UNIVERSITY OF NIŠ

ISSN 0354-2017 (Print)
ISSN 2406-0526 (Online)
COBISS.SR-ID 32415756

FACTA UNIVERSITATIS

Series
MEDICINE AND BIOLOGY
Vol. 20, N° 2, 2018

Hypertension

GH Use

GHD

Asthma

Smoking

Professional Stress

Metabolic Syndrome

Hypoplastic Maxilla

Statin Therapy

ISS

SGA

Short Stature

Crouzon syndrome

Caesarian Delivery

Craniosynostosis

Camouflage Therapy

Scientific Journal **FACTA UNIVERSITATIS**
UNIVERSITY OF NIŠ
Univerzitetski trg 2, 18000 Niš, Serbia
Phone: +381 18 257 095 Telefax: +381 18 257 950
e-mail: facta@junis.ni.ac.rs http://casopisi.junis.ni.ac.rs/

Scientific Journal FACTA UNIVERSITATIS publishes original high scientific level works in the fields classified accordingly into the following periodical and independent series:

Architecture and Civil Engineering
Automatic Control and Robotics
Economics and Organization
Electronics and Energetics
Law and Politics
Linguistics and Literature
Mathematics and Informatics
Mechanical Engineering
Medicine and Biology
Philosophy, Sociology, Psychology and History
Physical Education and Sport
Physics, Chemistry and Technology
Teaching, Learning and Teacher Education
Visual Arts and Music
Working and Living Environmental Protection

SERIES **MEDICINE AND BIOLOGY**

Publication frequency – one volume, two issues per year.
Published by the University of Niš, Serbia

Financial support: Ministry of Education, Science and Technological Development of the Republic of Serbia
Printed by "UNIGRAF-X-COPY" – Niš, Serbia

ISSN 0354-2017 (Print)
ISSN 2406-0526 (Online)
COBISS.SR-ID 32415756

FACTA UNIVERSITATIS

Series **MEDICINE AND BIOLOGY**
Vol. 20, No 2, 2018

UNIVERSITY OF NIŠ

AUTHOR GUIDELINES

Facta Universitatis, Series: Medicine and Biology (FU Med Biol) is the official publication of the University of Niš, Serbia. It publishes original scientific papers relating to medicine, biology and pharmacy (editorials, original experimental or clinical works, review articles, case reports, technical innovations, letters to the editor, book reviews, reports and presentations from (inter)national congresses and symposiums which have not been previously submitted for publication elsewhere). All manuscripts are assumed to be submitted exclusively unless otherwise stated, and must not have been published previously except in the form of an abstract or as parts of a published lecture and/or academic thesis.

Original papers may not exceed 10 printed pages, including tables and/or figures.

Reviews may not exceed 16 printed pages, including tables and/or figures.

Case reports/technical innovations/book reviews/presentations from congresses may not exceed four printed pages, including tables and/or figures. The figures should not occupy more than two-thirds of one printed page. The editor or co-editor reserves the right to make the decision if a manuscript qualifies to be corresponding type of the article.

Manuscripts are normally subject to the assessments of reviewers and the editor. Manuscripts that have been extensively modified in the editorial process will be returned to the author for retyping. All submitted articles will be reviewed by at least 2 reviewers, and when appropriate, by a statistical reviewer. Authors will be notified of acceptance, rejection, or need for revision within 4–5 weeks of submission.

Paper submitted for publication may be written exclusively in English. Authors for whom English is a second language should have the manuscript professionally edited before its submission.

Each author that participated sufficiently in the work has public responsibility for the content. This participation must include: a) Conception of design, or analysis and interpretation of data, or both, and b) Drafting the article of revising it for critically important intellectual content.

Text

The manuscript should contain following subdivisions:

(1) Title page (with short running page heading, title, authors names and affiliations, corresponding author with full address, e-mail, phone and fax).

Number all manuscript pages consecutively beginning with the title page. Do not hyphenate words at the end of the lines. Do not begin sentences with abbreviations.

(2) Abstract

The main abstract should be maximum 250 words, double-spaced and on a separate page. It should briefly describe respectively the problems being addressed in the study, how the study was performed, the salient results (without abbreviations) and what the authors conclude from the results.

2.1. Key words

Immediately after the Abstract, up to six topical key words for subject indexing must be supplied.

(3) Main body (Introduction, Material and methods, Results, Discussion, Conclusion)

The headings (Introductions; Material and Methods, etc.) should be placed on separate lines.

Abbreviations should be defined at first mention and used consistently thereafter.

The Introduction should give the pertinent background to the study and should explain why the work was done. References should be numbered in the order in which they appear (1, 2 or 1–3, etc.)

Each statistical method and/or other investigation should be described in the Material and Method section. All scientific measurements must be given in SI units.

The Results should present the findings of the research. They must be free of discussion. Results should be written in the past tense. Spell out the word Figure in the text except when it appears in parentheses: Figure 3 (Figs. 2–5). Always spell out numbers when they stand as the first word in a sentence: abbreviated units cannot follow such numbers. Numbers indicating time, mass, and measurements are to be in Arabic numerals when followed by abbreviations (e.g. 1 mm; 3 ml, etc.). All numbers should be given as numerals.

The Discussion should cover, but not simply repeat the new findings and should present the author's results in the broader context of other work on the subject interpreting them with a minimum of speculation.

(4) Acknowledgements

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

(5) References

The references should be limited to those relating directly to the content of the manuscript. Authors are responsible for ensuring that the information in each reference is complete and accurate. All references should be numbered in the order in which they appear in the text. Do not use footnotes or endnotes as a substitute for a reference list. At the end of the article the full list of references should give the name and initials of all authors unless there are more than six, when only the first three should be given followed by "et al". The authors' names should be followed by the title of the article, the title of the Journal abbreviated according to the style of Index Medicus, the year of publication, the volume number and page numbers. In case that more than one paper by the same author(s) published in the same year is cited, the letters a, b, c, etc., should follow the year, e.g. Bergmann (1970a) ― in the reference list.

Examples for journals and other sources are as follow:

Journals:

1. Kuroda S, Ishikawa T, Houkin K, Nanba R, Hokari M, Iwasaki Y. Incidence and clinical features of disease progression in adult Moyamoya disease. Stroke 2005; 36:2148–2153.
2. Papantchev V, Hristov S, Todorova D, et al. Some variations of the circle of Willis, important for cerebral protection in aortic surgery — a study in Eastern Europeans. Eur J Cardiothorac Surg 2007; 31:982–998.
3. Jovanović S, Gajić I, Mandić B, Mandić J, Radivojević V. Oral lesions in patients with psychiatric disorders. Srp Arh Celok Lek 2010; 138:564–569. (Serbian)
4. Valença MM, Martins C, Andrade-Valença LPA. Trigeminal neuralgia associated with persistent primitive trigeminal artery. Migrâneas cefaléias (Brasil) 2008; 11:30–32.
5. Belenkaya RM. Structural variants of the brain base arteries. Vopr neirokhir 1974; 5:23–29. (Russian)

Abstract:

6. Tontisirin N, Muangman SL, Suz P, et al. Early childhood gender in anterior and posterior cerebral blood flow velocity and autoregulation. In Abstract of Pediatrics 2007. (doi:10.1542/peds. 2006-2110; published online February 5).

