinsufficiency disorders are extremely variable, from extremely short stature due to homozygous deletion or mutation on both *SHOX* alleles, to milder ISS without other clinical characteristic. We have conducted genetic screening study for the assessment of *SHOX* deletion/mutation prevalence in a group of 40 Slovenian children with ISS. The *SHOX* gene region was analysed using two independent methods with a different mutation detection range: fluorescence in situ hybridisation to identify large deletions, and direct DNA sequencing to identify point mutations and small deletions or insertions. FISH analysis appears as an easy, appropriate, and inexpensive method for the detection of *SHOX* deletion (14). Cytogenetic chromosomal investigations in our group of patients with ISS did not reveal *SHOX* deletion. Sequencing analysis also found no pathogenic point mutations in coding region of *SHOX* gene. In contrast to FISH, this method appears as time consuming and expensive, and, most importantly, in general, it is covering a lower percentage of causative gene defects, as point mutations are less frequent compared to *SHOX* gene deletions (8).

Our estimated prevalence of *SHOX* molecular defect was lower than previously reported. The largest published study on 1608 patients with short stature revealed *SHOX* deficiency in 4.2% of the analysed individuals: complete gene deletion in 70%, partial deletion in 5.9% and point mutation in 23.5% (8). General estimates for prevalence in children with ISS ranked from 2 to 15% (2, 5, 14, 15). In contrast, studies that were performed on smaller groups, show conflicting results. One of the first published studies on *SHOX* deficiency using FISH analysis detected no deletions in a cohort of 36 patients with unexplained short stature (16). There are several possibilities explaining the discrepancies between our and reported findings. Different clinical criteria for short

stature between studies with different methodological approaches are just one of them. During recent years, the discovery of deletion downstream the *SHOX* and its functional characterization led to the identification of several enhancer elements. To date, 4 enhancers located downstream and 3 enhancers upstream of the *SHOX* gene inside PAR1 have been described (17). Among them, the recurrent PAR1 deletion downstream of the *SHOX* spanning 47543 bp with identical breakpoints in several patients was characterized and confirmed as regulatory enhancer element for *SHOX* transcription (18).

With this additional enhancers recognized and higher frequency of *SHOX* deletion compared to point mutation, multiple ligation probe amplification (MLPA) seems as the most logical method of choice in investigation of ISS genetic defects. MLPA analysis was already recognized as fast, simple and high throughput screening method in the group of short children, and is recommended to be used for large scale screening of *SHOX* deletions (19).

An estimated heritability of human height is about 80-90% (20). Recent genome-wide association studies for copy number variations (CNV) and SNP arrays showed that rare CNV and SNP are also a common cause of short stature (4, 20). Especially SNP rs1042725 in HMGA2 gene is a strong candidate for a height-associated allelic variant. Researchers analyzing large samples confirmed that rs1042725 C allele is associated strongly with increased human height (21, 22). Despite numerous height variations contributing only a small fraction under a polygenic model, finding the genetic cause in this frequent condition is very important.

5 CONCLUSIONS

Linear growth is one of the most sensitive indicators of health, and is affected by various pathophysiological mechanisms, including genetic variations in *SHOX* gene. In the present study of children with ISS, however, no deletions or pathogenic point mutation in the *SHOX* gene were identified. This data does not corroborate currently published results in other populations, where a higher incidence of genetic variations (especially deletions) in *SHOX* gene was determined. Therefore, we propose that, in children with ISS, first-tier genetic analysis should include one of molecular cytogenetic testing, either MLPA or FISH, and not *SHOX* mutations detection, since they are less frequent.

FUNDING

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

ETHICAL APPROVAL

Written informed consent was obtained from all participants or their parents. The study was approved by the Slovene Medical Ethics Committee.

REFERENCES

1. D'haene B, Hellemans J, Craen M, De Schepper J, Devriendt K, Fryns JP. et al. Improved molecular diagnostics of idiopathic short stature and allied disorders: quantitative polymerase chain reaction-based copy number profiling of SHOX and pseudoautosomal region 1. J Clin Endocrinol Metab 2010; 95: 3010-8.
2. Rappold GA, Fukami M, Niesler B, Schiller S, Zumkeller W, Bettendorf M. et al. Deletions of the homeobox gene SHOX (short stature homeobox) are an important cause of growth failure in children with short stature. J Clin Endocrinol Metab 2002; 87: 1402-6.
3. Chial H. Polygenic inheritance and gene mapping. Nature Educ 2008; 1: 17.
4. van Duyvenvoorde HA, Lui JC, Kant SG, Oostdijk W, Gijsbers AC, Hoffer MJ. et al. Copy number variants in patients with short stature. Eur J Hum Genet 2014; 22: 602-9.
5. Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. Horm Res Paediatr 2011; 75: 81-9.
6. Rappold G, Blum WF, Shavrikova EP, Crowe BJ, Roeth R, Quigley CA. et al. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. J Med Genet 2007; 44: 306-13.
7. Stuppia L, Calabrese G, Gatta V, Pintor S, Morizio E, Fantasia D. et al. SHOX mutations detected by FISH and direct sequencing in patients with short stature. J Med Genet 2003; 40: E11.
8. Lutchman N. Short stature in children. Continuing Med Educ 2006; 24: 372.
9. McEvoy BP, Visscher PM. Genetics of human height. Econ Hum Biol 2009; 7: 294-306.
10. Helena Mangs A, Morris BJ. The Human Pseudoautosomal Region (PAR): origin, function and future. Curr Genomics 2007; 8: 129-36.
11. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A. et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet 1997; 16: 54-63.
12. Amorim MR, Vargas FR, Llerena JC Jr, Pombo-de-Oliveira MS. DNA extraction from fixed cytogenetic cell suspensions. Genet Mol Res 2007; 6: 500-3.
13. Morizio E, Stuppia L, Gatta V, Fantasia D, Guanciali Franchi P, Rinaldi MM. et al. Deletion of the SHOX gene in patients with short stature of unknown cause. Am J Med Genet A 2003; 119A: 293-6.
14. Huber C, Rosilio M, Munnich A, Cormier-Daire V. French SHOX GeNeSIS Module. High incidence of SHOX anomalies in individuals with short stature. J Med Genet 2006; 43: 735-9.
15. Binder G, Schwarze CP, Ranke MB. Identification of short stature caused by SHOX defects and therapeutic effect of recombinant human growth hormone. J Clin Endocrinol Metab 2000; 85: 245-9.
16. Musebeck J, Mohnike K, Beye P, Tönnies H, Neitzel H, Schnabel D. et al. Short stature homeobox-containing gene deletion screening by fluorescence in situ hybridisation in patients with short stature. Eur J Pediatr 2001; 160: 561-5.

17. Benito-Sanz S, Aza-Carmona M, Rodríguez-Estevez A, Rica-Etxebarria I, Gracia R, Campos-Barros A. et al. Identification of the first PAR1 deletion encompassing upstream SHOX enhancers in a family with idiopathic short stature. Eur J Hum Genet 2012; 20: 125-7.
18. Benito-Sanz S, Royo JL, Barroso E, Paumard-Hernández B, Barreda-Bonis AC, Liu P. et al. Identification of the first recurrent PAR1 deletion in Léri-Weill dyschondrosteosis and idiopathic short stature reveals the presence of a novel SHOX enhancer. J Med Genet 2012; 49: 442-50.
19. Gatta V, Antonucci I, Morizio E, Palka C, Fischetto R, Mokini V. et al. Identification and characterization of different SHOX gene deletions in patients with Leri-Weill dyschondrosteosys by MLPA assay. J Hum Genet 2007; 52: 21-7.
20. Zahnleiter D, Uebe S, Ekici AB, Hoyer J, Wiesener A, Wieczorek D. et al. Rare copy number variants are a common cause of short stature. PLoS Genet 2013; 9: e1003365.
21. Weedon MN, Lettre G, Freathy RM, Lindgren CM, Voight BF, Perry JR. et al. Diabetes Genetics Initiative; Wellcome Trust Case Control Consortium, Davey Smith G, Groop LC, Hattersley AT, McCarthy MI, Hirschhorn JN, Frayling TM. A common variant of HMGA2 is associated with adult and childhood height in the general population. Nat Genet 2007; 39: 1245-50.
22. Yang TL, Guo Y, Zhang LS, Tian Q, Yan H, Guo YF, Deng HW. HMGA2 is confirmed to be associated with human adult height. Ann Hum Genet 2010; 74: 11-6.

ANNUAL PSYCHOLOGICAL SCREENING IN YOUTH AND YOUNG ADULTS WITH TYPE 1 DIABETES

LETNO PRESEJALNO PSIHOLOŠKO TESTIRANJE PRI MLADOSTNIKIH IN MLADIH ODRASLIH S SLADKORNO BOLEZNIJO TIPA 1

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ABSTRACT

Aim. Youth and young adults with type 1 diabetes are at a great risk for developing depression and diabetes specific distress, therefore, systematic psychological screening is recommended. Routine psychological screening was implemented in Slovene diabetes clinic for children, adolescents and young adults in 2012. One-year results are presented.

Methods. Adolescents and young adults (*N* = 159, aged 11 - 25 years), attending the obligatory yearly educational outpatient visit at University Children's Hospital, Ljubljana, Slovenia, were examined using questionnaires measuring depression (depression scale from Slovene version of Trauma Symptom Checklist for Children) and diabetes distress (Diabetes Distress Screening Scale). Six additional items were included to assess the fear of hypoglycemia and family support. Socio-demographic and diabetes-related data were collected. Questionnaires were analyzed by a psychologist, and the patients that scored above cut-off point were invited to an individual psychological assessment.

Results. Of the sample, 1.3 % reached the threshold for elevated depressive symptoms, and 32.7 % reported significant diabetes distress. The need for psychological support from a specialist was expressed by 5.0 %. There were statistically significant associations between all psychological variables; moreover, better glycemic control was associated with lower diabetes distress and better family support. Nine patients (5.7 %) started with psychological treatment according to the referrals after screening.

Conclusions. The results after one year of psychological screening in Slovene type 1 diabetes population displayed small rates of depression and a large proportion of diabetes distress. Only a small percentage of patients attended the offered individual psychological assessment.

Keywords:
depression, diabetes distress, fear of hypoglycemia, family support, glycemic control

IZVLEČEK

***Namen raziskave**. Pri mladih s sladkorno boleznijo tipa 1 je prisotno večje tveganje za razvoj depresivne motnje, pogosto pa ti bolniki poročajo tudi o obremenjenosti s sladkorno boleznijo. Zato se priporoča psihološko presejalno testiranje. V letu 2012 smo presejalno testiranje za mladostnike in mlade odrasle uvedli tudi v Sloveniji ter prve rezultate predstavili v tej raziskavi.*

***Metode**. Rednega letnega edukacijskega pregleda na Kliničnem oddelku za endokrinologijo, diabetes in bolezni presnove Pediatrične klinike se je udeležilo 175 mladostnikov in mladih odraslih, starih od 11 do 25 let. Od teh jih je 159 rešilo presejalni vprašalnik, ki je ocenjeval depresivne simptome (lestvica depresije iz vprašalnika o travmatiziranosti otrok in mladostnikov) in obremenjenost s sladkorno boleznijo (presejalna lestvica za oceno obremenjenosti s sladkorno boleznijo). Dodanih je bilo šest postavk za oceno strahu pred hipoglikemijo in podporo družine. Zbrali smo podatke o sladkorni bolezni in sociodemografskem ozadju. Psiholog je pregledal vprašalnike in podal kratko mnenje, mladostnike in odrasle, ki so presegli kritično število točk, pa smo povabili na posvet in dodatno obravnavo k psihologu.*

***Rezultati**. Rezultate, ki pomenijo povečano tveganje za depresijo, je doseglo 1,3 % udeležencev, 32,7 % udeležencev pa je poročalo o pomembni obremenjenosti s sladkorno boleznijo. Željo po psihološki obravnavi je izrazilo 5 % preiskovanih. Prisotna je bila statistično pomembna povezanost med vsemi psihološkimi parametri. Dobra presnovna urejenost je bila povezana z manjšo obremenjenostjo s sladkorno boleznijo in boljšo podporo družine. Devet (5,7 %) mladih je po presejalnem psihološkem testiranju pričelo obravnavo pri psihologu.*

***Zaključki**. Prvo leto presejalnega psihološkega testiranja mladih s sladkorno boleznijo tipa 1 je pokazalo nizek delež udeležencev z depresivnimi simptomi in visok delež pacientov, ki so izrazito obremenjeni s sladkorno boleznijo. Le majhen delež udeležencev se je odzval na povabilo na obravnavo pri psihologu in začel obravnavo.*

Ključne besede:
depresija, depresivne motnje, presejalno psihološko testiranje, hipoglikemija, podpora družine, sladkorna bolezen tipa 1, mladostniki

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1 INTRODUCTION

Youth and young adults with type 1 diabetes (T1DM) are at an increased risk for the development of depression and other psychiatric disorders (1). Moreover, distress, associated with the chronic illness, is an additional burden to patients, especially for adolescents who already have stronger emotions, poorer self-control, self-esteem problems and worse glycemic control (2-4). Especially adolescents who have more peer-conflicts, more negative diabetes-related emotions, less parental involvement in diabetes care and are less psychologically mature are at the risk for declines in the glycemic control (5-7). Early recognition of emotional problems and timely interventions are of major importance in order to help children and youth at risk.

Depression is one of the most commonly occurring comorbid conditions among youth with diabetes (1). The estimated prevalence of depression among children and adolescents with T1DM varies from 8% to 15.2 % (8-11), depending on the population studied and methodology used. Most of the studies found that the level of depressive symptoms was higher in adolescents with diabetes than in the general population, or when compared to healthy controls (9-12).

Comorbidity of T1DM and depression or diabetes-specific distress can lead to worse diabetes self-management, especially less frequent blood glucose monitoring (BGM), poorer glycemic control, and to an earlier onset of diabetes complications (8, 9, 13-17). Moreover, higher anxiety predicted higher glycosylated hemoglobin A1c (HbA1c) values in the future (17). Similarly, higher diabetes-specific distress reported by adolescents was associated with poorer glycemic control, depression symptoms and lower quality of life (2).

Fear of hypoglycemia (FoH) increases psychological distress associated with diabetes, and also has a negative impact on the diabetes management and glycemic control (18, 19). Even though FoH is not directly associated with glycemic control, the elevated fear may motivate some patients to take counter actions to prevent hypoglycemia at the expense of experiencing unhealthy high glucose levels (18, 20-22).

Family support is another factor that contributes to youth wellbeing and treatment compliance (23). Parental involvement in diabetes management supports more frequent BGM (24). Shared responsibility for diabetes management is associated with a better psychological health, good self-care behavior, and a better glycemic control (25), while perceived 'over-involvement' of parents is related to poorer glycemic control (26). Family communication and conflict resolution skills have also been found as strong predictors of the diabetes outcome variables (27). Moreover, frequent conflicts between the child and his parents have been associated with poorer adherence and glycemic control (28, 29), and, nonetheless, with the child's depression (9).

American Association for Diabetes (ADA) and International Society for Pediatric and Adolescent Diabetes (ISPAD) recommend routine annual screening for depression of all young patients with T1DM above the age of ten years (30, 31). Additionally, youth with difficulties achieving treatment goals, or with recurrent diabetes ketoacidosis, should be screened on psychiatric disorders, psychosocial functioning, especially on depression and family coping. Patients with positive screening should be referred promptly for treatment (30). No special instruments or screening tools are recommended by the mentioned guidelines.

Although psychological screening is recommended, it is rarely formally conducted due to the barriers of its implementation. Butwitcka and her colleagues compared the diagnostic accuracy and time expenditure models of screening models for mood disorders among children with T1DM (32). The results of this study showed that the use of HbA1c levels as a first screener (with threshold at 8.7%), followed by Children's Depression Rating Scale, was more time-efficient and accurate procedure to screen for mental disorders than screening with HbA1c levels, followed by the Children Depression Inventory (CDI) or CDI alone. Corathers and her team screened adolescents with T1DM with the electronic version of CDI (16). Elevated CDI scores (≥ 16) were found in 8 % of sample and suicidal ideation was reported by 7 % of the sample. For those patients, a referral to a social worker was arranged on the same day of screening and outpatient psychological service on the next day. Both patients and staff reported acceptance of screening, while authors evaluated it challenging, but feasible.

The presented program therefore aimed to screen for patients with emotional problems, namely, depression, diabetes distress, FoH and a lack of family support, and to provide them fast psychological intervention. Preliminary results after one year of screening are presented.

2 METHODS

2.1 Subjects

University Children's hospital Ljubljana is the only center for childhood diabetes in Slovenia. Currently, 650 children, adolescents and students up to the age of 25 years are regularly visiting the outpatient clinic for diabetes.

Adolescents and young adults with T1DM in the age range between 11 and 25, attending the yearly regular educational outpatient visit at the Department of Pediatric Endocrinology, Diabetes and Metabolism, University Children's Hospital, Slovenia, were enrolled in the study

between March 2012 and June 2013. The inclusion criteria were the presence of T1DM for at least two years, and a minimum age of 11. The participation was voluntary; of the 175 invited patients, 159 answered the Questionnaire for psychological screening (the response rate was 90.8 %). One hundred and forty-one (88.7 %) participants used continuous subcutaneous insulin infusion (insulin pump), and the rest (18, 11.3%) multiple daily injections.

All patients signed an informed consent prior to enrolment. The study protocol was approved by the National Medical Ethics Committee (Approval No. 76/03/13).

2.2 Measures

2.2.1 Questionnaire for Psychological Screening

A screening questionnaire was developed to identify the patients at risk and in need of psychological support. Items measuring depressive symptoms, diabetes distress, FoH and family support were included. Each domain was evaluated separately. The patients were also asked about the school performance (possible answers: 1 – very poor, 2 – poor, 3 – average, 4 – good, 5 – very good). Moreover, they answered the question if they would like to have psychological support from an expert (yes/no).

2.2.1.1 Depressive Symptoms

The depression scale from the adapted Slovenian version of Trauma Symptom Checklist for Children (TSCC) was used to assess depressive symptoms (33). Normative data for Slovene population between age of 10 and 18 are provided. The depression scale contains 9 items (with answer options on 4 point Likert scale: 0 – never, 1 – sometimes, 2 – often, 3 – always) and has a high internal consistency (Cronbach's alfa = 0.85 in healthy population and 0.89 in a clinical sample). There are high correlations between depression scale of TSCC and scores on CDI (r = 0.68–0.73) (33, 34) and between TSCC depression and Beck Depression Inventory (r = 0.81) (35). Cut-off point for elevated depressive symptoms was set at scores that correspond to *T*-scores at and above 65 (1.5 standard deviations above the mean), that are generally considered clinically significant.

2.2.1.2 Diabetes Distress

Diabetes-specific distress was evaluated using The Diabetes Distress Screening Scale (DDS2) (36). DDS2 includes two items from the Diabetes Distress Scale – DDS17, and showed a high level of accuracy (96.7%), good sensitivity (95 %), good specificity (85 %) and 3.3 % of false-positive results when assessing diabetes-specific distress. DDS2 uses a 6 point Likert scale with each item scored from 1 (no distress) to 6 (serious distress), concerning distress experienced over the last month. Those patients whose average of the 2 screening items was ≥ 3, were included in further procedures.

2.2.1.3 Fear of Hypoglycemia

FoH was assessed with three questions regarding having worries of not recognizing hypoglycemia (1), not having supplies to treat blood sugars in case of hypoglycemia (2), and fear that no one could help when having hypoglycemia (3). Patients answered them on 5 point Likert scale from 0 (never) to 4 (always). Those patients who scored 3 (often) or 4 (always) on any of the three items were included in further procedures.

2.2.1.4 Family Support

Family support, family conflicts and appropriate involvements of parents in diabetes, were assessed using the following three statements: *There are a lot of conflicts between me and my parents due to diabetes*. (1), *My parents are overprotective regarding diabetes*. (2), and *My family does not give me enough support with my diabetes self-management*. (3). Patients answered the items on 5 point Likert scale from 0 (never) to 4 (always). Patients with scores 3 (often) or 4 (always) on any of the three items, were included in further procedures.

2.2.2 Adherence to the Treatment

An average number of BGM per day over past three or four weeks was downloaded from insulin pumps to assess one of the components of patients' adherence to the diabetes regiment. Next to the pump downloads, diabetes diaries were checked also. For patients that used multiple daily injections insulin therapy, the average number of BGM per day was assessed from their diabetes diaries; moreover, the data from glucometers was downloaded for verification.

2.2.3 Glycemic Control

Glycemic control was assessed using HbA1c, measured with the DCA 2000 + analyzer (Bayer Diagnostics, Tarrytown, NY). The measures were obtained at outpatient visits.

2.3 Procedures

The patients attending regular yearly educational visits were invited to complete the Questionnaire for psychological screening. Health and specific diabetes-related variables (such as HbA1c, type of diabetes treatment, age of diabetes onset, insulin delivery method) were collected. A psychologist analyzed the questionnaires and wrote a short report for the patient, parents and diabetologist. The patients that scored above the cut-off point for depressive symptoms, or expressed any need for psychological support, were invited to a scheduled psychological treatment for further diagnostic procedures and cognitive behavior therapy. Parents were

also invited to this session if the patient was below the age of 18 years. The patients that reached the cut-off scores on any other of the three domains (diabetes distress, FoH or family support), were invited to call (or make an e-mail contact) and schedule their appointment with a psychologist as well.

2.4 Statistical Analysis

Analyses included descriptive statistics to characterize the sample, and the *t*-test or Mann-Whitney test for assessing the between-group differences. Spearman's and Pearson's coefficients of correlation were used to evaluate the association between psychological, socio-demographics and health-related variables. The model of multivariate linear regression analysis was built to evaluate the predictors of diabetes distress.

The level of significance was set at 0.05. Statistical analyses were performed using the statistical software package PASW® Statistics 18.0.0 (37) and G*Power 3.1 for statistical power analyses (38).

3 RESULTS

Baseline characteristic and diabetes-related variables are summarized in Table 1. Average test scores of psychological domains on the Questionnaire for psychological screening are presented in Table 2. Only two patients reported elevated depressive symptoms, but nearly one third (32.7 %) of the patients reported significant diabetes distress.

The need for a psychological support from a mental health specialist was expressed by eight patients (5.0 %), whereas four patients (2.5 %) did not answer this question. The patients that expressed the need for support reported higher depressive symptoms (*M-W* = 248, $p = 0.005$) and a higher diabetes distress (*M-W* = 244.5, $p = 0.005$). Most of them were girls (seven vs. one boy).

Table 1. Baseline (socio-demographic and health-related) characteristics of study participants (*N* = 159).

Variable	*N* (%)	Mean ± *SD*	*Min - Max*
Age (years)		17.97 ± 3.30	11-25
Age of diabetes onset (years)		8.64 ± 3.93	1-19
Female sex	67 (42.1)		
School performance* (N = 156)		3.87 ± 0.91	1-5
HbA1c (%)		8.02 ± 1.04	5.9 - 11.9
BGM (measures per day)		5.00 ± 1.86	0.4 - 10.5
Diabetes treatment regimen			
Continuous subcutaneous insulin infusion	141 (88.7)		
Multiple daily injection	18 (11.3)		

* - possible answers: 1 - very poor, 2 - poor, 3 - average, 4 - good, 5 - very good.

Table 2. Average test scores of psychological domains on Questionnaire for psychological screening (*N* = 159).

Variable	Mean ± *SD*	Median	*Min - Max*	*N* of patients above cut-off points (%)
Depressive symptoms	0.34 ± 0.36	0.22	0.00-2.22	2 (1.3)
Diabetes Distress	2.29 ±1.08	2.00	1.00-6.00	52 (32.7)
Fear of Hypoglycemia	0.87 ± 0.74	0.66	0.00-4.00	14 (8.8)
Family support*	0.88 ± 0.79	0.66	0.00-3.67	37 (23)

*- Higher score means less family support.

3.1 Associations Between Psychological Variables, Age, Glycemic Control and BGM

The correlation (Spearman's rhos) between psychological variables, age, glycemic control and BGM is presented in Table 3. There were statistically significant associations between all psychological variables, among which the strongest associations were displayed between diabetes distress, depressive symptoms and lack of family support. Good glycemic control was associated with lower diabetes distress, better family support and more frequent BGM (r = -0.22, p = 0.009).

Moreover, older patients reported less depressive symptoms, had better glycemic control (r = -0.20, p = 0.013) and more BGM (r = -0.24, p = 0.003).

Table 3. Associations between psychological variables, age, glycemic control and BGM (N = 159).

Variable	Depressive symptoms	Diabetes Distress	Fear of Hypoglycemia	Family support*
Depressive symptoms		**0.50 (<0.001)**	**0.30 (<0.001)**	**0.36 (<0.001)**
Diabetes Distress			**0.22 (0.006)**	**0.56(<0.001)**
Fear of Hypoglycemia				**0.24 (0.003)**
Glycemic control (HbA1c)	0.12 (0.13)	**0.37 (<0.001)**	0.04 (0.60)	**0.32 (<0.001)**
BGM (measures per day)	0.04 (0.68)	-0.14 (0.09)	0.06 (0.49)	-0.07 (0.39)
Age	**-0.21 (0.009)**	-0.04 (0.65)	-0.12 (0.13)	-0.15 (0.056)
Age of diabetes onset	-0.04 (0.60)	0.06 (0.46)	-0.09 (0.28)	-0.11 (0.16)

Table presents Spearman's rho, level of statistical significance is written in parentheses.
* - Higher score means less family support.