Books:

7. Patten MB. Human embryology, 3rd edn. McGraw-Hill: New York, 1968.
8. Marinković S, Milisavljević M, Antunović V. Arterije mozga i kičmene moždine—Anatomske i kliničke karakteristike. Bit inžerenjering: Beograd, 2001. (Serbian)

Chapters:

9. Lie TA. Congenital malformations of the carotid and vertebral arterial systems, including the persistent anastomoses. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology, vol. 12. North Holland: Amsterdam, 1972; pp 289–339.

Unpublished data:

10. Reed ML. *Si-SiO_2 interface trap anneal kinetics*, PhD thesis. Stanford University: Stanford, 1987.

Online document:

11. Apostolides PJ, Lawton MT, David CA, Spetzler RF. Clinical images: persistent primitive trigeminal artery with and without aneurysm. Barrow Quarterly 1997; 13(4). http://www.thebarrow.org/Education_And_Resources/Barrow_Quarterly/204843
12. Cerebrovascular embryology, in: power point; 2000. http://brainavm.oci.utoronto.ca/staff/Wallace/2000_curriculum/index.html

(6) Tables

(7) Figure legends

Table and figure legends should be included within the text file and contain sufficient data to be understood without reference to the text. Each should begin with a short title for the figure. All symbols and abbreviations should be explained within the legend. If the magnification is quoted in a sentence, it should appear in parentheses, e.g. (x400); at the end of legend it should appear, e.g., "... fibrils. X20 000".Tables must be typed on separate pages. All tables should be simple without duplicating information given in the text. Numerical results should be expressed as means with the relevant standard errors and/or statistically significant differences, quoting probability levels (p-value).

Illustrations

All figures should be cited in the paper in a consecutive order and submitted in separated files. All figures, photographs, line drawings and graphs, should be prepared in electronic form and converted in TIFF or JPG (max quality) file types, in 300 dpi resolution, for superior reproduction. Figures, line drawings and graphs prepared using elements of MS Drawing or MS Graph must be converted in form of pictures and unchangeable. All illustrations should be planned in advance so as to allow reduction to 8 cm in column width or 16.75 cm in page width. Please review all illustrations to ensure that they are readable.

The manuscripts in above form should be submitted to the Editor-in Chief or Co-editor of the journal in electronic form prepared by MS Word for Windows (Microsoft Word 1997–2003, *.doc or higher Word, *.docx) ― font Times New Roman (12-point). The manuscript should be typed double-spaced on one side only of A4 paper. The manuscript in electronic form should be submitted through the submission portal: http://casopisi.junis.ni.ac.rs/index.php/FUMedBiol.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. i–i

Editorial

Dear Readers,

Facta Universitatis Series Medicine and Biology is attracting more and more readers and authors. Number of submissions for Facta is growing, as well as the quality of manuscripts. Editorial Team is especially proud of managing to attract some key opinion leaders and distinguished scientists to write for Facta in the period of past three years. This resulted in plenty of outstanding publications for whom we have great expectation regarding citations and improving future impact of Facta.

This time of year gives me also the opportunity to wish you Happy New Year for your personal and professional life.

Editor-in-Chief

Ljiljana Šaranac

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 35–39

UDC 616.43-008.6-053.2:615.357
https://doi.org/10.22190/FUMB190119001S

Opinion Article

THE UNBEARABLE LIGHTNESS OF PRESCRIBING GROWTH HORMONE

Ljiljana Šaranac

Faculty of Medicine, University of Niš, Niš, Serbia

Abstract. *Short stature is the most visible and prominent physical characteristic and one of the commonest reason for referral to pediatric endocrinologist. It is assumed as disabling condition with psychosocial consequences that seeks treatment. Behind short stature severe pathology could be hidden, although not necessarily of endocrine origin. Even in the new millennium, many diagnostic pitfalls and dilemmas persist in confirmation of growth hormone deficiency (GHD), the first indication that fits in endocrine paradigm: to replace the missing hormone. In 1985 FDA approved recombinant human GH (hGH) as the treatment of pediatric patients who have growth failure due to inadequate secretion of endogenous GH. Availability of hGH in unlimited amounts enhanced the number of indications of GH use. Pediatric endocrinologist started to promote and to apply use of hGH for height in short, but otherwise healthy children, with hormonal normalcy. Reasonable criticism of such praxis arises in the light of recent safety alerts. The unbearable lightness of prescribing growth hormone to every apparently or really short child is reaching epidemic progress in Serbia. When authorities in this low income country (estimated as developing), approved hGH for use in SGA (small for gestational age) children, use and misuse of hGH exploded. Socially acceptable height, the term applied by some endocrinologists actually means that society does not accept short people. The pharmaceutical companies go even further offering, besides growth acceleration, better and brighter future for potential patients, in whom the self-confidence is measured by centimeters of height. However, benefits of such treatment on quality of life were never confirmed. Children with severe growth failure and documented GHD should be treated undoubtedly, but use of hGH for height, so called cosmetic endocrinology, needs critical appraisal. Children and their parents should be informed about height prediction and long-term consequences.*

Key words: *Growth Hormone Use, GHD, SGA, ISS, Short Stature, Cosmetic Endocrinology.*

"Even in the most wary mariner may easily lose his way as he seeks to steer his bark amid the glandular temptations whose siren voices have proved the downfall of many who have gone before" wrote Boyd in 1943, according to Bailey [1]

Introduction

Growth failure due to growth hormone deficiency (GHD) is the easiest endocrine disorder to treat and the hardest to diagnose [2]. Short stature is the most visible and prominent physical characteristic. It is assumed as disabling condition with psychosocial consequences that seeks treatment. However, benefits of such treatment on quality of life were never confirmed. Behind short stature severe pathology could be hidden, although not necessarily of endocrine origin. Neglecting growth monitoring in primary and secondary health care centers results in delayed diagnosis of severe diseases or plenty of inappropriate referrals. A high frequency of diagnostic delays is reported for so-called classical target conditions, such as Turner syndrome, GHD, Crohn's disease, celiac disease and cystic fibrosis. On the other hand, inadequate growth monitoring can result in unnecessary diagnostic cumbersome investigation [3].

It was hoped that the discovery by Evans and Long in 1921 of a mammalian pituitary gland extract capable of producing growth in laboratory animals, would solve the problem of short stature in children. However, it was not until 1956, when growth hormone from human pituitary glands was extracted by Raben. In 1957, the effective treatment was available for the small percentage of short children with growth hormone deficiency. GH substitution therapy in hypopituitary man, published in NEJM by Raben in 1962 was an example of the first personalized therapy use [4]. In late 1984, a 20-year-old man who was previously treated with cadaveric pitGH died from Creutzfeldt-Jakob disease, which led to abrupt withdrawal of pitGH in United States. In 1985 FDA approved recombinant human GH (hGH) as the treatment of pediatric patients who have growth failure due to inadequate secretion of endogenous GH, and shortly after hGH was approved in Europe. Availability of hGH in unlimited amounts since 1985, enhanced the number of indications of its use (Table 1).

Correspondence to: Ljiljana Šaranac, M.D., Ph.D.
Faculty of Medicine, 81 Zoran Đinđić Blvd., 18000 Niš, Serbia
Phone: +381 62 82 42 161
E-mail: endoljilja@yahoo.com
Received January 19th, 2019

Table 1 Approved Indications for hGH use in EU, Serbia and USA

European Union and Serbia	United States of America
▪ GHD in children and adults	▪ GHD* in children and adults
▪ Turner Syndrome	▪ Turner Syndrome
▪ Small for Gestational Age	▪ Small for Gestational Age
▪ Prader-Willi Syndrome	▪ Prader-Willi Syndrome
▪ Chronic Renal Failure	▪ Chronic Renal Failure
	▪ Idiopathic Short Stature (ISS)
	▪ SHOX deficiency
	▪ Noonan Syndrome
	▪ Short Bowel Syndrome
	▪ AIDS wasting in Adults

* GHD – Growth Hormone Deficiency

For prescribers the availability of abundant amounts of recombinant human growth hormone led to introduction of hGH for height treatment, and not only for substitution of missing hormone or restoring physiological hormonal normalcy. The diagnosis of GHD was widen and more inclusive by arbitrary raising cutoff diagnostic values in provocative testing. The old diagnostic criterion, commonly used in the past for GHD in children was peak level during stimulation less than 3 ng/mL (very restrictive and limited to severe or persistent and true GHD, also called „certain" or „definitive GHD"). Paradoxically, as GH assays grew more specific, criterion for failing a GH stimulation test grew even more generously. Cutoffs rose from 3 to 7 ng/mL to the current 10 ng/mL level [5]. MRI findings are more cosistent with clinical characteristics of GHD and more predictive of response to hGH therapy than are peak GH responses in provocative testing. Structural abnormalities of the hypothalamic-pituitary region have been described on MRI in up to 90% of patients with multiple pituitary hormone deficiency (MPHD), suggesting that brain MRI may represent the first-line investigation for diagnosing GHD, especially in infancy and early childhood [5,6]. In 2003, the FDA expanded hGH use to the treatment of ISS (Idiopathic short stature), also called non-GH-deficient short stature, defined by height standard deviation score (SDS) <–2.25 (<1.2nd percentile). The height cutoff of –2.25 SD (1.2nd percentile) corresponds in adults to 160 cm for men and 150 cm for women [7–9]. The concept of of ISS is controversial, artificial, arbitary and heterogeneous, including normal and patients with pathology, but having one thing in common: our ignorance to obtain an etiopathogenic diagnosis [5, 8].

Short stature is one of the commonest reasons for referral to most pediatric endocrine clinics. The unbearable lightness of prescribing growth hormone to every apparently or really short child is reaching epidemic progress in Serbia. When authorities in this low income country (estimated as developing), approved hGH for use in SGA (small for gestational age) children, use and misuse of GH exploded. Without any serious estimation of pro and contra reasons, many times under pressure of parents, children get GH. Even children with failure to thrive because of gluten intolerance or caloric restriction due to poverty, get hGH therapy, as result of superficial investigation or misdiagnosis. Medical doctors without any international reference travel across the planet sponsored by pharmaceutical industry, get a big honoraria promoting that bigger is better and even healthier. But, children are not so happy with their daily injections. The height gain is variable as myriad of reasons influencing growth are in game. The average increase in adult height after multiple years of treatment ranged from 3.56 to 7.56 cm. The question arises whether an average height gain of 5cm should be considered as satisfactory taking into account the cost of 70 000 $ for such gain (or 10,000$-60,000$ per patient year), besides pain of daily injections and discomfort of frequent periodic clinical investigations [9–11].

Thus the arrival of recombinant hGH shifted basic endocrine paradigm in endocrinology: to substitute deficient hormone and to suppress hyperfunction or; physiological hGH replacement to pharmacological hGH therapy for height enhancement [9]. This new statement is not free of potential severe consequences. As key opinion leader, Allen DB emphasized that there is increasing uncertainty about the real benefits and potential risks of hGH therapy for many thousands of short, but otherwise healthy children [9,10]. Applying hGH in children with hormonal normalcy as in ISS is common decision of parents and doctors. Laventhal et al. [11] describe this complex relationship as a tango for three (patients and their parents, doctors willing to prescribe and regulators ready to approve and to pay for its use). As De Vries noticed [12] they do not mention a fourth dancer: the pharmaceutical companies. Marketing strategies of these companies result in intense and close doctor-industry relationships and could influence doctors' prescribing practices [13–15]. Prescribers of hGH became "investigators" whose institution or even they personally, received per-patient compensation for detection, treatment and follow-up and reporting hGH-treated patients [10, 15]. Recent articles published in Hormone Research in Pediatrics and in The New England Journal of Medicine summarize all important issues in this area. They attracted my attention by reasonable criticism of cosmetic endocrinology and also revealed new, brilliant explanation of such phenomenon, lurking behind the scene [9, 10,15].

GH Effects beyond Height

Many teenagers with GHD have poor bone strength, fatigue and lack of stamina, as well as depression, lack of concentration, poor memory and anxiety problems. As a mother of one GH deficient child said: "GHD isn't just about growth, as lack of growth hormone impacts the child in many ways, such as lack of strength and they can find it difficult to keep up physically with their peers. It impacts the child self-esteem as they are often treated as being much younger, because of their size" [16]. GH treatment allows the child to growth to their genetic potential. Majority of short people have life-

long problem in finding appropriate clothes and shoes, or purchase in children's stores, because shops do not carry their size [17]. Severe short stature is no doubt, physically debilitating requiring special accommodations for reaching shelves or driving [15].

Components of GH-IGF-1 axis make an important contribution to the development, function, regeneration and neuroprotection of the CNS. GH plays well established roles in neurogenesis, axonal elongation and formation of oligodendrocytes, astrocytes and glial cells, as well as in angiogenesis, synaptogenesis, and synaptic transmission and prevention of neuronal apoptosis [18,19]. Indeed in patients with congenital classic triade syndrome, we were informed by parents about improvement in maturity and positive behavioral changes. Namely, true GHD shows not only impairment in psychological well-being but also defects in attention, memory and executive function, with evidence of improvement in cognitive function after GH replacement [18–20]. This effect is more evident in "definite GHD" and it is obtained at least partly by stimulating T4 to T3 conversion with great impact on neurodevelopment.

The discontinuation of treatment in adolescents with GHD after completion of linear growth may be associated with adverse effects on body composition, lipid profile, and cardiac morphology [18].

While people tend to attribute significantly less favorable characteristic to short persons, research repeatedly in majority of studies fails to detect an excess of psychosocial adaptation problems among short-statured youths [15]. Socially acceptable height, the term used by some endocrinologists actually means that society does not accept short people. The pharmaceutical companies go even further and offer, not only growth acceleration and improvement of final height as result of GH therapy, but also better and brighter future for potential recipients in whom the self-confidence is measured by centimeters of height. As Allen DB noticed: „It is too good to be all good" [15]. Is hGH „panacea or Pandora's box?" he asked [21]. A trend of exaggeration of hGH use for height, „heightism" [21,22] remainds me of sociopathic virus of fear of aging („ageism"), also imported in Europe from USA. Justification of hGH use for height needs confirmation that this treatment is safe and free of serious short term and long term adverse events. These phantastic references also led me to publications of Samaras concerning of logevity regarding height. Within generally healthy conditions short people live longer. Most centenarians tend to be short or small. It is best to be short and lean and to restrict protein intake. One mechanism supporting the "shorter and smaller is better" thesis is somatic cell duplication potential. Bigger bodies use up more cell replications during their maturation, because they contain more cells. It takes more cells to build and maintain a bigger body, and more cells increase the risk in cancer in taller people. Big people cost more [23–25]. Disruption of the GH-IGF-1 axis may result in increased longevity [26].