3.2 Characteristics of Patients at Risk for Diabetes Distress

Almost one third of patients scored over the cut-off point on diabetes distress questions. The ones over the threshold were significantly more depressed (M-W = 4252, p < 0.001), reported more worries about hypoglycemia (M-W = 3292, p= 0.034), had poorer family support (M-W = 4334, p < 0.001) and poorer glycemic control (T-test = 5.276, p < 0.001). Patients did not differ in age or in BGM according to diabetes distress.

The model of multivariate regression analysis was built to define the strongest predictors. The model was statistically significant (R^2 = 0.49, p < 0.001, Table 4). Power of the model (1-β) was > 0.999. The strongest predictors of higher diabetes distress, all being statistically significant, were lack of family support, more depressive symptoms, poorer glycemic control, older age and female sex (Table 4).

Table 4. Predictors of higher risk for diabetes distress assessed with DD2 (N = 156).

Predictor	*B*	*SE*	Beta (ß)	*p*	*R*	R^2	*p*
(Constant)	-1.95	0.83		0.021	0.70	0.49	< 0.001
Depressive symptoms	**0.10**	**0.02**	**0.30**	**< 0.001**			
Fear of Hypoglycemia	0.11	0.09	0.08	0.243			
Family support*	**0.43**	**0.10**	**0.31**	**< 0.001**			
Glycemic control (HbA1c)	**0.22**	**0.07**	**0.22**	**0.002**			
BGM (measures per day)	-0.02	0.04	-0.04	0.601			
Age	**0.07**	**0.02**	**0.21**	**0.002**			
Sex	**0.44**	**0.15**	**0.21**	**0.003**			

Multiple linear regression analysis. B is regression coefficient, SE is standard error of coefficient, β is standardized regression coefficient, R is multiple correlation coefficient, R^2 is proportion of variation in dependent variable explained by regression model, p is level of statistical significance.
* - Higher score means less family support.

3.3 Sex Differences

Girls had significantly higher scores of depressive symptoms (*M-W* = 4426, $p < 0.001$) and diabetes distress (*M-W* = 4304.5, $p < 0.001$) than boys, but there were no differences in the FoH or family support. Girls had also higher HbA1c (8.28 vs. 7.84, *T*-test = -2.708, $p = 0.008$).

3.4 Outcomes of Screening

All patients received a short report written by a psychologist. Sixty-one (38.1 %) patients should have been invited to call or write and schedule the appointment with a psychologist. As six (3.8 %) patients were already attending psychological treatment, the invitation was sent to 55 patients. In the year after the invitation, six of those patients (3.8 %) started to visit a psychologist.

Based on the screening, ten (6.3 %) patients should have received the report with date and hour of session with a psychologist. Since two patients were already included in a psychological treatment, it was sent to eight patients. Of those eight patients, three (1.9 %) patients attended the appointment with the psychologist.

From the remaining group of patients that were not at risk for emotional problems, according to psychological screening (88 patients, 55.3 %), only one patient was already included in psychological treatment and no one additionally joined.

4 DISCUSSION

Youth and young adults with diabetes are at high risk for the development of depressive symptoms and experiencing diabetes specific distress, which results in a poorer glycemic control. Systematic screening is therefore recommended, although it is rarely formally conducted. Psychological outpatient screening was implemented in Slovene national diabetes center to provide help for the youth and young adults at the greatest risk.

One-year results showed that 1.3 % of the patients screened positively on elevated depressive symptoms. The percentage of patients with elevated depressive symptoms in the present sample was relatively small, compared to the findings of the previous studies (8-11, 16), therefore there is a great chance of falsely negative results and the patients that were not detected. Low detection rate can be due to social desirability bias or to the threshold that was set according to *T*-scores (≥ 65) in a general population, and could be set too high. Low detection rate at patients with diabetes on general depression questionnaires has been observed before, therefore the use of different cutoff thresholds was recommended when screening for depression in patients with somatic diseases (39). On the other hand, many screening tools for depression demonstrated low positive predictive values at patients with diabetes, meaning high rates of false positive results (40).

One third of patients reported symptoms considered as significant diabetes distress. The results are in accordance with the previous studies in adult patients with diabetes (39). The burden of the disease combined with general characteristics of adolescence are likely to contribute to this observation. The high frequency of observed diabetes distress puts in question the usefulness of screening and presenting these results to the patients and carers. Presenting results about elevated diabetes distress to the patients could enhance anxiety, whilst inappropriate referrals to a psychologist can have negative effects on the clinical practice. The use of more specific measures with a higher threshold may be of value for screening for the diabetes distress. Perhaps it would be useful to use the HbA1c level as the first screening tool to capture the patients with the worse glycemic control as recommended by Butwicka et al. (32), or to implement prevention programs for lowering diabetes distress for all the patients.

Although it is important to note that the screening tools are not intended for the assessment of the severity of symptoms, the presented results displayed a statistically significant associations between all psychological variables. Patients with diabetes distress were significantly more depressed, reported more worries about hypoglycemia, had less family support, and their glycemic control was less satisfactory. The strongest predictors of higher diabetes distress were lack of family support, more depressive symptoms, poorer glycemic control, older age and female sex. These findings support the results of the previous studies, which demonstrated associations between diabetes-specific distress, and depressive, glycemic control and family factors (2, 9, 25-29).

In accordance with the previous studies are also the findings that girls had significantly higher scores of depressive symptoms, diabetes distress and poorer glycemic control (2, 4, 9). Interestingly, of the eight patients that expressed the need for psychological treatment, seven were girls.

The need for psychological support was expressed by eight (5.0 %) patients, for whom psychological sessions were scheduled. Fifty-five (34.6 %) patients received an invitation to schedule an appointment with the psychologist by themselves. Altogether, nine patients joined psychological treatment after the psychological screening had been performed. Of the two patients that were positively screened on elevated depressive symptoms, one was already receiving treatment, while the other one did not reply to the invitation. Confirming the diagnosis of clinical depression was therefore not feasible. Other

ways of inviting patients at risk need to be considered (a telephone call or referral by his/her diabetologist), due to the low response rate to the referrals. Another study in adult diabetic population encountered similar responses from positively screened patients that were not interested in further diagnostic procedure or referrals to specialists, which raises the question of cost-effectiveness for such screening procedures (41).

Before we conclude, the limitations of the present study should be discussed. Firstly, the patients recruited in the study had a wide age range, especially when considering that TSCC scale for depression was designed and valuated for children and adolescents from ten to 18 years. Secondly, TSCC was not intentionally designed as a screening tool, and it was selected because of its availability in the Slovene language and normative data for Slovene population. Thirdly, items for measuring the FoH and family support were designed for the purposes of the screening based on the existing theory and clinical experiences, and were not otherwise tested.

According to our results, the following recommendations were designed. Firstly, there is an urgent need for adapting the screening tool for depression in Slovene language, with a wider age-range and with appropriate psychometric characteristics. WHO-5 or CES-D are two self-reported measures, both brief, with empirically supported cut-of scores to identify youth and young adults at risk for depression (42-45). Secondly, when screening for diabetes distress, a shorter version of Problem Areas in Diabetes Scale PAID-5 instead of DDS2 should be considered, since PAID-5 showed satisfactory sensitivity and specificity, and the correlation with PAID-20 was high (r = 0.92) (46). Thirdly, a previous study using self-report questionnaires on Slovene adolescents with T1DM showed that 35% ever experienced suicidal thoughts and almost 9 % attempted suicide (47). Therefore, suicidal ideation should be considered for screening, and possible interventions offered for those who score positively.

5 CONCLUSION

In conclusion, the results after one year of psychological screening in the young diabetes population in Slovenia, displayed low depressive symptoms and high diabetes distress rates. The patients with positive screening were invited or referred to individual psychological assessments for further diagnostic procedures or therapy, but only a small percentage of patients responded. Annual assessments of patients' psychological difficulties and referrals to a psychologist or another specialist may be of value for the young diabetes population, although specific screening tools for children and their parents need to be continuously improved or developed.

The value of the presented results for the future clinical diabetes care could be evaluated with prospective studies assessing whether patients with psychological distress would benefit from screening and referrals to psychological treatment. Whether a routine assessment of depression and diabetes distress in pediatric population with diabetes, followed by psychological treatment improves the patient's wellbeing and glycemic control, remains to be determined.

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

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LEGENDS

T1DM – Type 1 diabetes; BGM – blood glucose monitoring; HbA1c – glycosylated hemoglobin; FoH – fear of hypoglycemia; ADA – American Association for Diabetes; ISPAD – International Society of Pediatric and Adolescent Diabetes; CDI – Children Depression Inventory; TSCC – Trauma Symptom Checklist for Children; DDS2 – The Diabetes Distress Screening Scale.

REFERENCES

1. Kovacs M, Goldston D, Obrosky DS, Bonar LK. Psychiatric disorders in youths with IDDM: rates and risk factors. Diabetes Care 1997; 20: 36-44.
2. Weissberg-Benchell J, Antisdel-Lomaglio J. Diabetes-specific emotional distress among adolescents: feasibility, reliability, and validity of the problem areas in diabetes-teen version. Pediatr Diabetes 2011; 12: 341-4.
3. Steinberg L. A social neuroscience perspective on adolescent risk-taking. Dev Rev 2008; 28: 78-106.
4. Dovc K, Telic SS, Lusa L, Bratanic N, Zerjav-Tansek M, Kotnik P. et al. Improved metabolic control in pediatric patients with type 1 diabetes: a nationwide prospective 12-year time trends analysis. Diabetes Technol Ther 2014; 16: 33-40.
5. Helgeson VS, Snyder PR, Seltman H, Escobar O, Becker D, Siminerio L. Brief report: trajectories of glycemic control over early to middle adolescence. J Pediatr Psychol 2010; 35: 1161-7.
6. King PS, Berg CA, Butner J, Drew LM, Foster C, Donaldson D. et al. Longitudinal trajectories of metabolic control across adolescence: associations with parental involvement, adolescents' psychosocial maturity, and health care utilization. J Adolesc Health 2012; 50: 491-6.
7. Drotar D, Ittenbach R, Rohan JM, Gupta R, Pendley JS, Delamater A. Diabetes management and glycemic control in youth with type 1 diabetes: test of a predictive model. J Behav Med 2013; 36: 234-45.
8. Lawrence JM, Standiford DA, Loots B, Klingensmith GJ, Williams DE, Ruggiero A. et al. Prevalence and correlates of depressed mood among youth with diabetes: the SEARCH for diabetes in youth study. Pediatrics 2006; 117: 1348-58.
9. Hood KK, Huestis S, Maher A, Butler D, Volkening L, Laffel LM. Depressive symptoms in children and adolescents with type 1 diabetes: association with diabetes-specific characteristics. Diabetes Care 2006; 29: 1389-91.
10. Whittemore R, Urban AD, Tamborlane WV, Grey M. Quality of life in school-aged children with type 1 diabetes on intensive treatment and their parents. Diabetes Educ 2003; 29: 847-54.
11. Naar-King S, Idalski A, Ellis D, Frey M, Templin T, Cunningham PB. et al. Gender differences in adherence and metabolic control in urban youth with poorly controlled type 1 diabetes: the mediating role of mental health symptoms. J Pediatr Psychol 2006; 31: 793-802.
12. Moussa MA, Alsaeid M, Abdella N, Refai TM, Al-Sheikh N, Gomez JE. Social and psychological characteristics of Kuwaiti children and adolescents with type 1 diabetes. Soc Sci Med 2005; 60: 1835-44.
13. Johnson B, Eiser C, Young V, Brierley S, Heller S. Prevalence of depression among young people with Type 1 diabetes: a systematic review. Diabet Med 2013; 30: 199-208.
14. Ciechanowski PS, Katon WJ, Russo JE. Depression and diabetes: impact of depressive symptoms on adherence, function, and costs. Arch Intern Med 2000; 160: 3278-85.
15. McGrady ME, Laffel L, Drotar D, Repaske D, Hood KK. Depressive symptoms and glycemic control in adolescents with type 1 diabetes: mediational role of blood glucose monitoring. Diabetes Care 2009; 32: 804-6.
16. Corathers SD, Kichler J, Jones NH, Houchen A, Jolly M, Morwessel N. et al. Improving depression screening for adolescents with type 1 diabetes. Pediatrics 2013; 132: 1395-402.
17. Hilliard ME, Herzer M, Dolan LM, Hood KK. Psychological screening in adolescents with type 1 diabetes predicts outcomes one year later. Diabetes Res Clin Pract 2011; 94: 39-44.
18. Gonder-Frederick LA, Fisher CD, Ritterband LM, Cox DJ, Hou L, DasGupta AA. et al. Predictors of fear of hypoglycemia in adolescents with type 1 diabetes and their parents. Pediatr Diabetes 2006; 7: 215-22.
19. Gonder-Frederick L, Nyer M, Shepard JA, Vajda K, Clarke W. Assessing fear of hypoglycemia in children with Type 1 diabetes and their parents. Diabetes Manag 2011; 1: 627-39.
20. Wild D, von Maltzahn R, Brohan E, Christensen T, Clauson P, Gonder-Frederick L. A critical review of the literature on fear of hypoglycemia in diabetes: Implications for diabetes management and patient education. Patient Educ Couns 2007; 68: 10-5.
21. Cox DJ, Gonder-Frederick L, Antoun B, Clarke W, Cryer P. Psychobehavioral metabolic parameters of severe hypoglycemic episodes. Diabetes Care 1990;13: 458-9.
22. Shiu AT, Wong RY. Fear of hypoglycaemia among insulin-treated Hong Kong Chinese patients: implications for diabetes patient education. Patient Educ Couns 2000; 41: 251-61.
23. La Greca AM, Auslander WF, Greco P, Spetter D, Fisher EB, Jr., Santiago JV. I get by with a little help from my family and friends: adolescents' support for diabetes care. J Pediatr Psychol 1995; 20: 449-76.
24. Anderson B, Ho J, Brackett J, Finkelstein D, Laffel L. Parental involvement in diabetes management tasks: relationships to blood glucose monitoring adherence and metabolic control in young adolescents with insulin-dependent diabetes mellitus. J Pediatr 1997; 130: 257-65.
25. Helgeson VS, Reynolds KA, Siminerio L, Escobar O, Becker D. Parent and adolescent distribution of responsibility for diabetes self-care: links to health outcomes. J Pediatr Psychol 2008; 33: 497-508.
26. Cameron FJ, Skinner TC, de Beaufort CE, Hoey H, Swift PG, Aanstoot H. et al. Are family factors universally related to metabolic outcomes in adolescents with Type 1 diabetes? Diabet Med 2008; 25: 463-8.
27. Wysocki T. Associations among teen-parent relationships, metabolic control, and adjustment to diabetes in adolescents. J Pediatr Psychol 1993; 18: 441-52.
28. Miller-Johnson S, Emery RE, Marvin RS, Clarke W, Lovinger R, Martin M. Parent-child relationships and the management of insulin-dependent diabetes mellitus. J Consul Clin Psychol 1994; 62: 603-10.
29. Miller VA, Drotar D. Decision-making competence and adherence to treatment in adolescents with diabetes. J Pediatr Psychol 2007; 32: 178-88.
30. Silverstein J, Klingensmith G, Copeland K, Plotnick L, Kaufman F, Laffel L. et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. Diabetes Care 2005; 28: 186-212.
31. Delamater AM. Psychological care of children and adolescents with diabetes. Pediatr Diabetes 2009; 10: 175-84.
32. Butwicka A, Fendler W, Zalepa A, Szadkowska A, Mianowska B, Gmitrowicz A. et al. Efficacy of metabolic and psychological screening for mood disorders among children with type 1 diabetes. Diabetes Care 2012; 35: 2133-9.
33. Briere J, Žemva B, Boben D. Vprašalnik o travmatiziranosti otrok in mladostnikov: priročnik. Ljubljana: Center za psihodiagnostična sredstva, 2000.
34. Evans JJ, Briere J, Boggiano AK, Barrett M. Reliability and validity of the Trauma Symptom Checklist for Children in a normal sample. In: San Diego Conference on Responding to Child Maltreatment. San Diego, 1994.
35. Sadowski CM, Friedrich WN. Psychometric properties of the Trauma Symptom Checklist for Children (TSCC) with psychiatrically hospitalized adolescents. Child Maltreat 2000; 5: 364-72.
36. Fisher L, Glasgow RE, Mullan JT, Skaff MM, Polonsky WH. Development of a brief diabetes distress screening instrument. Ann Fam Med 2008; 6: 246-52.
37. PASW® Statistics 18.0.0 Somers, New York: SPSS, An IBM Company, 2009.
38. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 2009; 41: 1149-60.

39. Hermanns N, Kulzer B, Krichbaum M, Kubiak T, Haak T. How to screen for depression and emotional problems in patients with diabetes: comparison of screening characteristics of depression questionnaires, measurement of diabetes-specific emotional problems and standard clinical assessment. Diabetologia 2006; 49: 469-77.
40. Roy T, Lloyd CE, Pouwer F, Holt RI, Sartorius N. Screening tools used for measuring depression among people with Type 1 and Type 2 diabetes: a systematic review. Diabet Med 2012; 29: 164-75.
41. Fleer J, Tovote KA, Keers JC, Links TP, Sanderman R, Coyne JC. et al. Screening for depression and diabetes-related distress in a diabetes outpatient clinic. Diabet Med 2013; 30: 88-94.
42. Bech P, Olsen LR, Kjoller M, Rasmussen NK. Measuring well-being rather than the absence of distress symptoms: a comparison of the SF-36 Mental Health Subscale and the WHO-Five Well-Being Scale. Int J Methods Psychiatr Res 2003; 12: 85-91.
43. de Wit M, Pouwer F, Gemke RJ, Delemarre-van de Waal HA, Snoek FJ. Validation of the WHO-5 Well-Being Index in adolescents with type 1 diabetes. Diabetes Care 2007; 30: 2003-6.
44. Roberts R, Andrews J, Lewinsohn P, Hops H. Assessment of depression in adolescents using the Center for Epidemiologic Studies Depression Scale. Psychol Assess 1990; 2: 122-8.
45. Roberts RE, Lewinsohn PM, Seeley JR. Screening for adolescent depression: a comparison of depression scales. J Am Acad Child Adolesc Psychiatry 1991; 30: 58-66.
46. McGuire BE, Morrison TG, Hermanns N, Skovlund S, Eldrup E, Gagliardino J. et al. Short-form measures of diabetes-related emotional distress: the Problem Areas in Diabetes Scale (PAID)-5 and PAID-1. Diabetologia 2010; 53: 66-9.
47. Radobuljac MD, Bratina NU, Battelino T, Tomori M. Lifetime prevalence of suicidal and self-injurious behaviors in a representative cohort of Slovenian adolescents with type 1 diabetes. Pediatr Diabetes 2009; 10: 424-31.

CLINICAL, GENETIC AND IMMUNOLOGICAL CHARACTERISTICS OF PAEDIATRIC AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 1 PATIENTS IN SLOVENIA

KLINIČNE, GENETSKE IN IMUNOLOŠKE ZNAČILNOSTI OTROK IN MLADOSTNIKOV Z AVTOIMUNSKIM POLIGLANDULARNIM SINDROMOM TIPA 1 V SLOVENIJI

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ABSTRACT

Introduction. Autoimmune polyglandular syndrome type 1 (APS-1) is an autosomal recessive disorder, caused by mutations in the *AIRE* gene. The major components of APS-1 are chronic mucocutaneous candidiasis (CMC), hypoparathyroidism (HP) and Addison's disease (AD). Clinical, genetic and immunological characteristics of Slovenian paediatric APS-1 patients were investigated.

Methods. Existing medical records of 15 APS-1 patients were rewieved, when necessary, additional clinical and laboratory investigations were issued. *AIRE* gene analysis was performed to identify causative mutations, and autoantibodies against type I interferons were measured by luminescence immunoprecipitation system.

Results. Patients had one to eight different manifestations of the disease. CMC was present in all, HP in 12/15 (80 %) and AD in 8/15 (53 %) patients. Growth retardation, due to hyposomatotropism, growth hormone resistance, autoimmune thyroiditis, corticosteroid treatment, malabsorption or secretory failure of exocrine pancreas, was observed in altogether 7 (46 %) patients. Six different *AIRE* gene mutations were detected and p.R257X mutation was present in 63.3 % of pathological alleles. Antibodies against type I interferons were detected in all patients.

Conclusion. APS-1 is a rare disorder with a broad spectrum of clinical manifestations, which, if unrecognized or inadequately treated may be fatal. *AIRE* gene mutational analysis and autoantibodies against type I interferons are important in early identification of the disease. The aetiology of growth retardation was shown to be extremely diverse, frequently caused by less characteristic manifestations. APS-1 may affect patients' quality of life in numerous ways, and may cause great psychosocial burden leading to depression and suicidal thoughts even in paediatric patients.

Keywords: autoimmunity, APS-1, APECED, *AIRE*

IZVLEČEK

***Uvod**. Avtoimunski poliglandularni sindrom tipa 1 (APS-1) je redka avtosomno recesivna bolezen, povezana z mutacijami gena AIRE. Tri najpomembnejše komponente APS-1 so kronična mukokutana kandidiaza (CMC), hipoparatiroidizem (HP) in Addisonova bolezen (AD). Raziskali smo klinične, genetske in imunološke značilnosti slovenskih pediatričnih bolnikov z APS-1.*

***Metode**. Pregledali smo obstoječo medicinsko dokumentacijo 15 bolnikov z APS-1, v izbranih primerih smo opravili dodatne klinične in laboratorijske preiskave. Z genetsko analizo smo prepoznali vzročne mutacije gena AIRE, s sistemom luminiscenčne imunoprecipitacije (LIPS) pa smo določili avtoprotitelesa proti interferonom tipa 1.*

***Rezultati**. Bolniki so imeli izraženo eno do osem različnih komponent bolezni. CMC je bila prisotna pri vseh bolnikih, HP pri 12 od 15 (80 %) in AD pri 8 od 15 (53 %). Zastoj rasti je bil prisoten pri 7 (46 %) bolnikih zaradi hiposomatotropizma, rezistence proti rastnemu hormonu, avtoimunskega tiroiditisa, zdravljenja s kortikosteroidi, malabsorbcije ali sekretorne okvare eksokrinega pankreasa. Prepoznanih je bilo 6 različnih mutacij gena AIRE, najpogostejša mutacija p.R257X je bila opredeljena pri 63,3 % mutiranih alelov. Avtoprotitelesa proti interferonom tipa 1 so bila prisotna pri vseh bolnikih.*

***Zaključki**. APS-1 je redka bolezen s širokim naborom kliničnih značilnosti in je lahko smrtna, če ni prepoznana ali je neprimerno zdravljena. Za zgodnje odkrivanje sta ključnega pomena genetska analiza in določanje protiteles proti interferonom tipa 1. Pri bolnikih z APS-1 je zastoj rasti pogosta komponenta bolezni z različnimi vzroki, pogosto povezanimi z manj značilnimi manifestacijami. APS-1 na različne načine vpliva na kvaliteto življenja bolnikov in je veliko psihološko breme, ki lahko vodi v depresijo in samomorilne misli celo pri mladih bolnikih.*

***Ključne besede:** avtoimunost, APS-1, APECED, AIRE*

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1 INTRODUCTION

Autoimmune polyglandular syndrome type 1 (APS-1) also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED; OMIM 240300) is rare but devastating primary immunodeficiency disease. The range of clinical features is broad and very variable. The classic triad includes chronic mucocutaneous candidiasis (CMC), hypoparathyroidism (HP) and Addison's disease (AD). Patients may develop several other autiommune endocrine disorders (gonadal failure, type 1 diabetes, autoimmune thyroid disease) and nonendocrine disorders (ectodermal dystrophy, alopecia, vitiligo, chronic hepatitis…) (1). The disease usually begins in infancy with CMC, additional symptoms gradually appear later in life. Clinical diagnosis requires the presence of two of three major criteria (CMC, HP, AD). If a sibling has the syndrome, only one of the above manifestations is required. The prevalence is higher in genetically isolated populations; in Slovenia it was estimated to be 1:43,000 (2). APS-1 is caused by autosomal recessively inherited mutations in autoimmune regulator (*AIRE*) gene (3). *AIRE* gene is encoding transcriptional regulator principally expressed in medullary thymic epithelial cells (mTEC). *AIRE* plays an important role in maintaining self-tolerance and is involved in negative selection of autoreactive T-cells (4). The absence of *AIRE* results in impaired clonal deletion of self reactive thymocytes, which escape into the periphery and attack variety of organs resulting in different autoimmune diseases and presence of different organ specific autoantibodies (5). Additionally some non-organ specific auto-antibodies are characteristic to APS-1. Antibodies against type I interferons, especially α and ω subtypes are reported to be highly specific for APS-1 (6). More recently, CMC in APS-1 patients have been associated with autoantibodies against Th17-related cytokines, primarily against IL-22 and IL-17F (7) showing that also CMC in APS-1 has an autoimmune basis.

2 METHODS

Fifteen APS-1 patients from thirteen unrelated Slovenian families were identified and included into the study. Mutational and partial clinical characteristics of patients 1 to 12 were previously reported (2, 8). Those patients were reinvestigated for the purposes of this study. The study protocol followed the Declaration of Helsinki and was approved by the national Ethical Committee (nr. 22/09/09, 28/2/13). Written informed consent was obtained by all participants or parents of minors prior to the study.