Safety Issues

Occurrence of adverse events during hGH treatment as intracranial hypertension, glucose intolerance, a slipped capital femoral epiphysis is well known and its incidence is low [27]. Physician's discussions with families must involve honest and realistic appraisal of treatment expectations for height gain [11]. It is worrisome that serious adverse events usually are not discussed. Post marketing studies sponsored by pharmaceutical companies, manufacturers of hGH so far reported only short term adverse events. Short term effects are not long term effects, more precisely; long term effects are still unknown. This statement does not refer to GH deficient subjects on hGH substitution therapy, but to hGH applied for height in healthy children.

When the announcement on increased mortality in GH treated individuals reached the headlines a lot of anxiety was produced in professional and scientific community as well as in GH recipients and their families [28]. The study on the Safety and Appropriateness of GH treatments in Europe (SAGhE study) announced that that the observation of long-term outcomes of children treated with hGH between 1985 and 1996, revealed a significant excess mortality (93 deaths vs. 70 expected from an age-matched population in France) [29]. Additionally a follow-up of 30 000 low-risk children treated with hGH between 1980 and 1990 in 8 European countries, who had reached 18 years of age between 2009-2010 was performed. Patients were classified as low mortality risk if they were diagnosed as idiopathic GHD, SGA, or ISS without genetic syndrome or defects. Patients with renal failure, short stature syndrome, like Turner syndrome, and tumor survivors were excluded. A preliminary report from France on 6928 low-risk children with mean follow-up of 17.3±4.1 years from the start of therapy showed an increase in the overall standardized mortality ratio. The increase in all-cause mortality was associated with a higher hGH dose of >50 µg/kg/day, but not with the severity of GHD or the duration of total GH exposure. Increased mortality rate was due to bone tumors, cardiovascular events and cerebrovascular hemorrhage [28–30]. Results have not been reproduced by reports from other countries participating in the study. Among 21 deaths identified, 12 were due to accidents, four were suicides, and one patient each died from pneumonia, endocrine dysfunction, primary cardiomyopathy, deficiency of humoral immunity, and coagulation defect [31]. The question of whether GHD *per se* is associated with an increased mortality, taking into account that pituitary produces LIF (Leukemia Inhibiting Factor) is for debate. It is strongly recommended by health authorities that hGH should be given to those who need it and the dose should be kept in the range given in the clinical trials that led to the registration of the medication [7,10,28].

The Situation in Serbia

Pediatric endocrinologists and pediatricians in Serbia embraced hGH use ever since its approval. We have published in 2004, Guidelines for early detection and treatment of children with congenital and acquired GHD, Turner syndrome and SGA. The publication in Serbian is result of team work, in accordance with Growth Hormone Research Society Consensus Guidelines for the treatment of GHD. Although not free of imperfections and confounders this recommendations contributed a lot in clarifying this complex area [32,33]. A plenty of periodical lectures are given, targeting pediatricians in Primary Health Care as a first line doctors, supposed to recognize and to refer short stature patients to Pediatric clinics with certified pediatric- endocrinologists. And this strategy works with regard to number of GHD children on hGH therapy in Serbia. However, the diagnosis of GHD is still made too late; in prepubertal or pubertal age. Unfortunately some short patients are referred after achievement of final heght. Data from the International Outcome Study (IOS) confirmed late referral of Serbian GHD children. The mean age at treatment start in Serbia is 11.2±3.5 years in comparison to 8.2±4.1 years in Czech Republic [34]. And *vice versa* non GHD children are often diagnosed as deficient due to reliance on GH provocative tests as the main diagnostic criterion of GHD [5,7]. The auxology is used as tool for detecting candidates for hGH treatment and not to establish accurate and exact diagnosis. Many clinical doctors are crossed planet due to medical tourism sponsored by hGH manufacturers; namely it is hardly to find any point, where some prescribers of hGH have never been. It is worthy to say that some of us are active in presenting Serbian results in the field all over the world and that it would be impossible without sponsorship [35–45]. What should be Serbian pediatric endocrinologist devoted to, in the light of new safety alerts. We must take seriously recent warnings and be engaged in raising awareness about potential short term and long term harmful effects of hGH, with the same effort used in promotion of hGH use. At least we must inform our patients about new discoveries and to practice not only parent's informed consent, but also children's assent, as experts from Unated states and Europe suggest. Finally as mentioned above to avoid cosmetic endocrinology and to be cost-conscious of such treatment in all aspects [15,22]. We must resist to siren voices and unbearable lighteness of prescribing growth hormone. Diagnosis of short stature and its treatment is not for the amateurs. Serious life-threatening diseases could be part of pituitary dysfunction and investigation of adrenal and thyroid axes should be the first diagnostic step. Pituitary disorders that need urgent, life-saving diagnosis are, present even at birth [46]. Cosmetic endocrinologists, obsessed with „heightism" are not involved in this process. Some of them are unfortunately only „growth- hormonologists".

Even in this genocentric millennium nothing could replace simple and accurate height and weight measurements, staying in touch with phenotypes, clinical curiosity and honesty. Nothing compares to satisfaction get by normalization of child's growth. Growth monitoring of apparently healthy children aims at early detection of serious underlying disorders.

Acknowledgments: *Supported by grants from the Ministry of Education, Science and Technological Development of Republic of Serbia No 31060, No 41018 and Internal Project No 37 of Faculty of Medicine Nis, University of Niš.*

References

1. Bailey JD. Foreword. Clinics in endocrinology and metabolism 1982; 11:9–16.
2. Rosenfield RG. Ten axioms in the evaluation of growth failure. The Endocrinologist 1997; 7:148–152.
3. Scherdel P, Dunkel L, Van Dommelen P, Goulet O, Salaun JF, Brauner, et al. Growth monitoring as an early detection tool: a systematic review. Lancet Diabetes Endocrinol 2016, http://dx doi.org/10.1016/S2213-8587(15)00392-7
4. Raben MS.Growth hormone-clinical use of growth hormone. NEJM 1962; 266:82–86.
5. Gandrud LM, Wilson DM. Is growth hormone stimulation testing in children still appropriate? Growth Horm IGF Res 2014; 14:185–194.
6. Pampanini V, Pedicelli S, Gubinelli J, Scire G, Cappa M, Boscherini B, et al. Brain magnetic resonance imaging as first-line investigation for growth hormone deficiency diagnosis in early childhood. Horm Res Paediatr 2015; 84:323–330.
7. Grimberg A, DiVall SA, Polichronakos C, Allen DB, Cohen IE, Quintos , et al. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor deficiency. Horm Res Padiatr 2016; 86:361-397.
8. Argente J. Challenges in the management of short stature. Horm Res Paediatr 2016; 85: 2–10
9. Allen DB.Lessons learned from the hGH era. JCEM 2011; 96:3042–3042.
10. Allen DB. Short stature in childhood-challanges andchoises. NEJM 2013; 368:1220–1228.
11. Laventhal NT, Shuchman M, Sandberg DE. Warning about warnings: weighing risk and benefit when information is in a state of flux. Horm Res Pediatr 2013; 79:4–8.
12. De Vries MC. A tango for four: deciding on growth hormone therapy in idiopathic short stature. Horm Res Paediatr 2013; 79: 2–3
13. Campbell EG, Weissman JS, Ehringhaus S, Rao SR, Moy B, feibelman S, Goold SD. Institutional academic-industry relationships. JAMA 2007; 298:1779–1786.
14. Moynihan R, Health I, Henry D. Selling sickness: the pharmaceutical industry and disease monitoring. BMJ 2002; 324:886–891.
15. Allen DB. Growth promoting ethics and the challenge to resist cosmetic endocrinology. Horm Res Paediatr 2017; 87: 145-152
16. Adelman M. Gene test for growth hormone deficiency developped. Medicine&health/Medical research 2018 https://medicalxpress.com/news/2018-04-gene-growth-hormone-deficiency
17. Laron Z. Extensive personal experience. Laron syndrome (primary growth hormone resistence or insensitivity): the personal experience 1958-2003. J Clin Endocrinol Metab 2004; 89:1031–1044.
18. Alatzoglou KS, Webb EA, Tissier Pl, Dattani M. Isolated growth hormonedeficiency (GHD) in childhood and adolescence. Endocrine Rev 2014; 35:376–432.

19. Aberg D. Role of the growth hormone/insulin-like growth factor 1axis in neurogenesis. Endocr Dev 2010; 17:63–76.
20. Russo VC, Gluckman PD, Feldman EL, Werther GA. The insulin-like growth factor system and its pleiotropic functions in brain. Endocr Rev 2005; 26:916–943.
21. Allen DB. Fost NC. Growth hormone therapy for short stature: panacea or Pandora's box. J Pediatr 1990; 117:16–21.
22. Allen DB. Cost-concious growth-promoting treatment: when discretion is the better part of value. Horm Res Paediatr 2018; 90:146–150.
23. Samaras TT. We are too tall. Public Health Nutr 2009; 12: 439.
24. Samaras TT. Is height related to longevity? Life Sci 2003; 7:1781–1802.
25. Samaras TT. We are too tall (Letter to Editor). Public Health Nutrition 2017;12: 439–440.
26. Laron Z. The GH-IGF1 axis and longevity. Hormones 2008; 7:24–27.
27. Dardendeliler F, Kargiannis G, Wilton P. Headache, idiopathic intracranial hypertension and slipped capital femoral epiphysis during growth hormone treatment: a safety update from the KIGS database. Horm Res 2007; 68 Suppl 5:41–47.
28. Zadik Z. Growth hormone mortality: will history repeat itself? J Pediatr Endocrinol Metab 2012; 25:813–814.
29. Sante Adulte GH Enfant (SAGhE). http://www.afssaps.fr/Infos-desecurite/Cornmuniques-Points – presset/Hormone-de-Croissance-synthetiqu e-somatropinerecornbinante-Premiersresultats-de-l-etude-epidemiologique-sur-latolerance-a-longterme-Communique. December 2010.
30. Carel JC, Ecosse E, Landier F, Meguuellati-Hakkas D, Kaguelidou F et al. Long-term mortality after recombinant growth hormone treatment for isolited growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. J Clin Endocrinol Metab 2012; 97:416–425.
31. Savendahl L, Maes M, Albertsson-Wikland K, Borgström B, Carel JC, Henrard S et al. Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during childhood in Belgium, The Netherlands, and Sweden: preliminary report of 3 countries participating in the EU SAGhE study. J Clin Endocrinol Metab 2012; 97:213–217.
32. Growth Hormone Research Society; GH Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. J Clin Endocrinol Metab 2000; 85:3990–3993.
33. Fraiser D. Editorial: the treatment of childhood and adolescent growth hormone deficiency-consensus or confusion?J Clin Endocrinol Metab 2000; 85:3988–3989.
34. Polak M, Konrad D, Tonnes Pedersen B, Puras G, Snajderova M. Still too little, too late? Ten years ofgrowth hormone therapy baseline data from the NordiNet International Outcome Study. J Pediatr Endocrinol Metab 2018; 31:521–532.
35. Saranac L, Zivanovic S, Radovanovic Z, Kostic G, Markovic I, Miljkovic P. Hyperprolactinaemia: different clinical expression in childhood. Horm Res Pediatr 2010; 73;187–192.
36. Saranac L, Gucev Z. New insights in septo-optic dysplasia. Prilozi 2014; 35: 123–128.
37. Saranac Lj, Stamenkovic H, Stankovic T, Zivanovic S, Djuric Z. Growth in Children with Thyroid Dysfunction. In Potlukova E Ed. Current Topics in Hypothyroidism with Focus on Development. Rijeka: InTech 2013:119–134, available from: http://www.intechopen.com/articles/show/title/growth-in-children-with-thyroid-dysfunction.
38. Saranac Lj, Stamenkovic H. Different Faces of Chronic Autoimmune Thyroiditis in Childhood in Adolescence. In Springer D Ed. A New Look at Hypothyroidism. Rijeka: InTech 2012; 125–132, available from: http://www.intechopen.com/articles/show/title/different-faces-of-chronic-autoimmune-thyroiditis-in-childhood-and-adolescence
39. Saranac L, Bjelakovic B, Djordjevic D, Novak M, Stankovic T. Hypopituitarism occurring in neonatal sepsis. J Pediatr Endocrinol Metab 2012; 25;847–848.
40. Saranac L, Stamenkovic H, Radovanovic Z, Kostic G, Bjelakovic B. Microprolactinoma, an unusual cause of growth hormone deficiency in a prepubertal obese boy. Inernat J on Disab and Develop 2005; 4:147–149.
41. Saranac L, Gucev Z. The ghrelin system; beyond the role in energy homeostasis. Facta Universitatis Series Med and Biol 2016; 18:33–38
42. Saranac L, Djuric Z, Stojsic Z, Milenovic A, Lazarevic D. Severe growth failure in a prepubertal boy: what is behind the scene? Austin J Endocrinol Diabetes 2016; 3:1046–1047.
43. Saranac L, Djuric Z, Markovic I, Stamenkovic H, Lazarevic D, Cvetkovic V. Outstanding growth response to growth hormone replacement therapy in 3 different cases of growth hormone deficiency.54th Annual ESPE 2015, 01-03 Oct Barcelona: 84
44. Saranac L, Bjelakovic B, Zivanovic S, Novak M, Markovic I, Radovanovic Z. Pituitary dysfunction types and clinical presentation of pituitary microadenomas in childhood and adolescence. 13th European Congress of Endocrinology, Rotterdam 2011, 30 Apr-04 May; 26
45. Saranac L, Novak M, Zivanovic S. Growth hormone deficiency in patients with pituitary lesions. Horm Res 2009; 72:504.
46. Scommegna S, Galeazzi D, Picone S, Farinelli E, Agostino R, Bozzao A et al. Neonatal identification for pituitary aplasia, a life-saving diagnosis (review of five cases). Horm Res 2004; 62:10–16.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 40–46