The patients' data were collected from existing medical records. The diagnosis of HP, AD, primary hypothyroidism, primary gonadal failure, pituitary failure were based on typical biochemical and endocrinological findings as described by others (9). We diagnosed oral candidiasis by visible mucosal changes in combination with positive Candida albicans culture and nail candidiasis by typical clinical findings or with the help of experienced dermatologist. Malabsorption was diagnosed from a history of recurrent episodes of diarrhea, growth retardation and/or measurement of fecal fat and pancreatic enzymes in stool. The duodenal and short bowel biopsy with chromogranin staining for detection of enterochromaffin cells was performed. Chronic elevation of liver enzymes without evidence of viral or drug-induced hepatitis was considered as suspected of autoimmune hepatitis. Consequently, liver biopsy and detection of autoantibodies against cytochrome P4501A2 (CYP1A2) were issued. Enamel hypoplasia was defined as defects in enamel not due to caries and the patients were evaluated by clinical examination and an orthopantomogram by an experienced dentist.

Genomic DNA was isolated from venous blood samples and *AIRE* gene exons were individually PCR amplified (2). Sequencing was performed using BigDye Terminator v.3.1 Cycle Sequencing Kit and 3500 Genetic Analyzer capillary electrophoresis system (Life Technologies, Foster City, USA). Identified variants were confirmed by the sequencing of at least two independent PCR products. Anti-IFN-α2, anti-IFN-α8, anti-IL-22, anti-IL-17A and anti-IL-17F autoantibodies were measured in 12 out of 15 patients by luminescence immunoprecipitation system (LIPS) as previously described (10).

3 RESULTS

3.1 Clinical Characteristics

In surveys performed in 2005 and again in 2012 we were able to identify fifteen patients from thirteen families. Their clinical manifestations and the age of onset are shown in Table 1. All recruited patients but one had either two of the three major clinical manifestations or one in combination with blood relative with established APS-1. Mutational analysis confirmed the diagnosis in the patient with CMC as the only clinical manifestation.

Table 1. Clinical and mutational characteristics in APS-1 patients (patients with growth retardation are marked with #; mutations in bold were novel when detected, HP-hypoparathyroidism; AD-Addison's disease; CMC-mucocutaneous candidiasis; Al-alopecia; ED-ectodermal dystrophy; KK-keratoconjunctivitis; Vi-vitiligo; T1D-type 1 diabetes, AH-autoimmune hepatitis; Ht-hypothyroidism; Ma-malabsorption; Hg-hypogonadism).

Patient/ Family	Gender	Year of birth	Clinical characteristics												*AIRE* mutation
			HP	A	CMC	Al	ED	KK	Vi	T1D	AH	Ht	Ma	Hg	
1/A	M	1979	14	10	12										p.[R257*];[R257*] c.[769C>T];[769C>T]
2/A	M	1980	6,3		17,5	7,0									p.[R257*];[R257*] c.[769C>T];[769C>T]
3/B#	F	1990	7,8	7,9	7,9								13,5	20,9	p.[R257*];[R257*] c.[769C>T];[769C>T]
4/C#	M	1990	6,5	6,4	6,5		6,5	10,3				11,1		14,8	p.[R257*];[R257*] c.[769C>T];[769C>T]
5/D	M	1986	11,1	10,9	11,1			13,1				25			p.[R257*];[R257*] c.[769C>T];[769C>T]
6/E#	M	1997	3,7	14,9	3,7	3,7	3,7					9,7	6,3		p.[R257*];[T16M] c.[769C>T];[47C>T]
7/E#	F	1992	5,5		11,4		5,5						15,9		p.[R257*];[T16M] c.[769C>T];[47C>T]
8/F	M	1977		7,8	23,3		7,8					26,3		21,3	p.[R257*];[R15fs] c.[769C>T];[21-43dup23]
9/G	M	1976	9,4		24,5	9			11,6	21		24,5			p.[R257*];[**G180fs**] c.[769C>T];[**540delG**]
10/H#	M	1992		8,5	3,6	3,6					3,6			17,8	p.[R257*];[R257*] c.[769C>T];[769C>T]
11/I#	F	1998	3,8	4,0	4,6			12,5						11,7	p.[R257*];[?] c.[769C>T];[**653-7_-5delCTC**]
12/J#	M	1990	12,9		0,3		5,5					5,5	14,5	20,7	**p.[Q358fs];[Q358fs]** **c.[1064-1068dupCCCGG];** **[1064-1068dupCCCGG]**
13/K	M	1998			1,8										p.[R15fs];[R15fs] c.[21-43dup23];[21-43dup23]
14/L	M	2004	7,9		5,8										p.[R15fs];[R15fs] c.[21-43dup23];[21-43dup23]
15/M	F	2009	3,3		2,5										p.[R257*];[R257*] c.[769C>T];[769C>T]

Among 11 boys and 4 girls, the onset of the disease ranged from 1,8 years to 10,9 years (mean 6,1 yr). HP was the first clinical manifestation in seven patients, AD in five, autoimmune hepatitis, autoimmune thyroiditis and CMC each in one. In three patients with HP or autoimmune thyroiditis as first manifestation, review of the available clinical data revealed that MC was present much earlier.

The patients had one to eight clinical manifestations. CMC was present in all patients, HP in 12 (80 %), AD in 8 (53 %), autoimmune thyroiditis and hypogonadism each in 6 (40 %). Other clinical manifestations appeared less often (Figure 1). AD was diagnosed in all patients in the first decade of life. Mineralocorticoid deficiency preceded hypocorticism in 3/8 patients (5/D, 6/E, 10/H). Hypogonadism was diagnosed in 2 girls and 4 boys during or after pubertal development. One male patient (4/C) had secondary hypogonadism in the course of autoimmune hypophysitis, all other patients had primary hypogonadism. Keratoconjuctivitis was diagnosed in three patients. In one of (4/C) them the changes improved only after long term systemic cyclosporine treatment.

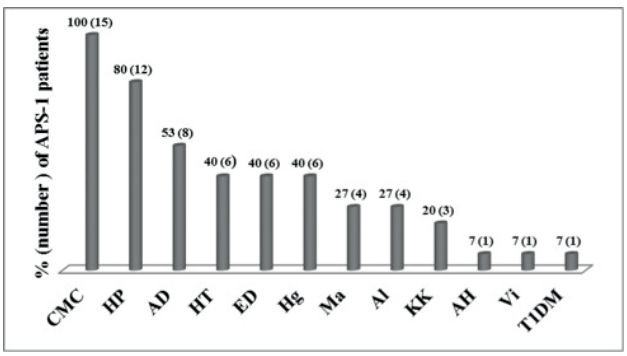

Figure 1. Number of patients affected by each manifestation of APS-1 (abbreviations as in Table 1).

Seven patients (46 %) had growth retardation from different causes shown in Table 2. Growth hormone deficiency was confirmed only in patient 4/C with secondary hypogonadism and several other clinical manifestations. In the female patient 11/I exaggerated response during growth hormone stimulation test and normal growth factors were observed.

Table 2. Etiology of growth retardation in APS 1 patients.

Growth hormone deficiency
Growth hormone resistance
Chronic corticosteroid therapy
Hypothyroidism (autoimmune thyroiditis)
Malabsorption:
• Massive duodenal colonisation with Giardia lamblia
• Massive gastrointestinal colonisation with Candida albicans
• Exocrine pancreatic insufficiency
• Enteroendocrine cell loss

Autoimmune thyroiditis with myxoedema and severe growth retardation was the first clinical manifestation of APS-1 in patient 12/J at the age of 5,5 years. After starting treatment with L-thyroxine, his growth velocity increased and he reached normal growth curves. Growth failure reappeared at the age of 14 years when steatorrhea and pancreatic insufficiency was diagnosed. Chronic corticosteroid treatment of autoimmune hepatitis caused growth delay in patient 10/H.

Depression was observed in three (4/C, 7/E, 11/I) patients who needed psychiatric support. During the study period, two young adult patients (8/F, 9/G) died, both with severe psychological dysfunction. One died due to addisonian crisis, after having neglected therapy for a long time. The other patient most probably died because of insulin overdose.

3.2 Mutational and Immunological Characteristics

Six different *AIRE* gene mutations were detected among 15 APS-1 patients (Table 1), three of which were novel at the time (2). The most prevalent mutation in Slovenian APS-1 population was p.R257* (c.769C>T) present in 63.3 % of pathological alleles, followed by p.R15fs (c.21-43dup23) mutation in 16.7 %. Anti-IFN-α2, anti-IFN-α8 and anti-IL-22 autoantibodies in high titres were detected in all analysed patients (Table 3). Anti-IL-17A were detected in 41,7 % and anti-IL-17F in 91,7 % of APS-1 patients.

Table 3. Immunological characteristics of the APS-1 patients (the values represent times over the mean of healthy control values; negative values are in italic).

	Clinical characteristics	anti-IFN-α2	anti-IFN-α8	anti-IL-22	anti-IL-17A	anti-IL-17F
1/A	3	133,2	69,2	382,4	*0,7*	13,7
3/B	5	pos	pos	pos	pos	pos
4/C	7	139,4	112,8	170,5	*1,6*	14,0
5/D	5	175,5	145,6	769,7	144,9	62,7
6/E	7	278,3	68,0	512,1	*0,7*	25,1

	Clinical characteristics	anti-IFN-α2	anti-IFN-α8	anti-IL-22	anti-IL-17A	anti-IL-17F
7/E	4	149,0	126,1	210,5	*0,7*	*0,8*
10/H	5	92,3	149,0	362,5	15,1	110,9
11/I	5	101,1	132,0	605,8	*2,2*	201,7
12/J	6	242,4	190,8	996,6	*1,5*	86,8
13/K	1	280,9	66,3	353,8	114,6	7,7
14/L	2	103,6	81,1	533,4	76,1	131,8
15/M	3	73,6	127,2	411,0	*3,8*	159,9
		100%	100%	100%	41,7%	91,7%

4 DISCUSSION

While the majority of reports are focusing on adult APS-1 patients, so far there is only one report describing clinical and genetic characteristics of paediatric North Irish APS-1 patients (11). Here, we report extensive clinical, genetic and immunological overview of Slovenian paediatric APS-1 cohort.

Whereas all but one patient in our study group met standard diagnostic APS-1 criteria, there were striking clinical variations, even between siblings with identical *AIRE* mutations. Clinical manifestations showed similar prevalence and follow-up as in those reported in other studies (11-13). Especially as in Finnish patients, with whom the majority of Slovenian patients share the common p.R257* (c.769C>T) mutation (1). CMC was detected in all Slovenian and Finnish patients, while it was present in less than 20 % of Iranian Jews patients, probably due to *AIRE* mutation present only in this community (14). On the contrary, type I diabetes was observed in only one Slovenian patient, but it is more frequent among Finnish patients (1).

The APS-1 patients were usually first admitted to our clinic because of clinical symptoms of hypoparathyroidism, Addison's disease, or symptoms of other endocrine or organ dysfunction, while it is possible that Candida albicans infection was present much earlier. At clinical examination, Candida infection was detected in 6 out of 15 (40 %) patients by the age of 5, and in all of them by the age of 25 years old. This is comparable to the Finnish data, where 50 % had candidiasis by the age of 5 and 97 % by the age of 30 (15).

Only individual cases of growth failure in APS-1 have been published to date (11, 16, 17). Nevertheless, growth failure as clinical presentation of various endocrine and nonendocrine dysfunctions, was observed in 46 % of Slovenian patients. The patient 4/C with growth hormone deficiency had hypersensitivity to growth hormone, which has not been reported in APS-1 so far. During growth hormone treatment, systemic allergic reaction occurred and the treatment was discontinued. In the patient 11/I with growth hormone resistance, the treatment with high doses of growth hormone did not improve her growth velocity, despite a significant increase in the growth factors level. We speculated that antibodies against growth factors or growth factor receptors may be responsible, but further investigations are needed.

APS-1 should be considered in the differential diagnosis of gastrointestinal symptoms, such as malabsorption, constipation, watery diarrhea or steatorrhea, as they are reported in almost 24 % of the APS-1 patients (17). Several studies demonstrated that gastrointestinal dysfunction (GD) in APS-1 patients is associated with severe or complete loss of enteroendocrine cells (18-20). Patients develop autoantibodies against tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of serotonin, having a critical role in enteric function (19). The identification of mucosal antibodies and repeated clinical remission achieved with immunosuppressive treatment in a patient with recurrent episodes of diarrhea with steatorrhea clearly support the idea that GD is due to *AIRE* deficiency (21). We observed GD in four patients (3/B, 6/E, 7/E, 12/J). All had intestinal candidiasis treated with systemic antifungal therapy, one of them additional massive duodenal colonisation with Giardia lamblia (7/E). Three patients (6/E, 7/E, 12/J) had recurring diarrhea and steatorrhea with exocrine pancreatic insufficiency, in two absence of enteroendocrine cells in intestinal biopsy was found.

Chronic endocrine disorders are known to impair patients' quality of life. APS-1 patients not only cumulate multiple endocrine disorders, requiring daily oral and lifelong hormonal replacement therapy, but the disease affects the quality of life in numerous ways: by life-threatening situations, disfiguring ectodermal manifestations, vision impairment, infertility and fear of developing additional components during life. Impairment of general health, emotional well-being and vitality were the most diminishing aspects of quality of life, with depressive

symptoms affecting 29 % of the adult APS-1 Finnish patients (22). Among Slovenian paediatric APS-1 patients, three developed depression and two young adults died. This is indicating the extent of the disease burden even in younger patients.

Over 95 different *AIRE* mutations have been identified, but two of them are found in the great majority of the patients. The p.Cys322fs (c.967_979del13) mutation is characteristic of North American, British and Norwegian patients (23), p.R257* (c.769C>T) is present in more than 85 % of the Finnish patients and common among Central and Eastern European patients (12,24). Mutational spectrum of Slovenian APS-1 patients was consistent with other populations in the region, since p.R257* mutation was present in 63,3 % of pathological *AIRE* alleles. The second most prevalent mutation was p.R15fs (c.21-43dup23bp). This mutation was reported only in one Austrian and one Hungarian patient (24), and it seems to be restricted to the region. Other mutations are much rarer and present in isolated families.

A great variability in the prevalence of autoimmune hepatitis in different populations is reported, namely: 5 % in Norwegian (25), 12 % in Finish (9) and 27 % in Sardinian patients (26). These populations differ highly in their *AIRE* gene mutational spectrum (23-25), and it seems that the prevalence of autoimmune hepatitis might be related to it. A great majority of Slovenian and Finnish APS-1 patients carry the same *AIRE* mutation, and the prevalence of autoimmune hepatitis in both cohorts is comparably low.
The hallmark of APS-1 is a variety of different autoantibodies that develop in association with the disease, where numerous organ specific, but also non-organ specific, autoantibodes may be present. Among the latter, antibodies against α and ω subtypes of type I interferons are present in all patients with APS-1, and, additionally, only in patients with thymoma with associated myasthenia gravis, but not in other autoimmune disorders (27). The autoantibodies are reported to be present before the onset of the disease (6). In our group of patients, anti-IFN-α2 and anti-IFN-α8 autoantibodies were present in all analysed patients, even in the patient 13/K, whose only symptom was CMC, and who had an uncommon *AIRE* mutation. This was confirming their diagnostic value in children with incomplete APS-1 clinical presentation.

Chronic mucocutaneous candidiasis is a persistent or recurrent infection of nail beds, skin or mucosa. It is the most prevalent disease manifestation associated with APS-1, present in all Slovenian patients. CMC in APS-1 have been associated with autoantibodies against Th17-related cytokines, revealing the link between CMC and *AIRE* deficiency. Neutralizing autoantibodies against IL-22 are present in 91 %, against IL-17F in 75 % and/or against IL-17A in 41 % of more than 150 analysed patients (7). They are highly specific for CMC in APS-1, and are present only in thymoma patients, but not in numerous other autoimmune diseases. Interestingly, all investigated Slovenian patients had anti-IL-22 autoantibodies present. Anti- IL-17F autoantibodies were present in 91 % of the patients, but anti-IL-17A were, as expected, less prevalent.

5 CONCLUSION

The phenotypical expression of the APS-1 shows a wide variability even among siblings with the same genotype. In view of this heterogeneity, an early diagnosis of APS-1 can be challenging and often leading to a considerable diagnostic delay. Therefore, clinicians should be aware that the presence of even a minor component of APS-1 in children should prompt a careful investigation for other signs and symptoms of the disease, including genetic and immunological testing, thus allowing an early diagnosis and prevention of severe and life-threatening events. Growth failure in presented population was common, while its aetiology may be diverse and frequently caused by less characteristic manifestations of the syndrome. Psychological burden of the disease was evident and requiring professional psychosocial support in the selected cases.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

The study was approved by the Slovene National Ethical Committee (Nr. 22/09/09, 28/2/13).

REFERENCES

1. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Clin Endocrinol Metab 2006; 91: 2843-50.
2. Podkrajsek KT, Bratanic N, Krzisnik C, Battelino T. Autoimmune regulator-1 messenger ribonucleic acid analysis in a novel intronic mutation and two additional novel AIRE gene mutations in a cohort of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. J Clin Endocrinol Metab 2005; 90: 4930-5.
3. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M. et al. Positional cloning of the APECED gene. Nat Genet 1997; 17: 393-8.

4. Liston A, Lasage S, Wilson J, Peltonen L, Goodnow CC. Aire regulates negative selection of organ-specific T-cells. Nat Immunol 2003; 4: 350-4.
5. Peterson P, Peltonen L. Autoimmune polyglandular syndrome type 1 (APS1) and AIRE gene: new views on molecular basis od autoimmunity. J Autoimmun 2005; 25: 49-55.
6. Meager A, Visvalingam K, Peterson P, Möll K, Murumägi A, Krohn K. et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 2006; 3: e289.
7. Kisand K, Bøe Wolff AS, Podkrajsek KT, Tserel L, Link M, Kisand KV. et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 2010; 207: 299-308.
8. Podkrajsek KT, Milenković T, Odink RJ, Claasen-van der Grinten HL, Bratanic N, Hovnik T. et al. Detection of a complete autoimmune regulator gene deletion and two additional novel mutations in a cohort of patients with atypical phenotypic variants of autoimmune polyglandular syndrome type 1. Eur J Endocrinol 2008; 159: 633-9.
9. Ahonen P, Myllärniemi S, Sipilä I, Perheentupa J. Clinical variation of autoimmune –polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 1990; 322: 1829-36.
10. Kärner J, Meager A, Laan M, Maslovskaja J, Pihlap M, Remm A. et al. Anti-cytokine autoantibodies suggest pathogenetic links with autoimmune regulator deficiency in humans and mice.Clin Exp Immunol 2013; 171: 263-72.
11. Millar S, Carson D. Clinical phenotypes of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy seen in the Northern Ireland paediatric population over the last 30 years. Ulster Med J 2012; 81: 118-22.
12. Betterle C, Greggio NA, Volpato M. Autoimmune polyglandular syndrome type 1. J Clin Endocrinol Metab 1998; 83: 1049-55.
13. Wolf ASB, Erichsen MM, Meager A, Magitta NF, Myhre AG, Bollerslev J. et al. Autoimmune polyendocrine syndrome type 1 in Norway: phenotypic variation, autoantibodies, and novel mutations in autoimmune regulator gene. J Clin Endocrinol Metab 2007; 92: 595-603.
14. Zlotogora J, Shapiro MS. Polyglandular autoimmune syndrome type 1 among Iranian Jews. J Med Genet 1992; 29: 824-6.
15. Husebye ES, Perheentupa J, Rautemaa R, Kampe O. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. J Intern Med 2009; 265: 514-29.
16. Ward L, Paquette J, Seidman E, Huot C, Alvarez F, Crock P. et al. Severe autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy in an adolescent girl with a novel AIRE mutation: response to immunosuppressive therapy. J Clin Endocrinol Metab 1999; 84: 844-52.
17. Pun T, Chandurkar V. Growth hormone deficiency, short stature, and juvenile rheumatoid arthritis in a patient with autoimmune polyglandular syndrome type 1: case report and brief review of the literature. ISRN Endocrinology 2011: 462759.
18. Högenauer C, Meyer RL, Netto GJ, Bell D, Little KH, Ferries L. et al. Malabsorption due to cholecystokinin deficiency in a patient with autoimmune polyglandular syndrome type I. N Engl J Med 2001; 344: 270-4.
19. Scarpa R, Alaggio R, Norberto L, Furmaniak J, Chen S, Smith BR. et al.Tryptophan hydroxylase autoantibodies as markers of a distinct autoimmune gastrointestinal component of autoimmune polyendocrine syndrome type 1. J Clin Endocrinol Metab 2013; 98: 704-12.
20. Posovszky C, Lahr G, von Schnurbein J, Buderus S, Findeisen A, Schröder C. et al. Loss of enteroendocrine cells in autoimmune-polyendocrine-candidiasis-ectodermal-dystrophy (APECED) syndrome with gastrointestinal dysfunction. J Clin Endocrinol Metab 2012; 97: E292-300.
21. Padeh S, Theodor R, Jonas A, Passwell JH. Severe malabsorption in autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy syndrome successfully treated with immunosuppression. Arch Dis Child 1997; 76: 532-4.
22. Kluger N, Jokinen M, Krohn K, Rankl A. What is the burden of living with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) in 2012? A health-related quality-of-life assessement in Finnish patients. Clin Endocrinol 2013; 79: 134-41.
23. Scott HS, Heino M, Peterson P, Mittaz L, Lalioti MD, Betterle C. et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. Mol Endocrinol 1998; 12: 1112-9.
24. Cihakova D, Trebusak K, Heino M, Fadeyev V, Tiulpakov A, Battelino T. et al. Novel AIRE mutations and P450 cytochrome autoantibodies in Central and Eastern European patients with APECED. Hum Mutat 2001; 18: 225-32.
25. Myhre AG, Halonen M, Eskelin P, Ekwall O, Hedstrand H, Rorsman F. et al. Autoimmune polyendocrine syndrome type 1 (APS I) in Norway. Clin Endocrinol 2001; 54: 211-7.
26. Meloni A, Willcox N, Meager A, Atzeni M, Wolf ASB, Husebye ES. et al. Autoimmune poyglandular syndrome type 1: an extensive longitudinal study in Sardinian patients. J Clin Endocrinol Metab 2012; 97: 1114-24.
27. Wolff AS, Sarkadi AK, Maródi L, Kärner J, Orlova E, Oftedal BE. et al. Anti-cytokine autoantibodies preceding onset of autoimmune polyendocrine syndrome type I features in early childhood. J Clin Immunol 2013; 33: 1341-8.

CHILDHOOD OSTEOPOROSIS AND PRESENTATION OF TWO CASES WITH OSTEOGENESIS IMPERFECTA TYPE V

OSTEOPOROZA V OTROŠKI DOBI IN PREDSTAVITEV DVEH BOLNIKOV Z OSTEOGENESIS IMPERFECTA TIPA V

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ABSTRACT

Introduction. Osteogenesis imperfecta (OI) is etiologically heterogeneous disorder characterized by childhood osteoporosis. A subtype OI type V is caused by the same c.-14C>T mutation in the *IFITM5* gene. Nevertheless, there is a marked interindividual phenotypic variability in clinical presentation; however, response to bisphosphonates is reported to be good.

Methods. Two individuals with OI type V had multiple recurrent fractures with hypertrophic calluses, scoliosis and ossifications of the forearm interosseous membranes. Sequencing of *IFITM5*, genotyping of variants rs2297480 in farnesyl diphosphate synthase gene (*FDPS*), and rs3840452 in geranylgeranyl diphosphate synthase 1 gene (*GGPS1*), both involved in bisphosphonate metabolism, was performed.

Results. In patient 1 BMD reached normal values during bisphosphonate treatment and remained normal four years after the treatment discontinuation. In patient 2 no increase in BMD after five years of bisphosphonate treatment was observed and callus formation continued. The c.-14C>T *IFITM5* mutation in heterozygous state was detected in both individuals. Additionally, both patients carried *FDPS* variant rs2297480 in homozygous state, and were heterozygous for *GGPS 1* variant rs3840452.

Conclusions. The paper presents a short overview of childhood osteoporosis with a special emphasis on OI type V by presenting two cases. Both OI type V patients had identical disease-causing mutation, but marked interindividual phenotypic variability. The striking failure in response to bisphosphonate treatment in one of the patients could not be explained by the variants in genes involved in bisphosphonate metabolism.

Keywords:
bone mineral density, hypertrophic callus, *IFITM5* gene, bisphosphonates

IZVLEČEK

***Uvod**. Osteogenesis imperfecta (OI) je vzročno heterogena bolezen, katere značilnost je osteoporoza v otroštvu. Pri vseh opisanih bolnikih s podtipom OI tipa V je vzrok bolezni ista mutacija c.-14C>T gena IFITM5. Kljub temu med bolniki obstaja izrazita fenotipska variabilnost v klinični sliki, toda opisan je le dober odgovor na zdravljenje z bisfosfonati.*

***Metode**. Oba bolnika z OI tipa V sta imela ponavljajoče se zlome kosti s hipertrofičnimi kalusi, skoliozo in zakostenelo membrano med podlahtnico in koželjnico.*
Opravili smo sekvenčno analizo gena IFITM5 in genotipizacijo variant rs2297480 gena za farnesil difosfat sintazo (FDPS) in rs3840452 gena za geranilgeranil difosfat sintazo 1 (GGPS1), ki sta vpletena v presnovo bisfosfonatov.

***Rezultati**. Pri bolniku 1 se je ob zdravljenju z bisfosfonati mineralna kostna gostota povečala do normalnih vrednosti in ostala nespremenjena štiri leta po prenehanju zdravljenja. Pri bolniku 2 kljub pet let trajajočemu zdravljenju z bisfosfonati ni prišlo do izboljšanja mineralne kostne gostote, še naprej so se pojavljali zlomi kosti in hipertrofični kalusi. Pri obeh bolnikih smo ugotovili znano mutacijo c.-14C>T v genu IFITM5 v heterozigotni obliki. Oba imata v homozigotni obliki prisotno tudi varianto rs2297480 gena FDPS in v heterozigotni obliki varianto rs3840452 gena GGPS1.*

***Zaključek**. V članku je predstavljen kratek pregled osteoporoze v otroštvu s posebnim poudarkom na OI tipa V pri dveh bolnikih. Pri obeh bolnikih z OI tipa V, ki sta imela različno klinično sliko in potek bolezni, smo ugotovili mutacijo c.-14C>T v genu IFITM5. Z analizo genov encimov, vpletenih v presnovo bisfosfonatov, nismo mogli pojasniti neuspešnega zdravljenja z bisfosfonati pri enem od bolnikov.*

Ključne besede:
mineralna kostna gostota, hipertrofični kalus, gen IFITM5, bisfosfonati

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1 INTRODUCTION

Osteoporosis is a skeletal disorder characterized by decreased bone mass and micro-architectural deterioration that leads to fragile bones and susceptibility to fracture.

Although osteoporosis is considered to be primarily a disorder of adults, there is evidence to suggest that its roots may actually lie in childhood. Adolescence represents a significant time period in bone formation and is described as the 'bone bank' of the future. Approximately 40% of bone mass is accumulated in adolescence and peak bone mass is attained in early adulthood (1, 2). Future bone health and the risk of osteoporosis in adulthood are significantly related to the bone mass that accumulates in adolescence through early adulthood.

The assessment of bone density can be an important and informative investigation in a child with suspected osteoporosis. The most frequently used modality is dual energy X-ray absorptiometry (DXA) due to its availability, speed of scan acquisition and low radiation dose (3).

The definition of osteoporosis in adults is based on bone mineral density measurements, primarily through DXA. A measurement that falls 2.5 standard deviations (SD) or more below the average value of a young healthy adult defines osteoporosis (4). The Pediatric Position Development of the International Society for Clinical Densitometry (ISCD) (2007) definition of osteoporosis is bone mass that is less than 2 SD (z-score) below the average value for age and gender-matched controls, and a significant fracture history, such as long bone fracture of the lower extremities, vertebral compression fracture and two or more long bone fractures of the upper extremities (5). DXA underestimates the true density of small bones and overestimates that of large ones, which is why a height-for-age z-score adjustment produces the least bias correction on areal BMD measures (6).

Fractures are common in healthy youth. By 16 years of age, almost one-half of boys and one-third of girls have sustained a fracture (7). The clinical challenge lies in the early discrimination of children with fractures due to underlying skeletal pathology.

Low BMD ascertained by DXA associates with fracture in youth (8), but a fracture threshold has not been as well established in children as it was in adults.

Multiple factors play a role in bone health, such as genetics, family history, nutrition, particularly calcium and vitamin D intake, hormonal status and physical activity.

Osteoporosis in children may be primarily the result of intrinsic bone abnormality. It is usually either genetic in origin, or secondary to an underlying medical condition and/or its treatment (Table 1).

Table 1. Classification of childhood osteoporosis (9).

Primary osteoporosis	
	Osteogenesis imperfecta
	Idiopathic juvenile osteoporosis
	Osteoporosis pseudoglioma syndrome
Secondary osteoporosis	
	Reduced mobility
	Cerebral palsy
	Spinal cord injury and spina bifida
	Duchenne muscular dystrophy
	Spinal muscle atrophy
	Head injury
	Inflammatory cytokines
	Juvenile idiopathic arthritis
	Systemic lupus erythematosus
	Dermatomyositis
	Inflammatory bowel disease
	Systemic glucocorticoids
	Rheumatologic conditions
	Inflammatory bowel disease
	Nephrotic syndrome
	Cystic fibrosis
	Leukaemia
	Organ and bone marrow transplantation
	Disordered puberty
	Thalassemia major
	Anorexia nervosa
	Gonadal damage due to radiotherapy / chemotherapy
	Turner syndrome
	Klinefelter syndrome
	Galactosemia
	Poor nutrition / low body weight
	Anorexia nervosa
	Chronic systemic disease
	Inflammatory bowel disease
	Cystic fibrosis
	Malignancy

Osteogenesis imperfecta (OI) is a heritable bone dysplasia, characterized by bone fragility and deformity and growth deficiency. The International Society of Skeletal Dysplasias suggested OI classification that differentiates five types based on clinical characteristics and severity (Table 2) (10). Currently, 15 genes are associated with OI, as reviewed by Valadares et al. and Marini et al. (11, 12). The

majority of patients with OI display autosomal dominant inheritance of the mutations in the type I collagen genes, *COL1A1* and *COL1A2*. During the past several years, a number of non-collagenous genes whose protein products interact with collagen have been identified as the cause of rare forms of OI (11, 12). The majority of the non-classical OI types have autosomal recessive inheritance. The exceptions are X-linked inheritance of the *PLS3* gene mutations associated with OI type 1 (13), and a unique dominant defect in the gene encoding interferon-induced transmembrane protein 5 (*IFITM5*), which is associated with OI type V (14).

Table 2. Classification of osteogenesis imperfecta, adapted from Valdares et al. (10).

Osteogenesis imperfecta	Inheritance	Genes
Nondeforming osteogenesis imperfecta (type I)	AD X-linked	*COL1A1, COL1A2* *PLS3*
Perinatal lethal (type II)	AD, AR	*COL1A1, COL1A2, CRTAP, LEPRE1, PPIB, BMP1, CREB3L1*
Progressively deforming (type III)	AD, AR	*COL1A1, COL1A2, CRTAP, LEPRE1, PPIB, FKBP10, SERPINH1, SERPINF1, WNT1*
Moderate (type IV)	AD, AR	*COL1A1, COL1A2, CRTAP, FKBP10, SP7, SERPINF1, WNT1, TMEM38B*
With calcification of the interosseous membrane and/or hypertrophic callus (type V)	AD	*IFITM5*

Legend: AD, autosomal dominant; AR, autosomal recessive.

Osteogenesis imperfecta type V, first described in 2000, is a distinct clinical entity with unique clinical, radiological, and histological features. Clinically, it is only moderately deforming. Patients have normal sclera and teeth. Radiologic diagnostic criteria include a triad of calcification of the radioulnar interosseous membrane, the presence of hypertrophic callus at fractures or post-operative sites, and radiodense metaphyseal band adjacent to growth plates (13). Histologically, it is distinguished by a mesh-like pattern of lamellation under polarized light microscopy in iliac bone samples. Ossification of the interosseous membrane of the forearm is a constant feature, which may vary in its extent, can severely limit movements of the forearm, and is associated with secondary dislocation of the radial head (14, 15). The formation of hypertrophic callus, if present, is the most conspicuous clinical symptom in OI type V (14, 16). Long bones are most often affected, particularly in the lower extremities (16). Hypertrophic callus can be precipitated either by a fracture or a surgery, or arises spontaneously, and can become very large or even mimic osteosarcoma (15, 17-19). Evolution of the lesions is variable, ranging from complete resolution to significant persisting morbidity (20).

OI type V is caused by a recurrent c.-14C>T mutation in the 5'- untranslated region of the *IFITM5* gene, encoding interferon induced transmembrane protein 5, detected in all so far described patients (21-25). Even though the disease-causing mutation is identical among patients with OI type V, the interindividual phenotypic variability is considerable (23, 24).

The present report describes long-term clinical and radiographic follow-up of two patients with OI type V caused by c.-14C>T in the 5'-UTR of *IFTM5* with markedly different response to bisphosphonate treatment. A possible cause for a different response to the therapy was investigated.

2 MATERIALS AND METHODS

2.1 Patients

2.1.1 Case Report 1

The boy was born after an uneventful pregnancy and delivery to non-consanguineous parents. None of his parents had OI. At 6 months of age, after a vaccination by injection into the thigh, a painful swelling appeared. Radiographically, an unexpected fracture of the mid-shaft of the femur with exuberant callus formation was seen. The fracture was treated conservatively and healed with time. Because of numerous fractures of the long bones in the following years, mainly of the lower extremities (3-4 per year), OI was suspected. Deformities presented as marked antecurvatures of both tibiae and fibulae with cortical thinning and osteopenia. The patient had normal sclerae, no hearing loss and no dental abnormalities.

At the age of 8 years, a fracture of the right femur that occurred after a minor trauma was surgically treated by internal fixation with intramedullary telescopic rod along with corrective osteotomy. Swelling of the right thigh appeared two months after the surgery, with excessive and irregular new bone formation in the bone and soft tissue at the site of the fracture on x-rays (Figure 1A). Computerized tomography of the same area showed a large, well defined, ossified soft tissue mass with some ill-defined cortical destruction. Histologically, the lesion was diagnosed as hyperplastic callus (26). It disappeared spontaneously 6 months after the procedure. Numerous

corrective surgical procedures and elongation of the left femur and tibia were performed during the follow-up with good functional and structural results. At 10 years of age, low bone mineral density at the lumbar spine was detected (L1-L4 z – 3.56). Normal bone density was reached (L1 - L4 z - 0,9) after 3 years of cyclic treatment with pamidronate 1mg/kg b.w./day for three consecutive days every three months, followed by pamidronate cycles every 6 months for 2 years, and once yearly in the last two years, before the therapy was discontinued at the age of 16 years. Bone mineral density remained normal until the last follow-up at 21 years. Before the introduction of the bisphosphonate treatment, he suffered 20 fractures, and after the introduction of the therapy only three, all three without developing hypertrophic calluses. He reached his final height at 153 cm at his target height of 174 cm (- 3.57 HSDS).

2.1.2 Case Report 2

The boy was born after a normal pregnancy and delivery to healthy non-consanguineous parents. There was no family history of OI. His development was normal. The first fracture occurred at the age of 2 years and 9 months, with ten additional subsequent fractures, most of them atraumatic. Several fractures were followed by the formation of hyperplastic callus: subperiosteal fracture of right tibia, fracture of the third metacarpal on the right hand, and subperiosteal fissure of the right radius.

He was admitted to the paediatric pulmonary department because of pneumonia at the age of 3 years. Radiographically, besides pulmonary infiltrates and hypoplastic lungs, a bell-shaped chest with thin dysplastic ribs was observed. The deformities of vertebra of thoraco-lumbar spine were present, which led to the suspected diagnosis of spondyloepiphyseal dysplasia. Because of recurrent pulmonary infections, he was admitted several times to the pulmonary department.

At the age of 15 years, he was admitted to the department of rheumatology because of painful swelling of the distal part of the left thigh. Height and weight were on the 25th and 10th centile, respectively. He did not have blue sclera or dentinogenesis imperfecta. The large thigh mass was hot, tender and hard on palpation. Skeletal survey showed thoracic kyphoscoliosis, calcified interosseus membrane of the forearms and large radio-dense mass surrounding distal part of the femur without visible fracture of the bone. Magnetic resonance imaging demonstrated a large lobulated mass of 18 - 20 cm, surrounded by a thick calcified shell (Figure 1B). Histological investigation was typical for hypertrophic callus. The callus never regressed completely.

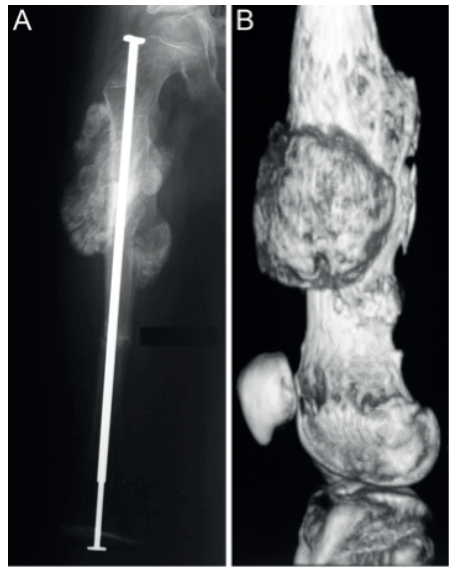

Figure 1A. Left: Patient 1. Hypertrophic callus formation appeared two months after a fracture of the right femur that was surgically treated by internal fixation with intramedullary telescopic rod along with corrective osteotomy.

Figure 1B. Right: Patient 2. Magnetic resonance imaging of a large lobulated mass of 18–20 cm in the distal femur surrounded by a thick calcified shell.

Bone densitometry showed low lumbar mineral density (L1 - L4 z – 3.1) and the diagnosis of OI type V was established. Cyclic treatment with pamidronate 1mg/kg b.w./day for three consecutive days every three months was started. He was switched to the treatment with zoledronate every 6 months at 20 years of age. Despite the bisphosphonate treatment, bone mineral density did not improve (Figure 2).

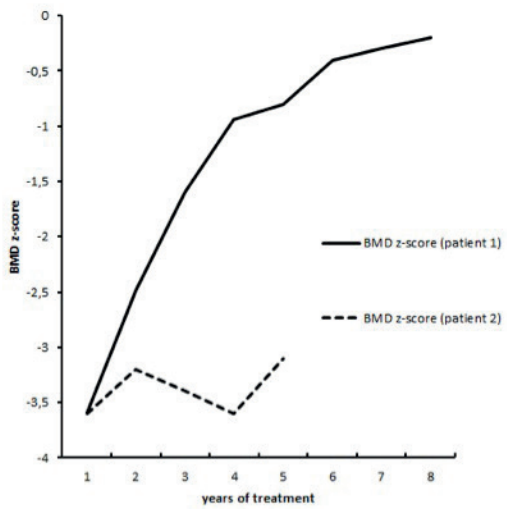

Figure 2. Bone mineral density in patients with OI type V during the bisphosphonate treatment.

During the course of the next 5 years, the patient had four more fractures, including the fractures of 4th, 5th and 6th rib on the right side, and a fracture of the left fibula with hypertrophic callus formation without regression. His final height was 168 cm (- 1.41 HSDS) (Table 3).

Table 3. Clinical findings in patients with OI type V caused by the c.-14C>T mutation in *IFITM5*.

Clinical characteristics	Patient 1	Patient 2
Age at diagnosis	*4*	*16*
Age at last visit	*21*	*21*
Height SDS	*- 3.57*	*- 1.41*
Δ Height SDS Height SDS – Target Height SDS	*- 2.97*	*- 1.24*
BMD z score (before treatment) (after treatment)	*- 3.59* *- 0.20*	*- 3.10* *- 3.60*
Number of fractures	*23*	*11*
Fractures at birth	-	*1*
Hyperplastic callus	+	+++
Interosseous membrane calcification	+	+
Radial subluxation/luxation	-	-

2.2 Mutational Analyses

Genomic DNA was isolated from venous blood samples using the FlexiGene DNA Kit 250 (Qiagen, Hilden, Germany), according to the recommended protocol. Both exons, exon-intron boundaries and 5'-UTR of *IFITM5* gene were PCR amplified using in-house designed primers (primer sequences available upon request). Sequencing was performed using BigDye Terminator v.3.1 Cycle Sequencing Kit and 3500 Genetic Analyzer capillary electrophoresis system (Life Technologies, Foster City, CA, USA). The obtained nucleotide sequences were compared to the normal *IFITM5* sequence (GenBank NG_03289.1).

In both patients, the same methodology of Sanger sequencing using specific in-house designed primers (primer sequences available upon request) was used for genotyping of variants rs2297480 in farnesyl diphosphate synthase gene (*FDPS*) and rs3840452 in geranylgeranyl diphosphate synthase 1 gene (*GGPS1*).

3 RESULTS

No pathological genetic variations were detected in the coding region and in exon-intron boundaries of the *IFITM* gene. However, the c.-14C>T *IFITM 5* mutation was detected in both individuals in heterozygous state. Both patients carried major *FDPS* variant rs2297480 in homozygous state and were heterozygous for *GGPS 1* variant rs3840452.

4 DISCUSSION

Both presented patients with clinical features of osteogenesis imperfecta type V were positive for the previously described 5'-UTR mutation of *IFTM5*, found in all so far described patients (21-25). The clinical diagnosis of OI type V was based on the presence of bone fragility, history of hyperplastic callus formation and calcification of the interosseous membrane of the forearm. None of our patients had radial head luxation.

Even though the disease-causing mutation is identical in patients with OI type V, the interindividual (23) and intrafamilial phenotypic variability is considerable (24, 27). Patient 1 with multiple recurrent fractures over the years developed only two hypertrophic calluses, both in the right thigh. Both lesions disappeared spontaneously. Patient 2 had 11 recurrent fractures of various bones, a majority of which developed hypertrophic calluses, also at the 3rd metacarpal and at the phalange of the index finger, and some never disappeared: the last one developed at the age of 21 years, after the final height has already been reached and pubertal signs fully developed. In the study of Lee DY et al., all patients have ossification of interosseus membrane of the forearm. The severity of ossification was significantly correlated with increasing age, but not with the range of forearm rotation (27). One of the index patients showed ossification of the interosseous membrane of the lower leg as well (27). Although the investigation was performed when both our patients were already adults, only an incomplete ossification was established with slight reduction of pronation and supination of the right arm in patient 2.

The phenotypic variability in OI type V is highlighted by the wide range of height and BMD results in this patient group (23, 24). It appears that some of it is due to gender differences, as males tend to have larger deficits in height and BMD (23). Our patients were both males, of the same age, and reached adulthood during the follow up. The severe growth failure in patient 1 may be related to several fractures with deformities, growth plate lesions and surgical correction of scoliosis at a relatively young age. On the other hand, patient's 2 final height was less than - 1SD below the mean for his target height despite being diagnosed and treated for osteoporosis only in late adolescence, but the fracture incidence was smaller.

Recent studies showed that intravenous pamidronate therapy has similar effects in OI type V as in the other OI types (28, 29). Successful treatment with intravenous zoledronate and ibadronate treatment in OI type V (24)

and other types of OI (30) was also observed. There was no correlation between phenotypic severity, the age at start of the treatment and treatment response in patients with OI types III and IV (31), as well as in patients with OI type V (28). Fracture incidence decreased in all patients (28, 29). Before treatment with bisphosphonates, the lumbar spine areal BMD was decreased in both our patients. Patient's1 BMD steadily increased until normal values after three years of cyclic pamidronate infusions, and remained in normal range 5 years after the discontinuation of the therapy. The second patient started treatment with bisphosphonates at the age of 16,5 years, before pubertal growth spurt and several years before final height was reached; however, no increase in BMD was observed and callus formation continued. Cheung MS et al. reported a patient with OI type V who has been treated by pamidronate for 9,5 years. During this time, he developed hyperplastic calluses at five different sites (20).

Farnesyl disphosphate synthase encoded by *FDPS* gene and geranyl-geranyl disphosphate synthase encoded by *GGPS1* gene are enzymes of the mevalonate pathway, which are inhibited by bisphosphonates leading to osteoclast apoptosis. Polymorphisms in *FDPS* and *GGPS1* genes are associated with a different response rate to bisphosphonate treatment in postmenopausal osteoporosis (32, 33). Analysed variants in those genes could not explain different response of bisphosphonate treatment in our patients, since both patients carried major *FDPS* variant rs2297480 and were heterozygous for *GGSP1* variant rs3840452.

In conclusion, two patients with OI type V due to recurrent c.-14C>T in the 5'-UTR of *IFTM5* mutation had a pronounced interindividual phenotypic variability in terms of fracture incidence and hypertrophic callus formation, and very different responses to bisphosphonate treatment during 17 years follow-up, which could not be explained by analysed variants in genes involved in bisphosphonate metabolism.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Patients and/or their parents signed written informed consent for genetic diagnostics and anonymous data publication.

REFERENCES

1. Bachrach LK, Hastie T, Wnag M-C, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, Black, and Caucasian youth: a longitudinal study. J Clin Endocrinol Metab 1999; 84: 4702-12.
2. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. N Engl J Med 1995; 332: 305-11.
3. Blake GM, Naeem M, Boutros M. Comparison of effective dose to children and adults from dual X-ray absorptiometry examinations. Bone 2006; 38: 935-42.
4. World Health Organization. WHO scientific group assessement of the osteoporosis at the primary care level. Geneva: WHO, 2004.
5. Bachrach L, Sills I. Clinical report-bone densitometry in children and adolescents. Pediatrics 2011; 127: 189-94.
6. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S. et al. Height adjustment in assessing dual energy X-ray absorptiometry measurements of bone mass and density in children. J Clin Endocrinol Metab 2010; 95: 1265-73.
7. Landin LA. Epidemiology of children's fractures. J Pediatr Orthop B 1997; 6: 79–83.
8. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM. More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 2000; 15: 2011-8.
9. Shaw NJ. Osteoporosis in paediatrics. Arch Dis Child Educ Pract Ed 2007; 92: 169-75
10. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M. et al. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A 2011; 155A: 943-68.
11. Valadares ER, Carneiro TB, Santos PM, Oliveira AC, Zabel B. What is new in genetics and osteogenesis imperfecta classification? J Pediatr (Rio J) 2014; 90: 536-41.
12. Marini JC, Reich A, Smith SM. Osteogenesis imperfecta due to mutations in non-collagenous genes: lessons in the biology of bone formation. Curr Opin Pediatr 2014; 26: 500-7.
13. van Dijk FS, Zillikens MC, Micha D, Riessland M, Marcelis CL, de Die-Smulders CE. et al. PLS3 mutations in X-linked osteoporosis with fractures. N Engl J Med 2013; 369: 1529-36.
14. Glorieux F, Rauch F, Plotkin H, Ward L, Travers R, Roughley P. et al. Type V osteogenesis imperfecta: a new form of brittle bone disease. J Bone Miner Res 2000; 15: 1650–8.
15. Rauch F, Glorieux F. Osteogenesis imperfecta. Lancet 2004; 363: 1377–85.
16. Cheung MS, Glorieux FH, Rauch F. Natural history of hyperplastic callus formation in osteogenesis imperfecta type V. J Bone Miner Res 2007; 22: 1181–6.
17. Vieira RL, Amaral DT, Jesus-Garcia FR, Saraiva G, Fernandes AR, Resnick D. Hyperplastic callus formation in osteogenesis imperfecta type V mimicking osteosacroma: 4-year follow-up with resolution. Skeletal Radiol 2006; 35: 402–5.
18. Rieker O, Kreitner KF, Karbowski A. Hyperplastic callus formation in osteogenesis imperfecta: CT and MRI findings. Eur Radiol 1998; 8: 1137–9.
19. Dobrocky I, Seidl G, Grill F. MRI and CT features of hyperplastic callus in osteogenesis imperfecta tarda. Eur Radiol 1999; 9: 665–8.

20. Cheung MS, Azouz EM, Glorieux FH, Rauch F. Hyperplastic callus formation in osteogenesis imperfecta type V: follow-up of three generations over ten years. Skeletal Radiol 2008; 37: 465-7.
21. Cho TJ, Lee KE, Lee SK, Song SJ, Kim KJ, Jeon D. et al. A single recurrent mutation in the 5'-UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Hum Genet 2012; 91: 343-8.
22. Semler O, Garbes L, Keupp K, Swan D, Zimmermann K, Becker J. et al. A mutation in the 5'-UTR of IFITM5 creates an in-frame start codon and causes autosomal-dominant osteogenesis imperfecta type V with hyperplastic callus. Am J Hum Genet 2012; 91: 349-57.
23. Rauch F, Moffatt P, Cheung M, Roughley P, Lalic L, Lund AM. et al. Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.-14C>T mutation in all patients. J Med Genet 2013; 50: 21-4.
24. Shapiro JR, Lietman C, Grover M, Lu JT, Nagamani SC, Dawson BC. et al. Phenotypic variability of osteogenesis imperfecta type V caused by IFITM5 mutation. J Bone Miner Res 2013; 28: 1523-30.
25. Balasubramanian M, Parker MJ, Dalton A, Giunta C, Lindert U, Peres LC. et al. Genotype-phenotype study in type V osteogenesis imperfecta. Clin Dysmorphol 2013; 22: 93-101
26. Lamovec J, Možina E, Baebler B. Hyperplastic callus formation in osteogenesis imperfecta. Ann Diag Pathol 2003; 7: 231-5.
27. Lee DY, Cho TJ, Choi IH, Chung CY, Yoo WY, Kim JH. et al. Clinical and radiological manifestations of osteogenesis imperfecta type V. J Korean Med Sci 2006; 21: 709-14.
28. Zeitlin L, Rauch F, Travers R, Munns C, Glorieux FH. The effect of cyclical intravenous pamidronate in children and adolescents with osteogenesis imperfecta Type V. Bone 2006; 38; 13-20.
29. Fleming F, Woodhead HJ, Briody JN, Hall J, Cowell CT, Ault J. et al. Cyclic bisphosphonate therapy in osteogenesis type V. J Pediatr Child Health 2005; 41: 147-51.
30. Vuorimies I, Toiviainen-Salo S, Hero M, Mäkitie O. Zoledronic acid treatment in children with osteogenesis imperfecta. Horm Res Paediatr 2011; 75: 346-53.
31. Zacharin M, Bateman J. Pamidronate treatment of osteogenesis imperfecta—lack of correlation between clinical severity, age at onset of treatment, predicted collagen mutation and treatment response. J Pediatr Endocrinol Metab 2002; 15: 163-74.
32. Marini F. Falchetti A, Silvestri S, Bagger Y, Luzi E, Tanini A. et al. Modulatory effect of farnesyl pyrophosphate synthase (FDPS) rs2297480 polymorphism on the response to long-term aminobisphosphonate treatment in postmenopausal osteoporosis. Curr Med Res Opin 2008; 24: 2609-15.
33. Choi HJ, Choi JY, Cho SW, Kang D, Han KO, Kim SW. et al. Genetic polymorphism in geranylgeranyl diphosphate synthase (GGSP1) predicts bone density response to bisphosphonate therapy in Korean women. Yonsei Med J 2010; 51: 231-8.