UDC 616.12-008.331.1 -057
https://doi.org/10.22190/FUMB180513007J

Original Article

THE INFLUENCE OF PROFESSIONAL STRESS IN PERMANENT WORKING CAPABILITY OF WORKERS WITH ARTERIAL HYPERTENSION AND ITS COMPLICATIONS

Jovica Jovanović[1,2], Ivana Šarac[3], Jovana Jovanović[1], Dušan Sokolović[1], Stefan Jovanović[1]

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]Institute of Occupational Health Niš, Niš, Serbia
[3]Institute for Medical Research, University of Belgrade, Center of Research Excellence in Nutrition and Metabolism, Belgrade, Serbia

Abstract. *Arterial hypertension is widespread in the population of workers. Stress at work has a significant impact on the emergence of many psychosomatic diseases including arterial hypertension. The aim of this research is to show the influence of professional stress on the occurrence of arterial hypertension, its complications and the permanent working ability of exposed workers in construction. The survey covered 1900 construction workers. The standardized questionnaire has assessed the level of stress and professional stressors in their workplaces. The survey group consisted of 1350 workers whose level of total professional stress index (OSI) was over 60, and a control group of 550 workers with an OSI level below 60. The prevalence of arterial hypertension and its complications is statistically significantly more frequent in the examined than in the control group. In the investigated group, a statistically significant number of workers were registered, who, due to their complete loss of working ability, were sent to the disability commission. With an increase in OSI values above 71, the number of workers with complete loss of working capacity is significantly increased in relation to the number of workers who are capable of working. Professional stress is a significant factor that affects the occurrence of arterial hypertension, its complications on vital organs, which lead to a complete permanent loss of working ability of exposed workers in construction.*

Key words: *professional stress, arterial hypertension, complications of arterial hypertension, work ability, construction.*

Introduction

Stress at work is expanding and it has a major impact on the emergence of many psychosomatic diseases including arterial hypertension [1–3]. Arterial hypertension is widespread in the population of workers [4–6] and represents a significant medical and economic problem [7,8]. Our previous research has shown a significant impact of stress on the work on temporary inability to work with workers with arterial hypertension [9]. Particularly important is the question of the impact of stress on the long-term ability of exposed workers.

Aim

The purpose of this research is to demonstrate the impact of professional stress on the occurrence of arterial hypertension, its complications and the permanent working ability of the exposed workers in construction industry.

Correspondence to: Jovica Jovanović, M.D., Ph.D.
Faculty of Medicine, 81 Zoran Đinđić Blvd., 18000 Niš, Serbia
Phone: +381 64 26 14 485
E-mail: joca@medfak.ni.ac.rs
Received May 13th, 2018, Accepted June 13th, 2018

Methodology

The survey covered 1900 construction workers. In all respondents, the use of a standardized questionnaire with the approval of the author was performed by an analysis of stress factors at the workplace and the calculation of the overall professional stress index (OSI). This method is based on the application of a workplace questionnaire containing 9 areas whose responses are graded with a scale of 0 to 2, where 0 means "not present", while 2 means "very present". The application of the computer program determined the total Occupational Stress Index (OSI score), which represents the total stress on stressful working conditions, as well as the burden on certain groups of professional stressors. A standardized questionnaire was written by Dr. Karen Belkić [10], who divided the stresses in the workplace into 7 groups (high job demands, rigor, conflict, subcontract, exposure to hazards, time limit, and exposure to noxiousness). The questionnaire encompasses 79 factors which are divided into a two-dimensional matrix, where in the vertical axis is the level of transmission of the information represented in a human (information reception, decision-making, operation and general level) and on the horizontal axis are the aspects of stress (sub-load, high requirements, time less, avoidance of danger and conflicts). Each element has a set of co-

ordinates so that it is possible to make a total sum of all aspects where the sum of the sums of all elements makes a total OSI score that quantifies the total burden of professional stresses. Each element counts from 0 to 2 points (0 – no presence, 2 – strong presence), so the biggest score is for the highest exposure in the workplace. The sum of all elements of each aspect of stress at a certain level of information transfer is calculated, as well as the total sum of a certain aspect of stress at all levels of information transfer. Aspect underload implies acceptance of similar signal, receiving rare signals, working alone without the need to communicate with other people, making decisions automatically based on the information received, the execution of the same, simple tasks, fixed salary regardless of our work, the inadequacy of wages for the environmental needs of workers and their family, failure to progress and lack of recognition for work, monotonous work. The high demand aspect includes the existence of several sources of information at the same time, diverse information, primary visual observation, high flow of incoming information, three sensory stimulus at the same time, the need for communication at work, complex and quick decisions affecting associates, pay for performance, lack of pause, shift and night work, and lack of holidays. The aspect of constraints (rigor) involves detecting all incoming signals, performing tasks according to strictly defined standards, fixed position during work, doing work in a cramped, skilled work space, limited influence on decision making with whom the job is shared. External time pressure (time limit) implies the impossibility of delaying decisions, reduced ability to control task execution, the need for speeding up the work, and the existence of a time limit for performing some work. Exposure to external noxious agents means exposure to blinding light, flashes, noise, vibrations, temperature extremes, gases, vapors, dust, and lifting loads. Avoidance of danger (an aversion) as an element of mental stress involves constant readiness to avoid possible serious consequences, presence visually disturbing scenes and events, work in the presence of flammable substances, an accident, testifying in court for an accident at work. Conflicts mean that there is an unclear difference between various incoming signals, the lack of essential information for decision making, the obtaining of contradictory information, the confrontation with unexpected events that require the change of the previous work plan, creating conflict and uncertainty in decision making, interruptions in the work of other people, working atmosphere burdened with interpersonal conflicts, lack of support and assistance from colleagues, inability to progress in the profession, work relationship at a certain time or threat of cancellation, violation of one's own norms and rules of conduct. The Professional Stress Index (OSI) is the main sum of the whole model and gives the characteristics and size of stress in the workplace. In relation to the value of OSI, the respondents in this study are divided into two groups. The examined group consisted of 1350 construction workers from several companies who were exposed to stress at work, with an OSI value of over 60, and were mainly workers working at height, handling construction machinery and exposed to increased psychophysical efforts and the effects of physical and chemical harm. The control group consisted of 550 administrative workers from several construction companies who worked at workplaces and in conditions where the OSI was below 60. The average age of the group was 53.8 ± 10.5 years and control group workers 54.7 ± 11,5 years, which does not represent a statistically significant difference ($p > 0.05$). In the examined group, 51.3% were smokers, and 48.9% of the workers constantly consumed cigarettes, which is not statistically significant ($p > 0.05$). Blood pressure was measured in all workers. Chronic elevations in blood pressure over 140/90 mm Hg were considered as arterial hypertension. In the case of workers with arterial hypertension, a detailed analysis of the health status and assessment of the work ability for the work that was performed at that moment was performed. In addition to work, personal, family and social history was gathered, data on problems including laboratory tests (sedimentation of erythrocytes, blood test results, urine examination, glycemia, cholesterol, triglycerides, electrolytes, urea, creatinine, acidum uricum), EKG, echocardiographic examination, physical exercise test with determination of the consumption of oxygen during the test, ultrasound examination of the kidney. In relation to complications of arterial hypertension, workers are classified into one of five groups:

1. Arterial hypertension without complications - in case that no complications are found on vital organs,
2. Arterial hypertension with heart complications - in case of heart rhythm disturbances, coronary disease, hypertrophy, or myocardial left ventricular dilation,
3. Arterial hypertension with complications in the eye bottom - in case of changes in blood vessels of the retina,
4. Arterial hypertension with complications on the central nervous system,
5. Arterial hypertension with complications on other organs - in the event of complications on other organs (kidney, peripheral blood vessels).

Based on the analysis of working conditions, working environment, health status and estimation of the functional capacity of the cardiovascular system, an opinion was given on the working ability of each respondent. The results are statistically processed and shown in the table.

Results

By analyzing the demands of jobs, the presence of noxious agents and the analysis of professional stressors in workplaces, it has been noticed that the workers of the investigated group were exposed to a statistically significantly higher level of the index of total professional stress (85.89 ± 4.21, min = 60.5, max = 90)

groups (55.53 ± 3.92, min = 45.5, max = 59.5). Values for all groups except the work stressors, stress from the group of a time limit, a statistically significantly higher in the experimental compared to control group (Table 1).

Arterial hypertension is statistically significantly more frequent among the workers in examined group (40.3%) compared to control group workers (24.1%) ($p < 0.05$). Arterial hypertension without complications on vital organs was statistically significantly more common among examined workers (30.9%) compared to control group workers (19.1%) ($p < 0.05$). Among the complications of this disease in both groups dominated by changes in the heart and the eyelid (Table 2).

By monitoring the prevalence of arterial hypertension in relation to the work experience in the examined group, an increase in prevalence with years of service is recorded as both arterial hypertension without complications and arterial hypertension with complications. The prevalence of arterial hypertension without complications but also with complications on vital organs statistically significantly increases in groups of workers with a working experience over 21 years (Table 3).

The analysis of the the results of the assessment of working capacity showed that in the tested group there is significantly higher number of workers sent to the disabled workers' committee because of a total loss of working ability in comparison to the control group. In the control group, there was a statistically significant increase in the number of workers who were able to continue their work in relation to the tested group of workers (Table 4).

By monitoring the results of the evaluation of the work ability in relation to the value of the index of professional stress, it is noticed that with the increase in the OSI level, the number of workers sent to the disabled workers' committee due to complete loss of working ability is increasing. The number of workers with complete loss of working ability is statistically significantly increased in the subgroups of workers who are exposed to OSI over 71, in which statistically significantly higher number of workers with complete loss of working ability are registered in relation to the number of workers able to work (Table 5).

Table 1 Values of professional stressors and the total index of professional stress at the control and examination groups.

Professional stressors	Examined group		Control group		p
	X	SD	X	SD	
High job requirements	19.34	5.15	10.05	4.27	<0.001
Strictness	15.42	2.02	10.13	3.68	<0.001
Conflict	16.57	4.74	9.38	2.58	<0.001
Underload	5.98	1.64	11.71	3.98	<0.001
Exposure to noxiousness	12.21	5.75	6.23	1.58	<0.001
Time limit	7.13	1.81	6.72	1.75	n.s.
Exposure to noxious agents	9.24	1.48	1.31	1.10	<0.001
OSI total	85.89	4.21	55.53	3.92	<0.001

n.s.– difference is not significant

Table 2 Prevalence of arterial hypertension in the examined and control group

	Examined group N=1350		Control group N=550		p
	Number	%	Number	%	
Arterial hypertension without complications	418	30.9	105	19.1	p<0.05
Arterial hypertension with complications on the heart	55	4.1	13	2.4	n.s.
Arterial hypertension with complications on the eyelet	50	3.7	12	2.2	n.s.
Arterial hypertension with complications on the central nervous system	15	1.1	2	0.4	n.s.
Arterial hypertension with complications on other organs	5	0.4	1	0.2	n.s.
Total	543	40.3	133	24.1	p<0.05

n.s.– difference is not significant

Table 3 Arterial hypertension in the examined and control group in relation to the work experience.

Working expirience (years)	Examined group					Control group					p 1	p2
	N	Hypertension without complications		Hypertension with complications		N	Hypertension without complications		Hypertension with complications			
		n	%	n	%		n	%	n	%		
<10	340	57	16.7	13	3.82	139	25	17.9	5	3.6	n.s	n.s
11–20	338	61	18.1	17	5.03	137	25	18.3	8	5.8	n.s	n.s
21–30	345	145	42.0	48	13.9	140	27	19.3	8	5.7	<0.05	<0.05
31–40	327	155	47.4	47	14.4	134	28	20.9	7	5.2	<0.05	<0.05
Total	1350	418	30.9	125	9.2	550	105	19.1	28	5.1	<0.05	<0.05

p1 – statistical significance of difference between test and control group in the presence of arterial hypertension without complications
p2 – statistical significance of difference between test and control group in the presence of arterial hypertension with complications

Table 4 Results of assessment of the working ability of workers with arterial hypertension in the examined and control group

	Examined group					Control group					p1	p2
	N	Capable for work		Referred to the Disability Commission		N	Capable for work		Referred to the disabled workers' committee			
		n	%	n	%		n	%	n	%		
Hypertension without complications	418	245	58.6	173	41.4	105	103	98.1	2	1.9	<0.05	<0.05
Hypertension with complications	125	11	8.8	114	91.2	28	27	96.4	1	3.6	<0.05	<0.05
Total	543	256	47.1	287	52.8	133	130	97.7	3	2.2	<0.05	<0.05

Table 5 Results of the evaluation of working capacity in relation to the value of OSI

OSI	Capable for work			Referred to the Disability Commission			p
	N	number	%	N	number	%	
60–70	481	203	42.2	181	78	43.1	n.s.
71–80	453	39	8.6	186	91	48.9	<0.05
81–90	416	14	3.4	183	118	64.5	<0.05