ATTACHMENT TO CAREGIVERS AND TYPE 1 DIABETES IN CHILDREN

NAVEZANOST NA STARŠE IN SLADKORNA BOLEZEN TIPA 1 PRI OTROCIH

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ABSTRACT

Attachment is a behavioral and physiological system, which enables individual's dynamic adaptation to its environment. Attachment develops in close interaction between an infant and his/her mother, plays an important role in the development of the infant's brain, and influences the quality of interpersonal relationships throughout life.

Security of attachment is believed to influence individual response to stress, exposing insecurely organized individuals to deregulated autonomic nervous system and exaggerated hypothalamic-pituitary-adrenal activity, which, in turn, produces increased and prolonged exposure to stress-hormones. Such stress responses may have considerable implications for the development of diverse health-risk conditions, such as insulin resistance and hyperlipidemia, shown by numerous studies.

Although the mechanisms are not yet fully understood, there is compelling evidence highlighting the role of psychological stress in the development of type 1 diabetes (T1D). One of the possible contributing factors for the development of T1D may be the influence of attachment security on individual stress reactivity. Thus, the suggestion is that insecurely attached individuals are more prone to experience increased and prolonged influence of stress hormones and other mechanisms causing pancreatic beta-cell destruction.

The present paper opens with a short overview of the field of attachment in children, the principal attachment classifications and their historic development, describes the influence of attachment security on individual stress-reactivity and the role of the latter in the development of T1D. Following is a review of recent literature on the attachment in patients with T1D with a conclusion of a proposed role of attachment organization in the etiology of T1D.

Keywords:
attachment, children, type 1 diabetes, etiology, stress reactivity

IZVLEČEK

Navezanost je vedenjski in fiziološki sistem, ki posamezniku omogoča dinamično prilagajanje na okolje. Navezanost se razvija pri sovplivu med dojenčkom in materjo, igra pomembno vlogo pri razvoju otrokovih možgan in vpliva na kvaliteto posameznikovih socialnih odnosov vse življenje.

Varnost ali oblika navezanosti vpliva na posameznikov odziv na stres (stresno reaktivnost). Tako pride pri negotovo navezanih posameznikih do slabše reguliranega avtonomnega živčnega sistema in pretirane reaktivnosti hipotalamo-hipofizno-suprarenalne osi, zaradi česar so ti v življenju pogosteje in dalj časa izpostavljeni delovanju stresnih hormonov. Tovrsten odziv na stres pa ima pomembno vlogo pri razvoju inzulinske rezistence, hiperlipidemije in drugih stanj, ki predstavljajo tveganje za zdravje.

Čeprav natančni mehanizmi še niso znani, je vedno več dokazov, da psihološki stres pomembno prispeva k razvoju sladkorne bolezni tipa 1 (SBT1). Eden od mehanizmov razvoja te bolezni bi lahko bil tudi vpliv oblike navezanosti na posameznikovo stresno reaktivnost. Tako so lahko negotovo navezani posamezniki pogosteje, dlje in v večji meri izpostavljeni delovanju stresnih hormonov, ki skupaj z drugimi dejavniki povzročajo uničenje beta celic trebušne slinavke.

Ta prispevek prikaže najprej kratek pregled področja navezanosti pri otrocih, glavne oblike navezanosti in njihov zgodovinski razvoj, oriše vpliv oblike navezanosti na posameznikovo stresno reaktivnost in vpliv te reaktivnosti na razvoj SBT1. Zaključi se s predlogom o vlogi oblike navezanosti pri razvoju SBT1 pri otrocih.

Ključne besede:
navezanost, otroci, starši, sladkorna bolezen tipa 1, etiologija, stresna reaktivnost

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1 INTRODUCTION

John Bowlby conceptualized attachment as an evolutionary behavioral system of comparable importance to the systems guiding feeding and reproductive behaviors (1). Contemporary theories describe it as a behavioral and physiological system with a biological basis in the orbitofrontal system of the right hemisphere and its cortical and subcortical connections (2). The infant or child dynamically adapts to its environment to regulate safety and survival by ensuring proximity of the caregiver or attachment figure in times of distress, using attachment behaviors that increase in their complexity with age. These include constant knowledge of the attachment figure's (an older person, most commonly the mother) whereabouts, using sounds to make her return when gone too far or fleeing to her if the distress or perceived danger rises to a level that is too high to endure on his own. Infant's attachment behavioral system is adapted to a complementary behavioral system of the attachment figure, namely, the care-giving system. This results in a behavioral interplay between the infant and the caregiver, in which the infant leaves 'the secure base' provided by the caregiver to explore, and returns back to 'the safe-haven' when in distress (tired, ill, hungry, alarmed or just too far) to be calmed down and ready to leave again. Over time, the repeated interactions between the caregiver and the infant are internalized by the infant and captured into implicit memory as 'internal working models' (2). These early models are thought to guide expectations and behaviors in a largely unconscious way, and are thought to remain relatively stable across the lifespan, provided the family environment and the wider ecology remain stable as well (3).

2 PATTERNS OF ATTACHMENT

According to Bowlby's and Piaget's theory, and in line with the current knowledge on brain development, the attachment to one principal (mostly the mother) and one or more secondary attachment figures is formed between the age of 7 months and 2 years, when the object's constancy (the knowledge that mother exists even when not present or within sight) has developed (2, 4, 5). Attachment continues to develop throughout childhood, depending on the stability or changes in the family environment (3, 4, 6). Bowlby's early observations of the striking behaviours of children subjected to separation from, and loss of, their attachment figures were further supported by Ainsworth's observational studies of individual differences in the quality of the interactions between infants and their mothers. What emerged was the critical importance of how sensitive and responsive mothers responded to their infants' attachment bids. These early observations paved the way for the development of a structured laboratory assessment, the Strange Situation Procedure, as a way of accessing children's internal working models of attachment relationships (6). These were thought to be reflected in their behavior in response to a series of separations from, and reunions with, their caregivers. Three organized attachment strategies were identified and, subsequently, Main and Solomon added a fourth attachment category, capturing attachment disorganization (7). These were:

1. Secure attachment: infant shows signs of missing mother during her absence, greets her actively, seeks comfort, settles and returns to play. The mother's behavior at home was tender, careful holding with contingent face-to-face pacing of interactions, and with sensitivity to the infant's signals in the first year of life.
2. Insecure resistant/ambivalent attachment: infant appears preoccupied with mother throughout procedure, is either markedly angry or markedly passive, alternately seeking and resisting the mother, fails to settle or return to play on reunion and continues to focus on the mother and cry. The mother's behavior was inept in holding, noncontingent in face-to-face interaction and unpredictable, but not rejecting.
3. Insecure avoidant: infant focuses on the toys, does not cry during separation, actively avoids and ignores mother on reunion, moves away, turns away, leans away when picked up. The mothers of these infants rejected attachment behavior and were particularly averse to tactual contact.
4. Insecure disorganized/disoriented: infant displays disorganized or disoriented behaviors in the parent's presence (freezing all movements, rocking on hands and feet, hands in air, rise and then fall prone at parent's entrance). Mostly infants whose parents behaved towards them in abusive manner (7).

Attachment to caregivers develops disregarding the way the child is treated by them, even when the child is maltreated (8). Attachment behavior, although in a different form, becomes activated in adults as well, in the way of monitoring the availability of attachment figures (most commonly romantic partners, parents or close friends) and seeking them as 'stronger and wiser' in times of stress (9). The patterns of attachment are manifested in different ways across the life span, using different, but related, methodologies. The focus shifted from the study of individual differences in behavior to the study of internal working models as reflected in narratives (8).

3 ATTACHMENT SECURITY AND STRESS REACTIVITY

Research findings drawn from diverse perspectives converge in showing that an individual's behavioral and

physiological response to a specific stressor is consistent throughout time and comparable in different species (10). The responses depend on the perception of the stressor as well as on the individual's stress reactivity and coping. Individual differences in stress response can be observed in different domains, namely: physiology, cognitive function, subjective experience and behavior (10). Within the physiological domain, changes responding to stressful experience can be divided into cardiovascular responses (indicated by blood pressure and heart rate), driven by autonomic nervous system activity, and metabolic responses produced by the output of the glucocorticoid hormone cortisol from the adrenal cortex, driven by hypothalamic-pituitary-adrenal (HPA) axis activity (10). Although physiological responses serve to meet metabolic demands posed by the stressor, prolonged exposure to the HPA axis hyperactivity may lead to insulin resistance, hyperlipidemia and other conditions with increased risk for health disturbance (11). Cognitive responses to stress include systems important in learning, memory as well as attentional processes (12). Within the subjective domain, the perception of the stressor in humans is believed to be determined within the orbitofrontal system of the right hemisphere (2), the most common emotional experience being fear and apprehension (13). Experiences that pose a risk are thought to activate the individual's attachment system and various physiological and behavioral mechanisms that are all directed towards homeostatically regulating the stress response, resulting in the deactivation of the attachment system (14). For example, the presence of a large dog will induce the feeling of fear in a small child and result in the child fleeing towards his/her mother, aiming to be lifted up away from the danger, held and comforted until the danger subsides. When this occurs and the child finds a secure base in the attachment figure, his/her physiological functions (increased heart rate, shiver) and the feeling of fear return to normal, and the child is able to continue with play.

Secure attachment is believed to provide resilience in the face of stress, 'which is expressed in the capacity to flexibly regulate emotional states via autoregulation and interactive regulation' (2), using internalized coping mechanisms and interaction with the attachment figure (2). Studies investigating stress reactivity in humans and animals have shown secure attachment to result in adaptive hypoactivity of the HPA axis, resulting in lower levels of stress hormones' release under the influence of psychological stress (15). Conversely, unresponsive or neglectful parenting and child maltreatment have been associated with blunted early morning cortisol levels, no diurnal decrease in cortisol and an exaggerated cortisol response to stressful situations, although the mechanisms behind are complex (16).

Various studies have shown that securely attached children, under stressful circumstances, maintain low levels of cortisol when in the presence of attachment figure, while insecurely attached (especially disorganized) children's cortisol responses were high (17-20).

4 THE ROLE OF STRESS IN THE DEVELOPMENT OF TYPE 1 DIABETES

The etiology of type 1 diabetes (T1D) has been extensively studied, with some clear findnings (21-24), but many questions have yet to be addressed (25). The mechanisms implied in the etiology of T1D are viral infection, seasonality, rapid growth, psychological stress, and many others, yielding conclusions of multi-factor origins of the disorder (21-24).

One of the proposed hypotheses on etiology of T1D assumed the destruction of pancreatic beta-cells was a result of functional overload of the cells, caused by overfeeding, accelerated growth at puberty, low physical activity as well as psychological stress (with elevations in cortisol, other stress hormones and autonomic nervous system imbalance), which all resulted in elevated needs for insulin production (24).

There is evidence for the role of diabetes-related autoimmunity in the destruction of pancreatic beta-cells and development of T1D. However, not all subjects with diabetes-specific autoantibodies develop clinical syndrome, and not all patients with T1D produce such antibodies. Sepa et al. conducted a wide prospective population-based study on more than 5000 newborns, with a follow-up of 1845 subjects after 2,5 years. Their results showed associations of serious life-events (such as divorce or death in the family) with diabetes-specific autoantibody status of babies without clinical diabetes. The association remained in the follow up sample, where 12,5% of children exposed to divorce developed autoimmunity, as compared to 3,8% of the children without the experience of divorce (26).

Veronique Mead elaborated on a hypothesis, which included the role of stressful life-events in the critical period of brain development, namely, experience-dependent maturation. She argues that 'disruptions in early bonding and attachment, including adverse events, such as traumatic stress, are capable of causing: (1) long-term imbalances in autonomic regulatory function and (2) relative dominance of sympathetic or parasympathetic activity.' The proposed mechanisms would again program an individual's autonomic nervous system and HPA axis, and expose the individual to higher insulin demands, as well as influence the immune system (27).

Taking all these into account, Ludvigsson proposed a unifying theory wherein various genetic, environmental and co-regulating factors were included as explanatory factors. In the proposed 'beta-cell stress hypothesis,' functional overload of the beta-cells as a result of multiple factors resulted in overexpression of specific antigens on the cells which mediated their destruction (28).

5 ATTACHMENT SECURITY AND DIABETES

There is only a scarcity of data on the attachment to caregivers or adults in close relationships in patients with diabetes. Ciechanowski et al. found dismissing attachment in the setting of poor patient-provider relationship to be associated with poorer treatment adherence in patients with type 1 and 2 diabetes. The study was performed using self-report measures of attachment security with 367 subjects (29). The same group then performed a cross-sectional study using self-report measures with 4095 adult primary care patients with diabetes type 1 or 2. Their results confirmed previous findings by showing that patients with dismissing attachment cooperated less well in their treatment than secure or preoccupied patients (lower levels of exercise, food care, diet and adherence to medications and higher rates of smoking), however, the patients with preoccupied attachment style were least likely to have worse metabolic control (not the securely attached). They concluded that insecure dismissing attachment is associated with worse diabetes outcomes, while secure and insecure preoccupied attachment predisposes to better outcomes (30).

Colton et al. studied disturbed eating behaviors in 106 girls with T1D in a one-year follow-up study using Children's Eating Disorder Examination Interview. They concluded that a more disturbed attachment to mother was one of the predictors of a new onset of eating disturbances (31). Rosenberg et al. performed a pilot study using self-report questionnaires on 31 families of adolescents with T1D, hypothesizing that attachment security to parents would be associated with metabolic control in adolescents. They were able to show only that maternal perceptions of more secure adolescents' attachment, not adolescents' reports, were associated with better glycemic control, although they concluded that the mechanism of the association was unclear (32).

Sepa et al. proposed that psychological stress could, via hormonal mechanisms, increase insulin resistance and trigger diabetes-related autoimmunity (33). They hypothesized that infants with diabetes-specific autoantibodies were more likely to have insecurely attached mothers than their antibody-negative peers. They interviewed 18 mothers of infants who were antibody-positive and 32 mothers of antibody-negative infants, using the Adult Attachment Interview (34). Although failing to reach statistical significance, the proportion of children with insecurely attached mothers was substantially larger in the antibody-positive group (33% compared to 19% and 33% compared to 20% for two types of diabetes-specific autoantibodies) (33).

6 CONCLUSIONS

Although not yet fully understood, growing evidence points to the role of psychological stress in the development of T1D. One of the possible mechanisms could be mediated through the child's attachment security and the influence of the attachment organization on stress reactivity, namely, the autonomous nervous system and the HPA axis reactivity. It is argued that insecurely attached, especially disorganized, children would be subjected to higher levels of stress hormones during exposure to stressful life-events. Insecure attachment and increased physiological stress reactivity would represent one of the factors contributing to the beta-cell overload, and, through the process of autoimmunity, peripheral tissue insulin resistance or some other as yet unknown process result in the destruction of beta-cells and clinical diabetes.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Not required.

REFERENCES

1. Bowlby J. Attachment and loss. Vol. 1: Attachment. London: PIMLICO, 1969.
2. Schore AN. Attachment and the regulation of the right brain. Attach Hum Dev 2000; 2: 23-47.
3. Waters E, Merrick S, Treboux D, Crowell J, Albersheim L. Attachment security in infancy and early adulthood: a twenty-year longitudinal study. Child Dev 2000; 71: 684-9.
4. Bowlby J. A secure base. London, New York: Routledge Classics, 2010.
5. Piaget J, Inhelder B. The psychology of the child. New York: Perseus Books Group, 1972.
6. Ainsworth MD. Patterns of infant-mother attachments: antecedents and effects on development. Bull N Y Acad Med 1985; 61: 771-91.
7. Main M, Solomon J. Procedures for identifying infants as disorganized/disoriented during the Ainsworth strange situation. In: Greenberg MT, Cichetti D, Cummings EM, editors. Attachment in the preschool years: theory, research and intervention. Chicago: University Press, 1990: 121-60.

8. Main M. Introduction to the special section on attachment and psychopathology: overview of the field of attachment. J Consult Clin Psychol 1996; 64: 237-43.
9. Bowlby J. Attachment and loss. Vol. 3: Loss - sadness and depression. London: PIMLICO, 1998.
10. Koolhaas JM, de Boer SF, Coppens CM, Buwalda B. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Front Neuroendocrinol; 31: 307-21.
11. Rosmond R, Bjorntorp P. The hypothalamic-pituitary-adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. J Intern Med 2000; 247: 188-97.
12. Arnsten AF. Catecholamine modulation of prefrontal cortical cognitive function. Trends Cogn Sci 1998; 2: 436-47.
13. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 2001; 24: 1161-92.
14. Nolte T, Guiney J, Fonagy P, Mayes LC, Luyten P. Interpersonal stress regulation and the development of anxiety disorders: an attachment-based developmental framework. Front Behav Neurosci 2011; 5: 55.
15. Gunnar M, Quevedo K. The neurobiology of stress and development. Annu Rev Psychol 2007; 58: 145-73.
16. Tarullo AR, Gunnar MR. Child maltreatment and the developing HPA axis. Horm Behav 2006; 50: 632-9.
17. Gunnar MR, Donzella B. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology 2002; 27: 199-220.
18. Kertes DA, Donzella B, Talge NM, Garvin MC, Van Ryzin MJ, Gunnar MR. Inhibited temperament and parent emotional availability differentially predict young children's cortisol responses to novel social and nonsocial events. Dev Psychobiol 2009; 51: 521-32.
19. Spangler G, Grossmann KE. Biobehavioral organization in securely and insecurely attached infants. Child Dev 1993; 64: 1439-50.
20. Hertsgaard L, Gunnar M, Erickson MF, Nachmias M. Adrenocortical responses to the strange situation in infants with disorganized/disoriented attachment relationships. Child Dev 1995; 66: 1100-6.
21. Oldstone MB, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell 1991; 65: 319-31.
22. Rothwell PM, Gutnikov SA, McKinney PA, Schober E, Ionescu-Tirgoviste C, Neu A. Seasonality of birth in children with diabetes in Europe: multicentre cohort study. European Diabetes Study Group. BMJ 1999; 319: 887-8.
23. Toniolo A, Onodera T, Yoon JW, Notkins AL. Induction of diabetes by cumulative environmental insults from viruses and chemicals. Nature 1980; 288: 383-5.
24. Dahlquist G. Can we slow the rising incidence of childhood-onset autoimmune diabetes? The overload hypothesis. Diabetologia 2006; 49: 20-4.
25. Ludvigsson J. Is it time to challenge the established theories surrounding type 1 diabetes? Acta Paediatr 2014; 103: 120-3.
26. Sepa A, Frodi A, Ludvigsson J. Mothers' experiences of serious life events increase the risk of diabetes-related autoimmunity in their children. Diabetes Care 2005; 28: 2394-9.
27. Mead VP. A new model for understanding the role of environmental factors in the origins of chronic illness: a case study of type 1 diabetes mellitus. Med Hypotheses 2004; 63: 1035-46.
28. Ludvigsson J. Why diabetes incidence increases-a unifying theory. Ann N Y Acad Sci 2006; 1079: 374-82.
29. Ciechanowski PS, Katon WJ, Russo JE, Walker EA. The patient-provider relationship: attachment theory and adherence to treatment in diabetes. Am J Psychiatry 2001; 158: 29-35.
30. Ciechanowski P, Russo J, Katon W, Von Korff M, Ludman E, Lin E. et al. Influence of patient attachment style on self-care and outcomes in diabetes. Psychosom Med 2004; 66: 720-8.
31. Colton PA, Olmsted MP, Daneman D, Rydall AC, Rodin GM. Natural history and predictors of disturbed eating behaviour in girls with Type 1 diabetes. Diabet Med 2007; 24: 424-9.
32. Rosenberg T, Shields CG. The role of parent-adolescent attachment in the glycemic control of adolescents with Type 1 diabetes: a pilot study. Fam Syst Health 2009; 27: 237-48.
33. Sepa A, Frodi A, Ludvigsson J. Mothers' attachment insecurity and diabetes-related autoantibodies in their infants. Ann N Y Acad Sci 2004; 1037: 110-3.
34. van IJzendoorn MH. Adult attachment representations, parental responsiveness, and infant attachment: a meta-analysis on the predictive validity of the Adult Attachment Interview. Psychol Bull 1995; 117: 387-403.

ENDOCRINE AND METABOLIC EFFECTS OF ADIPOSE TISSUE IN CHILDREN AND ADOLESCENTS

ENDOKRINA IN PRESNOVNA FUNKCIJA MAŠČOBNEGA TKIVA PRI OTROCIH IN MLADOSTNIKIH

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ABSTRACT

Adipose tissue is implicated in many endocrine and metabolic processes. Leptin was among the first identified adipose-secreted factors, which act in an auto-, para- and endocrine manner. Since leptin, many other adipose tissue factors were determined, some primarily secreted from the adipocytes, some from other cells of the adipose tissue.
So-called adipokines are not only involved in obesity and its complications, as are insulin resistance, type 2 diabetes and other components of the metabolic syndrome, but also in growth, reproduction, bone metabolism, immune response, cancer development and many other important biological processes. Research in the field of adipokines has revealed new insights into the physiological and pathophysiologal processes and opened new therapeutic possibilities. In the present article, a special emphasis is devoted to research in children and adolescents.

Keywords:
adipose tissue, obesity, endocrinology, adipokine, metabolic syndrome, child

IZVLEČEK

Maščobno tkivo ima vlogo pri številnih endokrinih in presnovnih procesih. Lepin je bil med prvimi odkritimi dejavniki iz maščobnega tkiva, ki delujejo avto-, para- in endokrino. Od opredelitve leptina so odkrili še številne druge dejavnike, od katerih se nekateri izločajo iz maščobnih celic, nekateri pa iz drugih celic maščobnega tkiva.
Tako imenovani adipokini niso povezani le z debelostjo in njenimi zapleti, kot so rezistenca proti inzulinu, sladkorna bolezen tipa 2 in druge komponente metabolnega sindroma, temveč tudi z rastjo, razmnoževanjem, presnovo kosti, imunskim odzivom, razvojem rakavih bolezni in mnogimi drugimi pomembnimi biološkimi procesi. Raziskave na področju adipokinov so opredelile nove fiziološke in patofiziološke procese in odprle nove možnosti zdravljenja. V tem prispevku poseben poudarek namenjamo raziskavam pri otrocih in mladostnikih.

Ključne besede:
maščobno tkivo, debelost, endokrinologija, adipokini, metabolni sindrom, otroci

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1 BACKGROUND

Adipose tissue was long considered to be an energy storage tissue only. Adipocytes store energy in the form of triglycerides when there is an excess of energy, and release it when energy is needed. By studying genetically obese and diabetic mice (ob/ob, db/db), it was determined that factors released from adipocytes are able to communicate with distant tissues and influence their function. Leptin was the first cytokine with such function to be determined. Since leptin, several additional factors with endocrine functions were determined. Some - as leptin and adiponectin - are released from the fat cells exclusively, whilst others are also released from others cells of the adipose tissue (macrophages, fibrocytes, endothelial cell), and other organs (liver, bone) (1, 2).

In the manuscript, we will discuss the role of the selected adipokines in obesity, and the development of components of metabolic syndrome, with an emphasis on their role in children and adolescents.

2 LEPTIN

The discovery of leptin caused a paradigm shift in the way adipose tissue is perceived. It is no longer regarded as an energy storage organ only, but also as an important endocrine organ with important effects on body metabolism. Leptin levels are increased in adipose tissue and circulation in human obese subjects, including children and adolescents (3-5). Mutations in the leptin gene or its receptor are associated with human morbid and early obesity (2, 6). Its levels are correlated with body mass index (BMI) and fat store content. They are decreased in subjects with decreased fat mass, such as lipodystrophy and anorexia (7, 8). Following weight loss, leptin levels decrease in both adults and children (9, 10). Leptin levels are higher in subcutaneous that visceral adipose tissue. They are higher in females as compared to males, and this dimorphism is present already in children (5). A mechanism described behind this dimorphism is the suppressive effect of androgens on leptin expression in adipocytes (11).

Central nervous system (CNS) leptin effects - particularly at the level of hypothalamus - are associated with energy homeostasis. Following secretion of leptin from fat stores into circulation, it is transported across the blood-brain barrier to CNS, where it stimulates processes that result in decreased food intake and increased energy consumption. In common obesity leptin resistance at the level of CNS, is a mechanism explaining continued energy intake despite severely increased circulating leptin levels (12).

In addition to CNS, leptin receptors are also present in peripheral tissues, where leptin decreases fat stores in the skeletal muscle and liver by stimulating fatty acid oxidation and glucose uptake. Peripheral leptin resistance (particularly in skeletal muscle) is also linked to insulin resistance (IR) in obesity (13, 14), and to the development of nonalcoholic fatty liver disease and metabolic syndrome in children (15, 16).

Besides its effects on energy homeostasis, leptin has several other important endocrine functions. The lack of leptins action at the level of CNS, is also associated with reduced reproductive function (6). Leptin is implicated in the regulation of immunologic and inflammatory processes (17). At the level of the bone, leptin has a dual and opposing role. On one hand, it stimulates osteoblasts, bone mineralization and growth, while, on the other hand, it suppresses bone development (18, 19). It has also been implicated in tumorigenesis, as leptin receptors can be found in certain cancer cells, possibly enabling leptin to stimulate growth of these cells under certain conditions (20).

Leptin has been successfully used in the therapy of leptin deficient subjects ameliorating hyperphagia, extreme obesity, hypogonadism and impaired cell immunity (6). Leptin is able to induce puberty in hypogonadotropic hypogonadism due to leptin deficiency, and to reduce liver steatosis associated with obesity due to leptin-deficiency (21, 22). In polygenic obesity, leptin therapy was not as successful, probably due to leptin resistance being the main feature in this condition (12). It could, however, be potentially used in subjects following weight loss. In these subjects, a decrease in fat content leads to decreased leptin levels and decreased energy expenditure, possibly preventing further weight loss or enabling a regain of lost weight. By administration of leptin at this stage, one could prevent a regain of weight in these subjects (23). In addition, leptin mimetics have been proposed to overcome leptin resistance, and co-administration of amylin with leptin was shown to positively modify leptin signalling (24).

3 ADIPONECTIN

Adiponectin is expressed in the mature adipocyte and is secreted into blood circulation, where it is present in 3 main oligomeric forms, high molecular weight (HMW) form being linked with most of the effects on peripheral tissues (25).

In contrast to leptin, and indeed most of the other adipokines, adiponectin blood levels are not increased, but decreased in obesity, including in children (26). There is a strong negative correlation between plasma adiponectin levels with body fat mass (27). Following weight loss, adiponectin levels increase in both adults and children (26, 28). The anti-obesity effect is associated

with the ability of adiponectin to increase body's energy expenditure and to decrease differentiation of adipocytes in experimental animals (29).

Adiponectin is, similarly to leptin, secreted in a gender dimorphic fashion, with circulating levels being higher in women than in men. Although stimulation of adipocytes with human male serum leads to a decrease in the expression of adiponectin, increasing concentrations of testosterone or estradiol do not influence either adiponectin mRNA expression or secretion, implicating, to date, unidentified serum gender specific factors (30).
In the peripheral tissues, adiponectin's actions are mediated through adiponectin receptor 1 or 2 (AdipoR1, AdipoR2). In skeletal muscles, adiponectin acts mainly through AdipoR1 and through AdipoR2 in the liver. Variations in the expression levels of these receptors at the level of the peripheral tissues, are in addition to circulating levels associated with adiponectin's effects (31).

Adiponectin acts as an insulin sensitizer in both experimental animals and humans. Insulin sensitising mechanism is linked to a reduction of hepatic gluconeogenesis and an increase of muscle glucose transport (32). Low levels of adiponectin, especially HMW form, are associated with the development of IR, type 2 diabetes (T2D), components of the metabolic syndrome and cardiovascular disease (33-35). This link is also present in children and adolescents (36, 37). Certain single nucleotide polymorphisms (SNP) in the adiponectin gene are associated with low adiponectin levels and T2D (38, 39). On the other hand, increased adiponectin levels are associated with the reduced risk of T2D (40), and therapy with insulin sensitizing drugs thiazolidinediones increases adiponectin (primarily HMW) levels (41). In children, lifestyle modifications also result in a beneficial increase in adiponectin levels, accompanied by increased insulin sensitivity (26).

Adiponectin has also anti-inflammatory and anti-oxidant properties. It inhibits tumor necrosis factor (TNF), alpha and superoxide radical generation in endothelial cells, and TNFalpha generation in adipose tissue (42). Low levels of adiponectin are also associated with nonalcoholic steatohepatitis independent of IR (43). In addition, low levels of adiponectin are linked to an increased risk of malignancies (44).

4 VISFATIN

Previously known as pre-B cell colony enhancing factor, visfatin is a controversial adipokine, whose levels were shown to be either increased, normal or decreased in adult human obesity (45-48). In children and adolescents, circulating visfatin levels and SNPs in visfatin gene were also inconsistently linked to obesity determined by BMI or waist circumference (49-51). Furthermore, it is controversial whether visfatin is predominantly expressed in human visceral or subcutaneous adipose tissue (45, 52, 53).

Visfatin binds to insulin receptor and was suggested to have insulin-like effects (52). It was determined to be a nicotinamide phosphoribosyltransferase implicated in promoting insulin secretion upon glucose stimulation (54, 55). Circulating visfatin levels are increased by hyperglycaemia in mouse models of T2D and in humans with type 1 diabetes (T1D) and T2D (52, 56). Again, these results were not confirmed in all studies, and SNPs in visfatin gene are not linked to T2D (54). In obese children, visfatin levels do not differ between those with and without IR (51). On the other hand, in children visfatin gene, SNPs are linked to higher visfatin levels, components of metabolic syndrome and low grade inflammation (51).

A decrease in body weight - following bariatric surgery - and exercise in T1D subjects lowers visfatin levels (47, 57). Treatment with insulin sensitizing drug rosiglitazone in humans does not lower visfatin levels (58).

Visfatin is implicated in the pathogenesis of chronic conditions, as are atherosclerosis and cardiovascular diseases (59). It is suggested to be a proinflammatory cytokine. As obesity is a state of chronic low-grade inflammation, this could be the common mechanism explaining some of the reported results; still, visfatin's role in obesity and its complications need to be further addressed.

5 RESISTIN

Although resistin is also expressed in adipocytes, the main source of resistin in humans are macrophages (60). Structurally, resistin is very similar to adiponectin including the formation of higher-order oligomerisation structures. In contrast to adiponectin, however, low-molecular structures are more physiologically active (61). Increased resistin levels were determined in human obesity in both adults and children, and a SNP in the resistin promoter is associated with obesity (62-65). More specifically, circulating levels of resistin were, in particular, associated with body fat mass in children (66). In animal obesity models, increased resistin levels are linked to IR, and resistin was described as a possible link between obesity and the development of T2D (67). Decreasing resistin levels or blocking its action, is linked to improved insulin sensitivity and glucose metabolism (67, 68). In humans, controversial results on the role of resistin in the development of IR, T2D and metabolic syndrome are described (63, 64, 69). Increased circulating levels are also reported in T1D subjects (70). A SNP in resistin promoter is, in addition to obesity, also associated with T2D (71).

Resistin is also linked with states of inflammation including low-grade inflammation in obesity (72, 73). Similarly as for visfatin, resistin's role in the development of obesity and its complications needs to be further addressed.

6 RETINOL BINDING PROTEIN 4 (RBP4)

Increased circulating and adipose tissue RBP4 levels are linked to obesity and visceral fat mass content (74, 75). Several studies, however, found no correlation between circulating RBP4 levels, the level of obesity, and the amount and distribution of adipose tissue (76-78).

RBP4 is suggested to be involved in early processes of atherosclerosis and cardiovascular diseases (79, 80). Circulating and adipose tissue RBP4 levels are associated with IR and T2D in both adults and in children and adolescents (81-83). RBP4 levels also correlate with other components of the metabolic syndrome (84-86). They decrease with lifestyle interventions - reduction of weight, increased exercise - in adults, children and adolescents (76, 87, 88). The associations between RBP4 levels and the development of obesity and its complications, such as IR, impaired glucose tolerance, T2D and certain components of the metabolic syndrome, have, however, not been found consistently in both adults and children (77, 78, 89-92).

Obesity is a state of low-grade inflammation. Several adipokines have been shown to be regulated by inflammatory factors (93). We therefore studied RBP4 expression in human adipocytes exposed to inflammatory milieu (culture media from activated machrophages), or selected proinflammatory cytokines interleukin 1 beta (IL-1beta) and TNFalpha, and determined that RBP4 expression in adipose tissue was consistently decreased in a proinflammatory environment (94). These results link inflammation and altered expression of RBP4 in adipose tissue, even though it seems that changes in RBP4 expression in adipose tissue are not directly related to the changes in circulating RBP4 levels that often precede the development of systemic IR (94, 95).

The levels of circulating RBP4 seem to be regulated in a sex-dependent manner. Males, including adolescents, showed higher levels of RBP4 compared to females (78, 96-99). Also, women with increased androgen levels had in increase in circulating RBP4 (78, 96-99). This sexual dimorphism was, however, not demonstrated in all studies (75, 100-102). Two classic adipokines, leptin and adiponectin, are regulated in a sex-dependent manner. In contrast to RBP4, their levels are lower in males compared to females, and further studies demonstrated that the male sex hormone testosterone inhibited the expression of leptin and adiponectin in adipocytes (11, 103).

We therefore decided to further explore the gender specific regulation of RBP4 expression in human adipocytes. As a model system, we used the human Simpson-Golabi-Behmel syndrome (SGBS) cell strain. These cells are characterized by a high capacity for adipogenic differentiation, and therefore provide a suitable cell system to study human adipocyte biology (104).

Effects of gender specific serum factors on RBP4 expression in human SGBS adipocytes were investigated. We collected serum samples from 10 healthy non-obese females (estradiol 89.99 pg/ml, testosteron 0.37 ng/ml, leptin 16.0 ng/ml) and 10 healthy non-obese males BMI- and age-matched volunteers (estradiol 34.58 pg/ml, testosterone 4.18 ng/ml, leptin 2.2 ng/ml). After pooling these samples, we added them to adipocyte cultures at a concentration of 10 % (vol/vol). As a control experiment, we first studied the expression of adiponectin. As expected from earlier studies, male serum was more efficient in downregulating adiponectin mRNA than female serum (Figure 1A) (30). Likewise, both female and male serum suppressed RBP4 mRNA expression, with male serum showing a significantly stronger inhibitory effect than female serum (Figure 1B). These results are in accordance with the data obtained from male and female adipose tissue explants, where significantly higher mRNA expression was determined in female adipose tissue when compared to male (100).

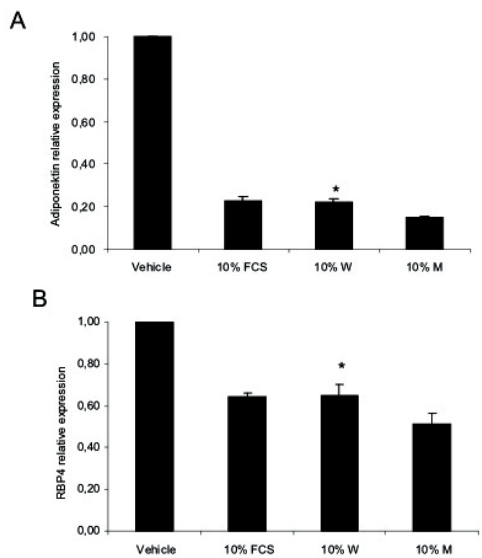

Figure 1A, 1B. Effects of pooled 10 % female (F) or male (M) serum on adiponectin (A) and retinol binding protein 4 (B) mRNA expression in SGBS adipocytes.

mRNA expression ratios were determined by qRT-PCR, using succinate dehydrogenase as a reference. Data are presented as mean ± SEM of 3 or more independent experiments, and are normalized to the expression or secretion in the vehicle (1% ethanol) treated samples. *p < 0.05 when compared to the vehicle.

7 CHEMERIN

Adipose tissue and liver are the main sources of chemerin, a chemo-attractant protein that acts through chemokine like receptor 1 (CMKLR1), which is located to adipocytes, endothelial cells and inflammatory cells (e.g. dendritic cells and macrophages). Chemerin is implicated in the process of adipogenesis, and its higher levels are associated with obesity, especially visceral, in both adults and children (105, 106). Interestingly, higher levels were determined in vitamin D deficient obese children, compared to vitamin D non-deficient obese children (107). Dysregulation of chemerin is associated with several metabolic abnormalities, such as increased blood pressure and total cholesterol, decreased HDL cholesterol, prediabetic state of IR and T2D (105). Chemerin levels positively correlate with leptin and negatively with adiponektin levels (108). In children, higher levels are associated with low-grade inflammation and endothelium dysfunction, as determined by markers of endothelial activation intercellular adhesion molecule-1 (ICAM-1) and E-selectin (109).

8 CONCLUSIONS

Adipose tissue is regarded an important endocrine tissue. Dysregulation of factors secreted from the adipose tissue – so-called adipokines – is not linked only to obesity and its complications, but has also important effects on bone metabolism, reproduction, immunity, cancer development, etc.

Several adipokines are considered biomarkers of patho-physiological states, in particular those linked to obesity. Some are still considered controversial due to inconsistent experimental results, and will have to be further studied in larger and well-controlled studies. In the future, disease-specific arrays of adipokines will possibly be used to determine, with higher specify and sensitivity, those at a significant risk of selected disease, or they will be used as monitoring tools to evaluate the efficacy of treatment. Of importance, noninvasive methods that will enable us to determine the origin of studied adipokines (e.g. visceral vs. subcutaneous adipose tissue) will bring the role of adipokines, as biomarkers, to a new and a higher level.

In addition, it is possible that the manipulation of the expression or actions of selected adipokines will be used therapeutically in the future. To this effect, leptin was the first adipokine used therapeutically in states of leptin deficiency (e.g. congenital leptin deficiency or certain lypodystrophies). As adipokines have increasingly important endocrine and metabolic effect also in non-obesity associated states, it is probable that adipokine therapy will also be used in non-obesity relates states. Delivery of therapeutic adipokine to a specific tissue, possibly with the use of combination therapy, would be of special benefit.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Not required.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.
2. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ. et al. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 1996; 84: 491-5.
3. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D. et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995; 269: 543-6.
4. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med 1995; 1: 950-3.
5. Caprio S, Tamborlane WV, Silver D, Robinson C, Leibel R, McCarthy S. et al. Hyperleptinemia: an early sign of juvenile obesity: relations to body fat depots and insulin concentrations. Am J Physiol 1996; 271: E626-30.
6. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C. et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002; 110: 1093-103.
7. Fiorenza CG, Chou SH, Mantzoros CS. Lipodystrophy: pathophysiology and advances in treatment. Nat Rev Endocrinol 2011; 7: 137-50.
8. Hebebrand J, Muller TD, Holtkamp K, Herpertz-Dahlmann B. The role of leptin in anorexia nervosa: clinical implications. Mol Psychiatry 2007; 12: 23-35.
9. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR. et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334: 292-5.
10. Holm JC, Gamborg M, Kaas-Ibsen K, Gammeltoft S, Ward L, Heitmann BL. et al. Time course and determinants of leptin decline during weight loss in obese boys and girls. Int J Pediatr Obes 2007; 2: 2-10.
11. Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W. et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest 1997; 100: 808-13.
12. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T. et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 1999; 282: 1568-75.

13. Steinberg GR, Parolin ML, Heigenhauser GJ, Dyck DJ. Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. Am J Physiol Endocrinol Metab 2002; 283: E187-92.
14. Steinberg GR, Smith AC, Van Denderen BJ, Chen Z, Murthy S, Campbell DJ. et al. AMP-activated protein kinase is not down-regulated in human skeletal muscle of obese females. J Clin Endocrinol Metab 2004; 89: 4575-80.
15. Yoshinaga M, Sameshima K, Tanaka Y, Wada A, Hashiguchi J, Tahara H. et al. Adipokines and the prediction of the accumulation of cardiovascular risk factors or the presence of metabolic syndrome in elementary school children. Circ J 2008; 72: 1874-8.
16. Kim IK, Kim J, Kang JH, Song J. Serum leptin as a predictor of fatty liver in 7-year-old Korean children. Ann Nutr Metab 2008; 53: 109-16.
17. Fantuzzi G. Three questions about leptin and immunity. Brain Behav Immun 2009; 23: 405-10.
18. Reseland JE, Gordeladze JO. Role of leptin in bone growth: central player or peripheral supporter? FEBS Lett 2002; 528: 40-2.
19. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT. et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 2000; 100: 197-207.
20. Lang K, Ratke J. Leptin and adiponectin: new players in the field of tumor cell and leukocyte migration. Cell Commun Signal 2009; 7: 27.
21. von SJ, Moss A, Nagel SA. et al. Leptin substitution results in the induction of menstrual cycles in an adolescent with leptin deficiency and hypogonadotropic hypogonadism. Horm Res Paediatr 2012; 77: 127-33.
22. von SJ, Moss A, Nagel SA, Muehleder H, Debatin KM, Farooqi IS. et al. Leptin substitution results in the induction of menstrual cycles in an adolescent with leptin deficiency and hypogonadotropic hypogonadism. Horm Res Paediatr 2012; 77: 127-33.
23. Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S. et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest 2005; 115: 3579-86.
24. Misra M. Obesity pharmacotherapy: current perspectives and future directions. Curr Cardiol Rev 2013; 9: 33-54.
25. Retnakaran R, Youn BS, Liu Y, Hanley AJ, Lee NS, Park JW. et al. Correlation of circulating full-length visfatin (PBEF/NAMPT) with metabolic parameters in subjects with and without diabetes: a cross-sectional study. Clin Endocrinol (Oxf) 2008; 69: 885-93.
26. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V. et al. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. J Clin Endocrinol Metab 2008; 93: 3051-7.
27. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996; 271: 10697-703.
28. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol 2002; 147: 173-80.
29. Bauche IB, El Mkadem SA, Pottier AM, Senou M, Many MC, Rezsohazy R. et al. Overexpression of adiponectin targeted to adipose tissue in transgenic mice: impaired adipocyte differentiation. Endocrinology 2007; 148: 1539-49.
30. Horenburg S, Fischer-Posovszky P, Debatin KM, Wabitsch M. Influence of sex hormones on adiponectin expression in human adipocytes. Horm Metab Res 2008; 40: 779-86.
31. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S. et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003; 423: 762-9.
32. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116: 1784-92.
33. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H. et al. Adiponectin and protection against type 2 diabetes mellitus. Lancet 2003; 361: 226-8.
34. Nakashima R, Kamei N, Yamane K, Nakanishi S, Nakashima A, Kohno N. Decreased total and high molecular weight adiponectin are independent risk factors for the development of type 2 diabetes in Japanese-Americans. J Clin Endocrinol Metab 2006; 91: 3873-7.
35. Fisher FM, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE. et al. Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia 2005; 48:1084-7.
36. Panagopoulou P, Galli-Tsinopoulou A, Fleva A, Pavlitou-Tsiontsi E, Vavatsi-Christaki N, Nousia-Arvanitakis S. Adiponectin and insulin resistance in childhood obesity. J Pediatr Gastroenterol Nutr 2008; 47: 356-62.
37. Murphy MJ, Hosking J, Metcalf BS, Voss LD, Jeffery AN, Sattar N. et al. Distribution of adiponectin, leptin, and metabolic correlates of insulin resistance: a longitudinal study in British children; 1: prepuberty (EarlyBird 15). Clin Chem 2008; 54: 1298-306.
38. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K. et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 2002; 51: 536-40.
39. Poitou C, Lacorte JM, Coupaye M, Bertrais S, Bedel JF, Lafon N. et al. Relationship between single nucleotide polymorphisms in leptin, IL6 and adiponectin genes and their circulating product in morbidly obese subjects before and after gastric banding surgery. Obes Surg 2005; 15: 11-23.
40. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA. et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 2002; 360: 57-8.
41. Kubota N, Terauchi Y, Kubota T, Kumagai H, Itoh S, Satoh H. et al. Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin-dependent and -independent pathways. J Biol Chem 2006; 281: 8748-55.
42. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun 2004; 315: 264-71.
43. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology 2004; 40: 46-54.
44. Kelesidis I, Kelesidis T, Mantzoros CS. Adiponectin and cancer: a systematic review. Br J Cancer 2006; 94: 1221-5.
45. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR. et al. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes 2005; 54: 2911-6.
46. Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R. et al. Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. J Clin Endocrinol Metab 2006; 91: 3165-70.
47. Haider DG, Holzer G, Schaller G, Weghuber D, Widhalm K, Wagner O. et al. The adipokine visfatin is markedly elevated in obese children. J Pediatr Gastroenterol Nutr 2006; 43: 548-9.
48. Jin H, Jiang B, Tang J, Lu W, Wang W, Zhou L. et al. Serum visfatin concentrations in obese adolescents and its correlation with age and high-density lipoprotein cholesterol. Diabetes Res Clin Pract 2008; 79: 412-8.
49. Belo VA, Souza-Costa DC, Lacchini R, Sertório JT, Lanna CM, Carmo VP. et al. Adiponectin associates positively with nitrite levels in children and adolescents. Int J Obes (Lond) 2013; 37: 740-3.
50. Li RZ, Ma X, Hu XF, Kang SX, Chen SK, Cianflone K. et al. Elevated visfatin levels in obese children are related to proinflammatory factors. J Pediatr Endocrinol Metab 2013; 26: 111-8.
51. Taskesen D, Kirel B, Us T. Serum visfatin levels, adiposity and glucose metabolism in obese adolescents. J Clin Res Pediatr Endocrinol 2012; 4: 76-81.

52. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K. et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005; 307: 426-30.
53. Varma V, Yao-Borengasser A, Rasouli N, Bodles AM, Phanavanh B, Lee MJ. et al. Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation. J Clin Endocrinol Metab 2007; 92: 666-72.
54. Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A. et al. Nampt/PBEF/ Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab 2007; 6: 363-75.
55. Spinnler R, Gorski T, Stolz K, Schuster S, Garten A, Beck-Sickinger AG. et al. The adipocytokine Nampt and its product NMN have no effect on beta-cell survival but potentiate glucose stimulated insulin secretion. PLoS One 2013; 8: e54106.
56. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. Diabetologia 2006; 49: 1909-14.
57. Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. J Clin Endocrinol Metab 2006; 91: 1578-81.
58. Pfutzner A, Marx N, Walcher D, Lobig M, Seidel D, Forst T. Impact of rosiglitazone on visfatin and adiponectin plasma concentrations in patients with type 2 diabetes and coronary artery disease. Clin Lab 2008; 54: 237-41.
59. Friebe D, Neef M, Kratzsch J, Erbs S, Dittrich K, Garten A. et al. Leucocytes are a major source of circulating nicotinamide phosphoribosyltransferase (NAMPT)/pre-B cell colony (PBEF)/ visfatin linking obesity and inflammation in humans. Diabetologia 2011; 54: 1200-11.
60. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C. et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003; 300: 472-6.
61. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C. et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003; 300: 472-6.
62. Nieva-Vazquez A, Pérez-Fuentes R, Torres-Rasgado E, López-López JG, Romero JR. Serum resistin levels are associated with adiposity and insulin sensitivity in obese Hispanic subjects. Metab Syndr Relat Disord 2014; 12: 143-8.
63. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV. et al. Resistin / Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes 2001; 50: 2199-202.
64. Sentinelli F, Romeo S, Arca M, Filippi E, Leonetti F, Banchieri M. et al. Human resistin gene, obesity, and type 2 diabetes: mutation analysis and population study. Diabetes 2002; 51: 860-2.
65. Azuma K, Oguchi S, Matsubara Y, Mamizuka T, Murata M, Kikuchi H. et al. Novel resistin promoter polymorphisms: association with serum resistin level in Japanese obese individuals. Horm Metab Res 2004; 36: 564-70.
66. Ortega L, Riestra P, Navarro P, Gavela-Perez T, Soriano-Guillen L, Garces C. Resistin levels are related to fat mass, but not to body mass index in children. Peptides 2013; 49: 49-52.
67. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM. et al. The hormone resistin links obesity to diabetes. Nature 2001; 409: 307-12.
68. Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y. et al. Regulation of fasted blood glucose by resistin. Science 2004; 303: 1195-8.
69. Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB, Sr., Wilson PW. et al. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. J Clin Endocrinol Metab 2008; 93: 3165-72.
70. Geyikli I, Keskin M, Kor Y, Akan M. Increased resistin serum concentrations in patientswith type 1 diabetes mellitus. J Clin Res Pediatr Endocrinol 2013; 5: 189-93.
71. Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H. et al. The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. Am J Hum Genet 2004; 75: 678-86.
72. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun 2003; 309: 286-90.
73. 73 gawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R. et al. Serum resistin (FIZZ3) protein is increased in obese humans. J Clin Endocrinol Metab 2003; 88: 5452-5.
74. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM. et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356-62.
75. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR. et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006; 354: 2552-63.
76. Janke J, Engeli S, Boschmann M, Adams F, Bohnke J, Luft FC. et al. Retinol-binding protein 4 in human obesity. Diabetes 2006; 55: 2805-10.
77. Yao-Borengasser A, Varma V, Bodles AM, Rasouli N, Phanavanh B, Lee MJ. et al. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. J Clin Endocrinol Metab 2007; 92: 2590-7.
78. Ulgen F, Herder C, Kuhn MC, Willenberg HS, Schott M, Scherbaum WA. et al. Association of serum levels of retinol-binding protein 4 with male sex but not with insulin resistance in obese patients. Arch Physiol Biochem 2010; 116: 57-62.
79. Bobbert T, Raila J, Schwarz F, Mai K, Henze A, Pfeiffer AF. et al. Relation between retinol, retinol-binding protein 4, transthyretin and carotid intima media thickness. Atherosclerosis 2010; 213: 549-51.
80. Sasaki M, Otani T, Kawakami M, Ishikawa SE. Elevation of plasma retinol-binding protein 4 and reduction of plasma adiponectin in subjects with cerebral infarction. Metabolism 2010; 59: 527-32.
81. Aeberli I, Biebinger R, Lehmann R, L'allemand D, Spinas GA, Zimmermann MB. Serum retinol-binding protein 4 concentration and its ratio to serum retinol are associated with obesity and metabolic syndrome components in children. J Clin Endocrinol Metab 2007; 92: 4359-65.
82. Reinehr T, Stoffel-Wagner B, Roth CL. Retinol-binding protein 4 and its relation to insulin resistance in obese children before and after weight loss. J Clin Endocrinol Metab 2008; 93: 2287-93.
83. Yeste D, Vendrell J, Tomasini R, Gallart LL, Clemente M, Simon I. et al. Retinol-binding protein 4 levels in obese children and adolescents with glucose intolerance. Horm Res Paediatr 2010; 73: 335-40.
84. Qi Q, Yu Z, Ye X, Zhao F, Huang P, Hu FB. et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. J Clin Endocrinol Metab 2007; 92: 4827-34.
85. Lim S, Yoon JW, Choi SH, Park YJ, Lee JJ, Park JH. et al. Combined impact of adiponectin and retinol-binding protein 4 on metabolic syndrome in elderly people: the Korean Longitudinal Study on Health and Aging. Obesity (Silver Spring) 2010; 18: 826-32.
86. Wu Y, Li H, Loos RJ, Qi Q, Hu FB, Liu Y. et al. RBP4 variants are significantly associated with plasma RBP4 levels and hypertriglyceridemia risk in Chinese Hans. J Lipid Res 2009; 50: 1479-86.
87. Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M. et al. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. J Clin Endocrinol Metab 2007; 92: 1168-71.
88. Balagopal P, Graham TE, Kahn BB, Altomare A, Funanage V, George D. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. J Clin Endocrinol Metab 2007; 92: 1971-4.

89. Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P. et al. Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. J Clin Endocrinol Metab 2007; 92: 4306-12.
90. von EM, Lepper PM, Liu D, Lang K, Baumann M, Nawroth PP. et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia 2007; 50: 1930-7.
91. Ribel-Madsen R, Friedrichsen M, Vaag A, Poulsen P. Retinol-binding protein 4 in twins: regulatory mechanisms and impact of circulating and tissue expression levels on insulin secretion and action. Diabetes 2009; 58: 54-60.
92. Chavez AO, Coletta DK, Kamath S, Cromack DT, Monroy A, Folli F. et al. Retinol-binding protein 4 is associated with impaired glucose tolerance but not with whole body or hepatic insulin resistance in Mexican Americans. Am J Physiol Endocrinol Metab 2009; 296: E758-64.
93. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol 2010; 314: 1-16.
94. Kotnik P, Keuper M, Wabitsch M, Fischer-Posovszky P. Interleukin-1beta downregulates RBP4 secretion in human adipocytes. PLoS One 2013; 8: e57796.
95. Sell H, Eckel J. Regulation of retinol binding protein 4 production in primary human adipocytes by adiponectin, troglitazone and TNF-alpha. Diabetologia 2007; 50: 2221-3.
96. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH. et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006; 29: 2457-61.
97. Jia W, Wu H, Bao Y, Wang C, Lu J, Zhu J. et al. Association of serum retinol-binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. J Clin Endocrinol Metab 2007; 92: 3224-9.
98. Kim IK, Lee HJ, Kang JH, Song J. Relationship of serum retinol-binding protein 4 with weight status and lipid profile among Korean children and adults. Eur J Clin Nutr 2011; 65: 226-33.
99. Tan BK, Chen J, Lehnert H, Kennedy R, Randeva HS. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. J Clin Endocrinol Metab 2007; 92: 2764-72.
100. Kos K, Wong S, Tan B, Kerrigan D, Randeva H, Pinkney J. et al. Human RBP4 adipose tissue expression is gender specific and influenced by leptin. Clin Endocrinol (Oxf) 2011; 74: 197-205.
101. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Schleicher E. et al. High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. Diabetes Care 2007; 30: 1173-8.
102. Takebayashi K, Suetsugu M, Wakabayashi S, Aso Y, Inukai T. Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. J Clin Endocrinol Metab 2007; 92: 2712-9.
103. Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H. et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes 2002; 51: 2734-41.
104. Wabitsch M, Brenner RE, Melzner I, Braun M, Moller P, Heinze E. et al. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. Int J Obes Relat Metab Disord 2001; 25: 8-15.
105. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD. et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem 2007; 282: 28175-88.
106. Shin HY, Lee DC, Chu SH, Jeon JY, Lee MK, Im JA. et al. Chemerin levels are positively correlated with abdominal visceral fat accumulation. Clin Endocrinol (Oxf.) 2012; 77: 47–50.
107. Reyman M, Verrijn Stuart AA, van Summeren M, Rakhshandehroo M, Nuboer R, de Boer FK. et al. Vitamin D deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. Int J Obes (Lond) 2014; 38: 46-52.
108. Chu SH, Lee MK, Ahn KY, Im JA, Park MS, Lee DC. et al. Chemerin and adiponectin contribute reciprocally to metabolic syndrome. PLoS One 2012; 7: e34710.
109. Landgraf K, Friebe D, Ullrich T, Kratzsch J, Dittrich K, Herberth G. et al. Chemerin as a mediator between obesity and vascular inflammation in children. J Clin Endocrinol Metab 2012; 97: E556-64.

TYPE 1 DIABETES IN THE YOUNG: ORGANIZATION OF TWO NATIONAL CENTERS IN ISRAEL AND SLOVENIA

SLADKORNA BOLEZEN TIPA 1 PRI OTROCIH IN MLADOSTNIKIH: ORGANIZACIJA DELA V DVEH NACIONALNIH CENTRIH V IZRAELU IN SLOVENIJI

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ABSTRACT

Type 1 diabetes is a chronic autoimmune disease that affects mainly young people. In the last 50 years, a steady increase of the T1D incidence in the young is reported worldwide, with an average 4 % increase annually. In addition, the mean age at the diagnosis is decreasing. Studies show that good metabolic control is important not only for delaying the chronic complications of diabetes but also for improving the quality of life of patients and their families. Continuous education, together with modern technology, is crucial in achieving these goals. Longitudinal data on glycated hemoglobin (HbA1c), along with the data on severe hypoglycemia and severe diabetic ketoacidosis, can describe the quality of care in a defined population. Two national reference diabetes centres taking care of children, adolescents and young adults with diabetes in Israel and Slovenia are described.

Keywords:
type 1 diabetes, children, metabolic control, reference centre, education, insulin pump, continuous glucose monitoring

IZVLEČEK

Sladkorna bolezen tipa 1 je kronično avtoimuno obolenje, ki najpogosteje prizadene mlade ljudi. Incidenca bolezni zadnjih 50 let narašča po vsem svetu, v povprečju je letni porast incidence ocenjen na 4 %, ob tem pa starost bolnikov ob času diagnoze pada. Raziskave kažejo, da je dobra presnovna urejenost bolnikov s sladkorno boleznijo zelo pomembna, saj je s tem mogoče odložiti pozne zaplete bolezni ter izboljšati kvaliteto življenja bolnikov in njihovih družin. Neprekinjena edukacija bolnikov skupaj z možnostmi moderne tehnologije lahko prispeva k izboljšanju njihove presnovne urejenosti. Presnovno urejenost spremljamo z določanjem glikoziliranega hemoglobina (HbA1c) ter s številom težkih hipoglikemij in ketoacidoz na populacijo pacientov v določenem časovnem obdobju. V prispevku sta opisana dva nacionalna referenčna centra za obravnavo otrok, mladostnikov in mladih odraslih s sladkorno boleznijo v Izraelu in Sloveniji.

Ključne besede:
sladkorna bolezen tipa 1, otroci, presnovna urejenost, referenčni center, edukacija, inzulinska črpalka, neprekinjeno merjenje glukoze v podkožju

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1 INTRODUCTION

Type 1 diabetes (T1D) is one of the most common chronic childhood diseases. In the last 50 years, a steady increase of the T1D incidence in children and adolescents is reported worldwide, with an average 4 % increase annually (1–5). In addition, the mean age at the diagnosis is decreasing (4–8). Accordingly, the educational and therapeutic approach of the diabetic team to a family with a child newly diagnosed with T1D is changing.

Already in the 1970s, the shortcomings of the accepted therapeutic approach handling diabetes strictly on the medical basis were recognized, noting the T1D treatment to be heavily burdensome for children (9).

Children and adolescents with T1D must not only learn to accept the fact that they differ from their peers, but also to coordinate the demands of their social framework (family, school, place of employment, leisure activities, sports) with those of the diabetic regimen (monitoring glucose levels, daily insulin administrations, and special diet maintenance). It soon became clear that a multidisciplinary team might best meet the needs of these young patients.

The changed approach resulted in the establishment of the Israel Counselling Centre for Juvenile Diabetics in 1976, introducing a comprehensive multidisciplinary approach within a specialized medical field to Israel. A similar approach led to the decision that all children with T1D should be treated centrally in Slovenia. The first child was treated in the University Children's Hospital Ljubljana (UCHL) in 1956, while all Slovene newly diagnosed children and adolescents have been treated in UCHL for the last 25 years. A multidisciplinary team and a structured educational program have been established (10, 11).

2 ORGANIZATION OF THE CLINICAL WORK

Today, the National Centre for Childhood Diabetes at the Institute of Endocrinology and Diabetes at Schneider Children's Medical Centre of Israel (SCMCI) is the national referral centre. It is the largest centre of the kind in Israel, and also one of the largest worldwide. The centre treats more than 1,600 patients with different types of diabetes annually, 100–120 of them newly diagnosed each year.

A holistic, multidisciplinary approach is practiced in the centre, accumulating valuable experience and expertise in the treatment of young people with diabetes.

The team is comprised of 8 senior pediatric endocrinologist, 3 fellows, 5 nurse educators, 4 dietitians, 2 social workers, and 3 psychologists. The staff is available on a 24-h basis, 7 days a week for calls. More than 6200 visits of patients with diabetes in the outpatient clinic are registered annually. The age of patients treated in this centre ranges between a few weeks and 30 years, with the same multidisciplinary team caring for children, adolescents, and young adults (Table 1).

Table 1. Descriptive data on patient population in Schneider's Children Medical Centre of Israel (SCMCI) and University Children's hospital Ljubljana (UCHL), Slovenia.

	SCMCI	UCHL
Incidence of T1DM (per 100.000)	12.2 (Jews), 8.9 (Arabs)	14.67
Average number of newly diagnosed children with T1D/year	120	60
Total number of patients	1600	650
Age range (years)	0-30	0-25
CSII users (%)	50	77
CGM users (%)	20	10
Medical team (number) physicians/nurses/psychologists/ dietitian/social worker	11/5/3/4/2	5/4/2/1/0.2
Education at diagnosis	Outpatient-based	In patient-based
Outpatient visits (number/year)	6200	3100
HbA1c (%) in 2000	8.3	9.26
HbA1c (%) in 2014	7.7	7.75

In Slovenia, the Department of Endocrinology, Diabetes and Metabolic Diseases at the UCHL, is the national centre for childhood diabetes. Currently, over 650 patients with T1D and other rarer forms of diabetes (T2D, cystic fibrosis (CF) related diabetes, MODY, neonatal diabetes) are treated. Annually, up to 65 children with newly diagnosed T1D are referred from all parts of Slovenia, in addition to approximately 5 children with T2D and 1 with CF related diabetes. At the time, 3 senior pediatric endocrinologists, 2 fellows, 4 nurse educators, 1 dietitian, and 2 psychologists, partly also a social worker, are taking care of the whole population of patients. A 24/7-telephone line is provided to help patients or/and other physicians in case of problems with disease management or/and in emergencies. Around 3100 visits of patients with diabetes in the outpatient clinic are registered annually, with an average patient visiting the centre four times per year. The age of patients ranges between few weeks and 25 years (Table 1).

3 CLINICAL MANAGEMENT

In Israel, newly diagnosed patients with diabetes ketoacidosis (DKA) are usually hospitalized in the pediatric unit or intensive care unit (children younger than 2 years or patients with severe DKA) until correction of the DKA. Patients without DKA and without dehydration are usually discharged from hospitalization 1–2 days after their admission and some (e.g. those of older age, those with a first family member with diabetes) are usually discharged on the day of admission. On the first day of admission, a pediatric endocrinologist evaluates the child's state, confirms the diagnosis, outlines the therapeutic approach and sees the patient and his/her parents. On the same day, a dietitian, a nurse, and a member of the psychological team see the family. This intervention is usually performed on an ambulatory basis. The fact that therapy can be applied on an ambulatory basis can itself reduce the anxiety of the patient, and it poses fewer problems for both the patient and the family. Attention is paid to the cultural and educational background of the patient's family and their psychosocial and economic state. Medical and educational counselling are given to the patient and his family members.

In Slovenia, the initial approach to newly diagnosed T1D patients is predominantly based on a five-day hospital stay, where one parent stays with the child. In this period of time, the insulin regimen is stabilized – toddlers start with insulin pump treatment (CSII) immediately, while older children start with multiple daily injections (MDI). Certified nurse educators take care of the educational program. Patients and family members visit a dietitian, with the help of which eating habits are assessed and a nutritional plan is prepared. The family receives psychological support and advice; a pediatric endocrinologist explains basic facts about diabetes and its chronic complications.

In both countries, the goals of intensive management of T1D were implemented after the Diabetes Control and Complications Trial (DCCT) (12); the management strategy follows international guidelines (13). Patients with T1D and their caregivers continue to be challenged with having to maintain blood glucose levels in the near-normal range, having to prevent sustained hyperglycaemia associated with long-term microvascular and macrovascular complications, and having to avoid recurrent episodes of hypoglycemia. Intensified treatment can be achieved by more frequent visits at the diabetes clinic, or increased communication between patients and their medical-care team (14, 15). Thus, one of the most important factors in the long-term management of diabetes is the regulation of follow-up visit frequency. Patients are seen at least once every 3 months or more frequently if needed (in the first months after the diagnosis, when changing treatment modality, and poorly controlled patients). The importance of the regular self-monitoring of blood glucose (SMBG) is repeatedly stressed to guide and monitor metabolic control. The SMBG should be performed at least 5–6 times a day. When a patient comes for the follow-up visits, the data from the glucometers and the insulin pump is downloaded to the hospital electronic record system. In the last years, the use of a web-based therapy management system (16), designed to simplify the information collection and assessment process and to help patients to communicate with their caregivers, is increasing.

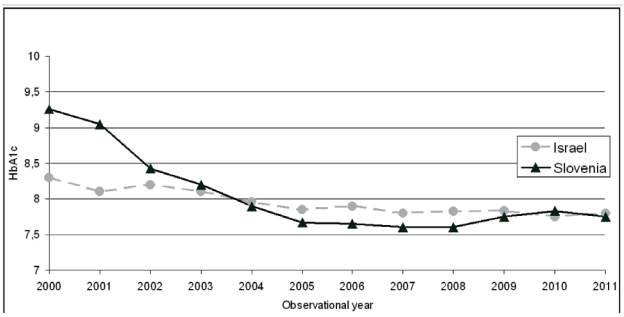
Adherence to an appropriate nutritional plan is another important part of the diabetes treatment regimen. The physician, nurse, and dietitian work together to attain an optimal diet, taking into account the age of the patient, her/his pubertal stage, amount of physical exercise, family habits and social/ethnic particularities (17). Patients/parents are instructed on how to calculate the carbohydrate and protein intake and how to adjust the insulin doses accordingly. In the first year after the diagnosis, patients consult the dietitian at every follow-up visit in Israel, while, in Slovenia, at least at the first follow-up visit and, subsequently, as needed. Thereafter, patients consult dietician twice a year or as required in Israel, while, in Slovenia, once yearly at a half-day follow-up visit, and possibly also during the summer camp.

One of the major obstacles in achieving and maintaining good metabolic control in T1D are the psychological problems that develop in both the affected child and his/her family members following the diagnosis of diabetes (18). Thus, in addition to the medical check-up, a psychologist and/or a social worker counsel the patient and his/her parents at least twice a year in Israel, and once a year in Slovenia. Additionally, a special intensified program was designed for children with suboptimal metabolic control, and a psychological support group for parents is available in Slovenia since 2009, where two psychologists lead group discussions on various important topics, such as partnership, living with a child with a chronic disease, emotional problems (19), etc. It seems that under the supervision and guidance of the multidisciplinary team, the child/adolescent with diabetes is able to lead a near-normal life in many, if not all, respects (20).

4 MONITORING GLYCEMIC CONTROL, SHORT- AND LONG-TERM COMPLICATIONS

Monitoring long-term glycaemic control is based on measuring HbA1c levels every 3 months in both centres. Capillary HbA1c is measured by an automated point-of-care immunoassay analyser (DCA Vantage Analyzer, Simens, Germany; reference range: 4.3–5.8%). Since the

beginning of the last decade, the annually measured average glycaemic control at the SCMCI has improved significantly with a decrease in the median HbA1c from 8.3% in 2000 to 7.7% in 2013 (21). Similarly, at UHCL, in the period between 2000 and 2012, the median HbA1c for the whole group of patients with all therapeutic regimens decreased from 9,26% to 7,75% (19) (Figure 1).



Legend:
Slovenia – University Children's Hospital Ljubljana
Israel – Schneider's Children Medical Centre of Israel
HbA1c – Glycated hemoglobin A1c

Figure 1. Metabolic control of children with type 1 diabetes over the last twelve years in the national reference centres in Slovenia and Israel.

Data on severe acute complications are recorded in the medical records. DKA is defined as an event requiring hospitalization and intravenous therapy, and severe hypoglycemia (SH) is defined as an event with a loss of consciousness and/or seizures, requiring hospitalization or glucagon injection, and/or intravenous glucose therapy (22, 23). At UHCL, the rates of these severe acute complications remain low at an average 1.54 per 100 patient-years for severe DKA, and 0.90 per 100 patient-years for SH (24).

In both centres, patients are followed annually for development of long-term microvascular complications by fundoscopic examination for detection of retinopathy and urinary analysis for microalbumin/creatine. Patients are also followed annually for the development of associated other autoimmune diseases (celiac, autoimmune thyroiditis).

5 STRUCTURED STANDARDIZED EDUCATIONAL PROGRAM

The program in UCHL is based on a standardized step-by-step education for patients, family members and caregivers. Each step is recorded in the medical record, and the attained knowledge is verified with several written tests, which are also saved in the record. Slide sets and brochures are available.

- At the diagnosis: Parents and patients have a structured educational in-hospital program for 5 days. Infants and toddlers start with the pump treatment immediately.
- At the first outpatient visits (after 3–5 weeks): The family meets a pediatric endocrinologist, a dietitian, and a nurse educator, where the educational process is repeated in a half-day session.
- At CSII start: Technical and medical education is given on an outpatient basis. Pump providers organize technical education, a dietitian is teaching carbohydrate and protein counting, and certified nurse educators provide structured standardized CSII education and training (a standard set of slides and a brochure). Most of the eligible children start with the pump treatment six to nine months after the diagnosis.
- At CGM (sensor) start: A proper insertion of the sensor is discussed and practiced, along with correct calibration of the sensor and the use of different alarms (sensor-specific written material is provided). The family returns to the outpatient after six days for another sensor insertion and pump download – if they are afraid to insert the second sensor at home –, and/or after one month to see the first sensor download and discuss different problems that appeared.
- Education for teachers and other professional caregivers is given on individual or group basis. In the last 5 years, more than 300 teachers and other caregivers came annually to learn about diabetes in a one-day course, where they met all members of the team. A medical plan for each individual child is agreed, and preferably signed, during these educational sessions.
- Education for the first graders – children who enter the primary school are invited to a half-day educational session, where they learn about diabetes with the hospital teacher and a certified nurse educator. At the same time, parents are discussing how to make the school start as simple as possible: a pediatric endocrinologist, a certified nurse educator and a psychologist are present.
- A special educational opportunity is the summer camp, where 120 children aged from 6 to 15 years participate for two weeks to learn about diabetes and its practical management; young adult patients with T1D help the diabetes team in education and day-to-day care. Children have practical lessons on carbohydrates counting, they learn how to prepare some simple meals, repeat how to react in the case of low or high blood sugar, how to recognize DKA, they swim every day and engage in other sport activities. The first camp was organized in 1967, and ever since annually; all costs are covered by the national health insurance system.

- Yearly meetings for the families of children with diabetes have been organized since 1971. A special publication called 'Sladkorčki' ('Sweeties') is published covering various topics about good diabetes management along with patients' stories, reports and poems.
- Web site: The Society for Children with Metabolic Diseases, which works in close collaboration with the diabetes team at UCHL, has a web page since 2005 (http://www.sladkorcki.si), on which a lot of information about diabetes can be found. Information about different events is posted, as well as cooking recipes, useful links and telephone numbers (25, 26).

An important aspect of SCMCI's diabetes team activities is fostering ties between the staff, patients and their parents.

- A meeting of the multidisciplinary team with parents of children diagnosed with diabetes during the previous 6-month period is organized every 6–12 months to provide relief to parents recently faced with diabetes.
- Prior to the onset of the school year, the institute initiates the meeting between children with diabetes who are about to enter the first grade, together with their parents. The team and parents are sharing information.
- A series of meetings is held for patients who have difficulties coping with the diabetes of their children.
- SCMCI's diabetes team also organizes meetings with teachers and nurses from the children's schools in order to promote the understanding of the issues facing the child with diabetes at school.

6 IMPLEMENTATION OF DIABETES-RELATED TECHNOLOGIES

6.1 Patients Treated with CSII – Insulin Pump Therapy

Intensified insulin delivery usually consists of either multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII), and is based on self-monitoring of blood glucose (SMBG) and, recently, also on real-time continuous glucose monitoring (CGM).

The use of CSII is steadily increasing. At SCMCI, approximately 800 (50%) patients are treated with CSII, while, at UCHL, approximately 500 out of 650 (77%). In both centers, the multidisciplinary diabetes team recommends CSII therapy to eligible patients and/or their caregivers. The decision to initiate pump therapy is individualized and guided by the recommended indications for CSII therapy (27). If the patient/parents have a preference for CSII treatment, the issue is discussed with the diabetes team of the individual patient, and if there is no contraindication, the patient can start with CSII therapy (28, 29). A certified diabetes nurse educator and a dietitian conduct a 3-day structured outpatients' education, along with technical training delivered by the pump provider. During the first month of CSII therapy, patients are asked to check blood glucose levels at least 8 times/day: before meals, 2 hours after meals, at bedtime, and at 2–3 a.m. Thereafter, the frequency is reduced to 6 times/day (without a regular night measurement). Insulin basal rates and correction boluses are adjusted as required at each follow-up visit, scheduled every 2–3 months, or during phone contacts. Patients are instructed to change infusion sets every 3 days, or more frequently if necessary.

6.2 Patients Treated with Continuous Glucose Monitoring (CGM)

Extensive evidence indicates that near-normalization of blood glucose levels is seldom attainable in children and adolescents after the remission period (30–32).Therefore, the development of CGM (33) may help children and adolescents with T1D improve their metabolic control (34, 35) and reduce the time spent in hypoglycemia (32, 36), as well as help their families to cope with the stress of day-to-day diabetes management (37–39).

At SCMCI, approximately 160 patients (20%) are treated with CGMs, while, at UCHL, roughly 60 patients (10%). The decision to use CGM is made jointly by the child/parents and diabetes team. The CGM is used either with MDI or CSII.

The connection to the CGM is usually done in patients with: evidence of severe hypoglycemic episodes, hypoglycemic unawareness, nocturnal hypoglycemia, and wide glucose excursions with suboptimal glycemic control (HbA1c exceeding the target range). In Israel, sensors are reimbursed for the whole pediatric population; in Slovenia, sensors are completely reimbursed for children bellow the age of six, who represent the largest group of CGM users, but most of them continue to use the sensor also above this age (30).

Prior to connection to the CGM, a patient/family gets initial informations about CGM. A visit for CGM start-up, including instructions for insertion, site care, alarm setting, trend analysis, data analysis, etc. by a nurse practitioner, follows. After the period of 2–3 weeks, the data of the CGM is discussed with the physician in order to make the required changes in the diabetes management.

7 RESEARCH PROGRAM

Long-term metabolic outcome depends not only on excellence in, and dedication to, clinical care, but also on continuous clinical and basic research (12).

Both centres are intensively engaged in clinical research, ensuring their stay on the cutting edge of the latest innovations and advanced treatment modalities. Clinical

research includes diabetes-prevention trials (40), introduction of new drugs (41, 42) and new technologies (43, 44) for the treatment of diabetes, along with psychological investigations (45–47). Additionally, applied basic research conducted in both centres contributes to the knowledge on disease mechanisms (48–52). Both centres also participate in international data collection and benchmarking within the Centre of Reference SWEET project (53).

Diabetes Technology Centre dedicated to develop artificial pancreas, by closing the loop between CGM systems and pumps for CSII, was established at SCMCI. The Diabetes Technology team has developed a long-term clinical evaluation of the MD-Logic Artificial Pancreas System (MDLAP), which is based on the model that imitates the logic of diabetes care givers and allows individualized automatic glucose regulation based on CGM and CSII. The Diabetes Technology Institute is collaborating with prominent centres in the USA and Europe in the artificial pancreas project, and multicentre studies, in the effort to close the loop, are being carried out with diabetes centres in Slovenia and Germany. The MDLAP was evaluated in clinical trials performed in hospital (54), camp (55), and home (56, 57) settings. A routinely available artificial pancreas system will improve metabolic control and considerably lessen the burden of day-to-day diabetes management.

8 CONCLUSIONS

With the assistance of a dedicated, skilled and qualified multidisciplinary team, devoted parents and caregivers, and with the help of modern diabetes-related technology, young patients with diabetes can live a normal, active, successful and happy live. However, the dream and hope for a cure for diabetes remains vivid.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Ethical approval for this report was not required. The National Medical Ethics Committee approved the maintenance of the national T1D registry in Slovenia.

REFERENCES

1. Bratina NU, Tahirović H, Battelino T, Krzisnik C. Incidence of childhood-onset Type I diabetes in Slovenia and the Tuzia region (Bosnia and Herzegovina) in the period 1990-1998. Diabetologia 2001; 44(Suppl 3): B27-31.
2. Patterson CC, Gyürüs E, Rosenbauer J, Cinek O, Neu A. et al. Trends in childhood type 1 diabetes incidence in Europe during 1989-2008: evidence of non-uniformity over time in rates of increase. Diabetologia 2012; 55: 2142-7.
3. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G, EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 2009; 373: 2027-33.
4. Radosevic B, Bukara-Radujkovic G, Miljkovic V, Pejicic S, Bratina N, Battelino T. The incidence of type 1 diabetes in Republic of Srpska (Bosnia and Herzegovina) and Slovenia in the period 1998-2010. Pediatr Diabetes 2013; 14: 273-9.
5. Blumenfeld O, Dichtiar R, Shohat T, Israel IDDM Registry Study Group (IIRSG). Trends in the incidence of type 1 diabetes among Jews and Arabs in Israel. Pediatr Diabetes 2014; 15: 422-7.
6. Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith GJ, Dabelea D. Childhood growth and age at diagnosis with Type 1 diabetes in Colorado young people. Diabet Med 2009; 26: 961-7.
7. Ehehalt S, Dietz K, Willasch AM, Neu A, DIARY-Group Baden-Wuerttemberg. Prediction model for the incidence and prevalence of type 1 diabetes in childhood and adolescence: evidence for a cohort-dependent increase within the next two decades in Germany. Pediatr Diabetes 2012; 13: 15-20.
8. Krasevec A. Newly discovered diabetes in children and adolescent in Slovenia: review and analyses of epidemiological and clinical data: master degree thesis. Ljubljana: Visoka šola za zdravstvo, 2013: 1-33.
9. Laron Z, Galatzer A, Amir S, Gil R, Karp M, Mimouni M. A multidisciplinary, comprehensive, ambulatory treatment scheme for diabetes mellitus in children. Diabetes Care 1979; 2: 342-8.
10. Kržišnik C, Bratanič N, Brus L. The current treatment of children and adolescents with insulin dependent diabetes in Slovenia. Zdrav Vest 1990; 59: 67-9.
11. Matajc L. Diabetes mellitus infantilis et juvenilis . Sladkorčki 1979: 1-49.
12. DCCT Research Group. The effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: the diabetes control and complications trial. J Pediatr 1994; 125: 177-88.
13. Danne T, Bangstad HJ, Deeb L, Jarosz-Chobot P, Mungaie L, Saboo B. et al. Insulin treatment in children and adolescents with diabetes. Pediatr Diabetes 2014; 15(Suppl 20): 115-34.
14. Holl RW, Swift PG, Mortensen HB, Lynggaard H, Hougaard P, Aanstoot HJ. et al. Insulin injection regimens and metabolic control in an international survey of adolescents with type 1 diabetes over 3 years: results from the Hvidore study group. Eur J Pediatr 2003; 162: 22-9.
15. Pihoker C, Badaru A, Anderson A, Morgan T, Dolan L, Dabelea D. et al. Insulin regimens and clinical outcomes in a type 1 diabetes cohort: the search for diabetes in youth study. Diabetes Care 2013; 36: 27-33.
16. Shalitin S, Ben-Ari T, Yackobovitch-Gavan M, Tenenbaum A, Lebenthal Y, de Vries L. et al. Using the internet-based upload blood glucose monitoring and therapy management system in patients with type 1 diabetes. Acta Diabetol 2014; 51: 247-56.
17. Smart CE, Annan F, Bruno LP, Higgins LA, Acerini CL. Nutritional management in children and adolescents with diabetes. Pediatr Diabetes 2014; 15(Suppl 20): 135-53.
18. Hoey H, Hvidoere Study Group on Childhood Diabetes. Psychosocial factors are associated with metabolic control in adolescents: research from the Hvidoere Study Group on Childhood Diabetes. Pediatr Diabetes 2009; 10(Suppl 13): 9-14.

19. Shorer M, David R, Schoenberg-Taz M, Levavi-Lavi I, Phillip M, Meyerovitch J. Role of parenting style in achieving metabolic control in adolescents with type 1 diabetes. Diabetes Care 2011; 34: 1735-7.
20. Wigert H, Wikström E. Organizing person-centred care in paediatric diabetes: multidisciplinary teams, long-term relationships and adequate documentation. BMC Res Notes 2014; 7: 72.
21. Shalitin S, Gil M, Nimri R, de Vries L, Gavan MY, Phillip M. Predictors of glycaemic control in patients with Type 1 diabetes commencing continuous subcutaneous insulin infusion therapy. Diabet Med 2010; 27: 339-47.
22. Wolfsdorf JI, Allgrove J, Craig ME, Edge J, Glaser N, Jain V. et al. Diabetic ketoacidosis and hyperglycemic hypersmolar state. Pediatric Diabetes 2014; 15(Suppl 20): 154-79.
23. Ly TT, Maahs DM, Rewers A, Dunger D, Oduwole A, Jones TW. Assessment and management of Hypoglycemia. Pediatric Diabetes 2014; 15(Suppl 20): 180-92.
24. Dovc K, Telic SS, Lusa L, Bratanic N, Zerjav-Tansek M, Kotnik P. et al. Improved metabolic control in pediatric patients with type 1 diabetes: a nationwide prospective 12-year time trends analysis. Diabetes Technol Ther 2014; 16: 33-40.
25. Swift PG. Diabetes education in children and adolescents. Pediatr Diabetes 2009; 10(Suppl 12): 51-7.
26. Bratina N, Battelino T. Insulin pumps and continuous glucose monitoring (CGM) in preschool and school-age children: how schools can integrate technology. Pediatr Endocrinol Rev 2010; 7(Suppl 3): 417-21.
27. Shalitin S, Lahav-Ritte T, Lebenthal Y, Devries L, Phillip M. Does the timing of insulin pump therapy initiation after type 1 diabetes onset have an impact on glycemic control? Diabetes Technol Ther 2012; 14: 389-97.
28. Tamborlane WV, Fredrickson LP, Ahern JH. Insulin pump therapy in childhood diabetes mellitus: guidelines for use. Treat Endocrinol 2003; 2: 11-21.
29. de Vries L, Grushka Y, Lebenthal Y, Shalitin S, Phillip M. Factors associated with increased risk of insulin pump discontinuation in pediatric patients with type 1 diabetes. Pediatr Diabetes 2011; 12: 506-12.
30. Phillip M, Danne T, Shalitin S, Buckingham B, Laffel L, Battelino T. et al. Use of continuous glucose monitoring in children and adolescents. Pediatr Diabetes 2012; 13: 215-28.
31. Nørgaard K, Scaramuzza A, Bratina N, Lalić NM, Jarosz-Chobot P, Kocsis G. et al. Routine sensor-augmented pump therapy in type 1 diabetes: the INTERPRET study. Diabetes Technol Ther 2013; 15: 273-80.
32. Shalitin S, Phillip M. Hypoglycemia in type 1 diabetes: a still unresolved problem in the era of insulin analogs and pump therapy. Diabetes Care 2008; 31(Suppl 2): S121-4.
33. Battelino T, Bolinder J. Clinical use of real-time continuous glucose monitoring. Curr Diabetes Rev 2008; 4: 218-22.
34. Battelino T, Conget I, Olsen B, Schütz-Fuhrmann I, Hommel E, Hoogma R. et al. The use and efficacy of continuous glucose monitoring in type 1 diabetes treated with insulin pump therapy: a randomised controlled trial. Diabetologia 2012; 55: 3155-62.
35. Battelino T, Phillip M. Real-time continuous glucose monitoring in randomized control trials. Pediatr Endocrinol Rev 2010; 7(Suppl 3): 401-4.
36. Battelino T, Phillip M, Bratina N, Nimri R, Oskarsson P, Bolinder J. Effect of continuous glucose monitoring on hypoglycemia in type 1 diabetes. Diabetes Care 2011; 34: 795-800.
37. Hommel E, Olsen B, Battelino T, Conget I, Schütz-Fuhrmann I, Hoogma R. et al. Impact of continuous glucose monitoring on quality of life, treatment satisfaction, and use of medical care resources: analyses from the SWITCH study. Acta Diabetol 2014; 51: 845-51.
38. Bode BW, Battelino T. Continuous glucose monitoring in 2012. Diabetes Technol Ther 2013; 15(Suppl 1): S13-23.
39. Battelino T, Bode BW. Continuous glucose monitoring in 2013. Diabetes Technol Ther 2014; 16(Suppl 1): S11-6.
40. Ludvigsson J, Krisky D, Casas R, Battelino T, Castaño L, Greening J. et al. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. N Engl J Med 2012; 366: 433-42.
41. Nimri R, Lebenthal Y, Shalitin S, Benzaquen H, Demol S, Phillip M. Metabolic control by insulin detemir in basal-bolus therapy: treat-to-target study in children and adolescents with type 1 diabetes. Pediatr Diabetes 2013; 14: 196-202.
42. Klein DJ, Battelino T, Chatterjee DJ, Jacobsen LV, Hale PM, Arslanian S. et al. Liraglutide's safety, tolerability, pharmacokinetics, and pharmacodynamics in pediatric type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Diabetes Technol Ther 2014; 16: 679-87.
43. Lebenthal Y, Lazar L, Benzaquen H, Shalitin S, Phillip M. Patient perceptions of using the OmniPod system compared with conventional insulin pumps in young adults with type 1 diabetes. Diabetes Technol Ther 2012; 14: 411-7.
44. Buse JB, Kudva YC, Battelino T, Davis SN, Shin J, Welsh JB. Effects of sensor-augmented pump therapy on glycemic variability in well-controlled type 1 diabetes in the STAR 3 study. Diabetes Technol Ther 2012; 14: 644-7.
45. Adolfsson P, Veijola R, Huot C, Hansen HD, Lademann JB, Phillip M. Safety and patient perception of an insulin pen with simple memory function for children and adolescents with type 1 diabetes--the REMIND study. Curr Med Res Opin 2012; 28: 1455-63.
46. Liberman A, Buckingham B, Phillip M. Diabetes technology and the human factor. Int J Clin Pract 2011; (Suppl 170): 83-90.
47. Radobuljac MD, Bratina NU, Battelino T, Tomori M. Lifetime prevalence of suicidal and self-injurious behaviors in a representative cohort of Slovenian adolescents with type 1 diabetes. Pediatr Diabetes 2009; 10: 424-31.
48. Hovnik T, Dolzan V, Bratina NU, Podkrajsek KT, Battelino T. Genetic polymorphisms in genes encoding antioxidant enzymes are associated with diabetic retinopathy in type 1 diabetes. Diabetes Care 2009; 32: 2258-62.
49. Battelino N, Sebestjen M, Keber I, Blagus R, Hovnik T, Bratina N. et al. Endothelial nitric oxide synthase T(-786)C polymorphism in children and adolescents with type 1 diabetes and impaired endothelium-dependent dilatation. Horm Res Paediatr 2011; 76: 248-53.
50. Gozlan Y, Tenenbaum A, Shalitin S, Lebenthal Y, Oron T, Cohen O. et al. The glucokinase mutation p.T206P is common among MODY patients of Jewish Ashkenazi descent. Pediatr Diabetes 2012; 13: e14-21.
51. Bratanic N, Smigoc Schweiger D, Mendez A, Bratina N, Battelino T, Vidan-Jeras B. An influence of HLA-A, B, DR, DQ, and MICA on the occurrence of Celiac disease in patients with type 1 diabetes. Tissue Antigens 2010; 76: 208-15.
52. Smigoc Schweiger D, Mendez A, Kunilo Jamnik S, Bratanic N, Bratina N, Battelino T. et al. Genetic risk for co-occurrence of type 1 diabetes and celiac disease is modified by HLA-C and killer immunoglobulin-like receptors. Tissue Antigens 2014; 84: 471-8.
53. Danne T, Lion S, Madaczy L, Veeze H, Raposo F, Rurik I. et al. Criteria for Centers of Reference for pediatric diabetes: an European perspective. Pediatr Diabetes 2012; 13(Suppl 16): 62-75.
54. Nimri R, Danne T, Kordonouri O, Atlas E, Bratina N, Biester T. et al. The "Glucositter" overnight automated closed loop system for type 1 diabetes: a randomized crossover trial. Pediatric Diabetes 2013; 14: 159-67.
55. Phillip M, Battelino T, Atlas E, Kordonouri O, Bratina N, Miller S. et al. Nocturnal glucose control with an artificial pancreas at a diabetes camp. N Engl J Med 2013; 368: 824-33.
56. Nimri R, Muller I, Atlas E, Miller S, Fogel A, Bratina N. et al. MD-Logic overnight control for 6 weeks of home use in patients with type 1 diabetes: randomized crossover trial. Diabetes Care 2014; 37: 3025-32.
57. Nimri R, Muller I, Atlas E, Miller S, Kordonouri O, Bratina N. et al. Night glucose control with MD-Logic artificial pancreas in home setting: a single blind, randomized crossover trial-interim analysis. Pediatr Diabetes 2014; 15: 91-9.

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Podatke o avtorju in soavtorjih vnesite kar se da natančno in popolno. Naveden naj bo odgovorni avtor (s polnim naslovom, telefonsko številko in elektronskim naslovom), ki bo skrbel za komunikacijo z uredništvom in ostalimi avtorji.

Jezik prispevka je angleščina. Objavljamo izvirne znanstvene članke, pregledne znanstvene članke, uvodnike, pisma uredništvu in recenzije knjig. Pri izvirnih in preglednih znanstvenih prispevkih morajo biti naslov, izvleček in ključne besede prevedeni tudi v slovenščino.

Naslov, ključne besede in izvleček se oddajajo dvojezično v angleščini in v slovenščini v strukturirana polja. Posebno polje za zapis v drugem jeziku obstaja le za izvleček, preostale podatke vnesite v obeh jezikih v ustrezno isto polje. Prvi izvleček je vselej v angleškem jeziku (do 250 besed - sistem vam besede sproti šteje), drugi pa v slovenskem jeziku (razširjen izvleček – do 400 besed).

Po vnosu strukturiranih podatkov oddajte še priponko – rokopis (od 1 Uvod naprej), ki ne sme zajemati podatkov, ki ste jih vnesli že pred tem v strukturirana polja. Ime datoteke ne sme vključevati avtorjevih osebnih podatkov, prav tako ne imen ustanov, vključenih v pripravo rokopisa. Grafično in slikovno gradivo je kot cel rokopis v angleškem jeziku. Vključite ga v besedilo na mesto, kamor le-to sodi in ga opremite z naslovom. Oddate torej le en sam dokument. V Wordu uporabite možnost Postavitev strani / Številke vrstic (tako bo na robu vsake vrstice dokumenta dodana številke vrstice).

Pri oddaji sledite napotkom, ki vam jih ponuja sistem, pomagate pa si lahko tudi z 'Editorial Manager's Tutorial for Autors'.

Sistem najbolje deluje, če uporabljate zadnjo različico Acrobata.

Če pri oddajanju rokopisa naletite na nepremostljive težave, se za pomoč obrnite na naslov uredništva: zdrav. var@nijz.si.

V nadaljevanju podajamo še nekaj natančnejših napotkov.

ROKOPIS

Besedila naj bodo napisana z urejevalnikom Word for Windows. Robovi naj bodo široki najmanj 25 mm. Znanstveni članki naj imajo naslednja poglavja: uvod, metode, rezultati, razpravljanje in zaključek. Ostale oblike člankov in pregledni članki so lahko zasnovani drugače, vendar naj bo razdelitev na poglavja in podpoglavja jasno razvidna iz velikosti črk naslovov. Poglavja in podpoglavja naj bodo številčena dekadno po standardu SIST ISO 2145 in SIST ISO 690 (npr. 1, 1.1, 1.1.1 itd.).

Priporočljiva dolžina prispevka je za uvodnik od 250 do 700 besed; za pismo uredništvu do 1500 besed, za recenzije knjig do 500 besed; za znanstveni članek od 2000 do 4500 besed z razpredelnicami in literaturo vred.

Naslov in avtorstvo

Naslov v angleškem in slovenskem jeziku naj bo kratek in natančen, opisen in ne trdilen (povedi v naslovih niso dopustne). Navedena naj bodo imena piscev z natančnimi akademskimi in strokovnimi naslovi ter popoln naslov ustanove, inštituta ali klinike, kjer je delo nastalo. Avtorji morajo izpolnjevati pogoje za avtorstvo. Prispevati morajo k zasnovi in oblikovanju oz. analizi in interpretaciji podatkov, članek morajo intelektualno zasnovati oz. ga kritično pregledati, strinjati se morajo s končno različico članka. Samo zbiranje podatkov ne zadostuje za avtorstvo.

Izvleček in ključne besede

Izvleček v angleškem in slovenskem jeziku naj bo pri znanstvenem članku strukturiran in naj ne bo daljši od 250 besed v angleščini in 400 besed v slovenščini, izvlečki ostalih člankov so lahko nestrukturirani in naj ne presegajo 150 besed. Izvleček naj vsebinsko povzema in ne le našteva bistvene vsebine dela. Izogibajte se kraticam in okrajšavam. Napisan naj bo v 3. osebi.

Izvleček znanstvenega članka naj povzema namen dela, osnovne metode, glavne izsledke in njihovo statistično pomembnost ter poglavitne sklepe (struktura IMRC – Introduction, Methods, Results, Conclusions).

Navedenih naj bo 3-10 ključnih besed, ki nam bodo v pomoč pri indeksiranju. Uporabljajte izraze iz MeSH - Medical Subject Headings, ki jih navaja Index Medicus.

Kategorija prispevka

Kategorijo prispevka predlaga z vnosov v ustrezno polje avtor sam, končno odločitev pa sprejme urednik na osnovi predlogov recenzentov. Objavljamo izvirne znanstvene članke, pregledne znanstvene članke, uvodnike, pisma uredništvu in recenzije knjig.

Reference

Vsako navajanje trditev ali dognanj drugih morate podpreti z referenco. Reference naj bodo v besedilu navedene po vrstnem redu, tako kot se pojavljajo. Referenca naj bo navedena na koncu citirane trditve. Reference v besedilu, slikah in tabelah navedite v oklepaju z arabskimi številkami. Reference, ki se pojavljajo samo v tabelah ali slikah, naj bodo oštevilčene tako, kot se bodo pojavile v besedilu. Kot referenc ne navajajte izvlečkov in osebnih dogovorov (slednje je lahko navedeno v besedilu). Seznam citirane literature dodajte na koncu prispevka. Literaturo citirajte po priloženih navodilih, ki so v skladu s tistimi, ki jih uporablja ameriška National Library of Medicine v Index Medicus. Uporabljajte numerično citiranje. Imena revij krajšajte tako, kot določa Index Medicus (popoln seznam na naslovu URL: http://www.nlm.nih.gov).

Navedite imena vseh avtorjev, v primeru, da je avtorjev šest ali več, navedite prvih šest avtorjev in dodajte et al.

Primeri za citiranje literature:

primer za knjigo:

Premik M. Uvod v epidemiologijo. Ljubljana: Medicinska fakulteta, 1998.

Mahy BWJ. A dictionary of virology. 2nd ed. San Diego: Academic Press, 1997.

primer za poglavje iz knjige:

Urlep F. Razvoj osnovnega zdravstva v Sloveniji zadnjih 130 let. In: Švab I, Rotar-Pavlič D, editors. Družinska medicina. Ljubljana: Združenje zdravnikov družinske medicine, 2002: 18-27.

Goldberg BW. Population-based health care. In: Taylor RB, editor. Family medicine. 5th ed. New York: Springer, 1999: 32-6.

primer za članek iz revije:

Barry HC, Hickner J, Ebell MH, Ettenhofer T. A randomized controlled trial of telephone management of suspected urinary tract infections in women. J Fam Pract 2001; 50: 589-94.

primer za članek iz revije, kjer avtor ni znan:

Anon. Early drinking said to increase alcoholism risk. Globe 1998; 2: 8-10.

primer za članek iz revije, kjer je avtor organizacija:

Women's Concerns Study Group. Raising concerns about family history of breast cancer in primary care consultations: prospective, population based study. BMJ 2001; 322: 27-8.

primer za članek iz suplementa revije z volumnom in s številko:

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102(Suppl 2): 275-82.

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996; 23(Suppl 2): 89-97.

primer za članek iz zbornika referatov:

Sugden K. et al. Suicides and non-suicidal deaths in Slovenia: molecular genetic investigation. In: 9th European Symposium on Suicide and Suicidal Behaviour. Warwick: University of Oxford, 2002: 76.

primer za magistrske naloge, doktorske disertacije in Prešernove nagrade:

Bartol T. Vrednotenje biotehniških informacij o rastlinskih drogah v dostopnih virih v Sloveniji: doktorska disertacija. Ljubljana: Biotehniška fakulteta, 1998.

primer za elektronske vire:

Mendels P. Textbook publishers extend lessons online. Pridobljeno 23.9.1999 s spletne strani: http://www.nytimes.com/library/tech/99/09.

Tabele

Tabele v angleškem jeziku naj bodo v besedilu prispevka na mestu, kamor sodijo. Tabele naj sestavljajo vrstice in stolpci, ki se sekajo v poljih. Tabele oštevilčite po vrstnem redu, vsaka tabela mora biti citirana v besedilu. Tabela naj bo opremljena s kratkim angleškim naslovom. V legendi naj bodo pojasnjene vse kratice, okrajšave in nestandardne enote, ki se pojavljajo v tabeli.

Slike

Morajo biti profesionalno izdelane. Pri pripravi slik upoštevajte, da gre za črno-beli tisk. Slikovno gradivo naj bo pripravljeno:

- črno-belo (ne v barvah!);
- brez polnih površin, namesto tega je treba izbrati šrafure (če gre za stolpce, t. i. tortice ali zemljevide);
- v linijskih grafih naj se posamezne linije prav tako ločijo med samo z različnim črtkanjem ali različnim označevanjem (s trikotniki, z zvezdicami...), ne pa z barvo;
- v grafih naj bo ozadje belo (tj. brez ozadja).

Črke, številke ali simboli na sliki morajo biti jasni, enotni in dovolj veliki, da so berljivi tudi na pomanjšani sliki. Ročno ali na pisalni stroj izpisano besedilo v sliki je nedopustno.

Vsaka slika mora biti navedena v besedilu. Besedilo k sliki naj vsebuje naslov slike in potrebno razlago vsebine. Slika naj bo razumljiva tudi brez branja ostalega besedila. Pojasniti morate vse okrajšave v sliki. Uporaba okrajšav v besedilu k sliki je nedopustna. Besedila k slikam naj bodo napisana na mestu pojavljanja v besedilu.

Fotografijam, na katerih se lahko prepozna identiteta bolnika, priložite pisno dovoljenje bolnika.

Merske enote

Naj bodo v skladu z mednarodnim sistemom enot (SI).

Kratice in okrajšave

Kraticam in okrajšavam se izogibajte, izjema so mednarodno veljavne oznake merskih enot. V naslovih in izvlečku naj ne bo kratic. Na mestu, kjer se kratica prvič pojavi v besedilu, naj bo izraz, ki ga nadomešča, polno izpisan, v nadaljnjem besedilu uporabljano kratico navajajte v oklepaju.

Uredniško delo

Prispelo gradivo z javnozdravstveno tematiko posreduje uredništvo po tehnični brezhibnosti v strokovno recenzijo trem mednarodno priznanim strokovnjakom. Recenzijski postopek je dvojno slep. Po končanem uredniškem delu vrnemo prispevek korespondenčnemu avtorju, da

popravke odobri in upošteva. Popravljen čistopis vrne v uredništvo po spletni aplikaciji Editorial Manager. Sledi jezikovna lektura, katere stroške krije izdajatelj. Med redakcijskim postopkom je zagotovljena tajnost vsebine prispevka. Avtor dobi v pogled tudi prve, t. i. krtačne odtise, vendar na tej stopnji upoštevamo samo še popravke tiskovnih napak. Krtačne odtise je treba vrniti v treh dneh, sicer menimo, da avtor nima pripomb.

V uredništvo se trudimo za čim hitrejši uredniški postopek. Avtorji se morajo držati rokov, ki jih dobijo v dopisih, sicer se lahko zgodi, da bo članek odstranjen iz postopka. Morebitne pritožbe avtorjev obravnava uredniški odbor revije.

Za objavo prispevka prenese avtor avtorske pravice na Nacionalni inštitut za javno zdravje kot izdajatelja revije (podpiše Izjavo o avtorstvu). Kršenje avtorskih in drugih sorodnih pravic je kaznivo.

Prispevkov ne honoriramo. Avtor dobi le izvod revije, v kateri je objavljen njegov članek.