Discussion

In this study it was determined that construction workers are exposed to high levels of occupational stress. Similar results have been found by other authors who have studied the working conditions of construction workers [11]. At workplaces construction workers registered with stress factors that could affect the occurrence, evolution and flow of arterial hypertension, which should be taken into account when making the final opinion of the working ability of these workers [12]. In this study, high prevalence of arterial hypertension among construction workers (40.3%) was established. The prevalence of arterial hypertension registered in this study is higher than in the results reported by other authors who have followed the risk factors for cardiovascular disease in construction workers in other environments. Thus, Prabhakaran [13] states that 32% of construction workers have arterial hypertension, and Sze Pui Pamela Tin states in his research the prevalence of arterial hypertension among construction workers in 22.6% [14]. These differences in prevalence can be explained by differences in age among the surveyed population groups of construction workers (in the Sze Pui Pamela Tin survey, the average age of construction workers was 43.1 ± 11.5 years, and in this survey the average age of construction workers is 53.8 ± 10.5 years) and different lifestyles and habits (51.3% of smokers among construction workers in this study while there were 39.1% smokers in Sze Pui Pamela Tin's research). There is convincing evidence in the literature that the factors of occupational exposure (stressed professional noxious agents and job requirements) can be favorable factors in the faster development of arterial hypertension [15–17]. Peters found that high-demand jobs and small controls over them, such as building workers, lead to a rise in blood levels of catecholamines and cortisols, which among other things results in an increase in blood pressure [18]. According to the literature, a significant correlation between the high demands of work, arterial hypertension and cardiovascular disease [19,20] was established. The restriction (strictness) of construction workers is reflected in the need to carry out jobs according to strictly defined standards and according to the results of previous research it may be a risk factor for the cardiovascular system [21,22]. The external time pressure that as a professional stressor in construction workers means that the worker has no influence on the pace of work and decision-making that cannot be postponed or indirectly involves long hours. Long working hours in epidemiological studies have been proven to be a stressful workplace and a factor contributing to the faster development of arterial hypertension and coronary disease [23–26]. Conflicts in the workplace, present in the subjects in this paper, arise when the work or work rhythm breaks up, leading to the occurrence of professional stress. There is an increase in arterial blood pressure and less satisfaction with work [27–28]. Unpredictable changes in the plan lead to the creation of new plans, which requires additional effort and stress [29]. One of the stressed noxious agents in the working environment of the construction workers is industrial noise. Clinical studies have shown that industrial noise is a significant factor contributing to the faster arterial hypertension in exposed workers [30–32], where the noise present in the community environment highlights the harmful effects of industrial noise on the blood pressure of exposed workers [33]. Noise can cause an increase in blood pressure and the occurrence of arterial hypertension by various mechanisms. Possible pathogenetic mechanisms are the influence of noise on the increase in circulating catecholamine concentrations released from the adrenal medulla as a result of activation of the adrenergic nervous system, the effect of higher adrenal steroid concentrations, the increased activity of the renin-angiotensin-al-

dosterone system under the effect of noise, and the effect of noise on the increase in total peripheral vascular resistance [34]. Neuroanatomic relationships in the central nervous system allow acoustic stimulus not only to affect the auditory area of the bark of the large brain, but also the limbic system, that is, the hypothalamus, leading to stimulation of the sympathetic and vasoconstriction on the periphery, resulting in an increase in peripheral resistance and an increase in blood pressure. Constantly repeated noise stimulation, in time, leads to chronic elevation of sympathetic tone, which can accelerate various structural changes on the vascular system and increase the peripheral resistance of the blood vessels leading to permanent arterial hypertension [35]. Over time, arterioles hypertrophy, thickness of the blood vessel wall increases and blood vessel sensitivity increases. Professional exposure to vibrations, the same pathogenetic mechanisms as noise, can favor a more frequent occurrence of arterial hypertension and coronary disease [36]. Working in the presence of inadequate air temperature of the working environment is a stress for the body of the worker, which in time leads to an increase in blood pressure and arterial hypertension [37–39]. This reaction of the cardiovascular system depends on the age of the worker, the level of workload and the genetic predisposition of the worker [40,41]. There is increasing evidence that long-term work in conditions of reduced outdoor air temperature can potentiate the development and occurrence of arterial hypertension in exposed workers [42,43]. A monotonous work involving repeatedly recurring activities is associated with a more frequent occurrence of arterial hypertension, coronary disease, diabetes mellitus, and hypercholesterolemia of exposed workers [44], which can be partly explained by a greater number of smokers among workers who perform monotonous activities [45,46]. Working in shifts and during the night, the happiness of the construction workers leads to the disruption of circadian rhythm and can be a significant favoring factor in the faster arterial hypertension [47,48]. Workers who work in shifts due to circadian rhythm disorders often suffer from arterial hypertension [49,50], which can be explained by the frequent occurrence of the habit of consuming cigarettes, alcohol and caloric food among "shift workers", and a more frequent occurrence of neuroses among workers working in shifts [51,52]. There is more and more convincing evidence that a chronic exposure to chemical agents at the workplace contributes to the faster arterial hypertension of exposed workers [53,54]. Some authors found a greater prevalence of ischemic heart disease, arterial hypertension, and kidney disease in residents living near lead smelters [55]. Simultaneous exposure to noise, lead, manganese and inadequate microclimate factors contributes to the more frequent occurrence of arterial hypertension of exposed workers [56]. Organic solvents lead to an increased release of catecholamines from the adrenal gland and to an acid-base disorder, which can negatively affect blood pressure regulation and circulation [57]. In a group of organic solvents, carbon dioxide takes a special place that leads to lipid metabolism disorders, favors the atherosclerosis process, suppresses the thyroid gland (can lead to hypothyroidism) and potentiates the development of arterial hypertension faster [58,59]. Constant exposure to vinyl chloride leads to an increase in the secretion of catecholamine and peripheral circulation disorders [60–62]. This study analyzed separately working ability of patients with arterial hypertension and its complications. The most important factors determining the working ability of workers with arterial hypertension are the biological demands of the workplace (in particular the level and type of workload, the presence of stressed nose and those harmful effects that affect the height of blood pressure and the occurrence of complications of arterial hypertension), the functional state of the cardiovascular system, arterial hypertension, age, year of work experience and employee motivation. Assessment of the working ability of patients with arterial hypertension and complications is done by aligning the biological functions of the organism with the requirements, risks and characteristics of the workplace. In assessing work ability, the psycho-physical and psychophysiological potentials first must be defined. Diagnostic procedures assess the health characteristics of the organism, the functional working capacity and the functional state of organs and organic systems. At the same time, an analysis of biological demands, loads and occupational risks in the workplace is carried out. In the end, the data obtained from previous actions are harmonized [63]. Opinion on working abilities is mostly based on the exercise of physical effort in the conditions of professional harmfulness. The results of this paper indicate high correlation between the presence of professional stressors and the complete loss of working ability. The correlation of permanent total loss of working ability and professional stressors can be explained by their impact on increased job dissatisfaction [64,65] and more frequent presence of risk factors for coronary disease in exposed subjects that lead to complications of arterial hypertension of the heart [66–68], stress caused by vascular complications in the central nervous system [69,70], cardiomyopathy [71], and atherosclerotic changes due to the action of professional stressors [72–74].

Conclusion

Professional stress is an important factor that affects the frequent occurrence of arterial hypertension and its complications in exposed workers. The prevalence of arterial hypertension and its complications increases with the duration of exposure to professional stress and in particular statistically significantly increases in workers with working experience over 21 years. Stress at work is a factor that significantly affects the total loss of working ability of exposed workers. The values of the index of professional stress over 71 significantly affect the complete loss of the permanent working capacity of the exposed workers in construction.

References

1. Siegrist J, Sies H. Disturbed Redox Homeostasis in Oxidative Distress: A Molecular Link From Chronic Psychosocial Work Stress to Coronary Heart Disease? Circ Res. 2017; 121(2):103–105.
2. Ushakov AV, Ivanchenko VS, Gagarina AA. Psychological Stress in Pathogenesis of Essential Hypertension. Curr Hypertens Rev 2016; 12(3):203–214.
3. Djindjić N, Jovanović J, Djindjić B, Jovanović M, Pesić M, Jovanović JJ. Work stress related lipid disorders and arterial hypertension in professional drivers - a cross-sectional study. Vojnosanit Pregl 2013; 70(6):561–568.
4. Manohar S, Thongprayoon C, Cheungpasitporn W, Mao MA, Herrmann SM. Associations of rotational shift work and night shift status with hypertension: a systematic review and meta-analysis.J Hypertens 2017; 35(10):1929–1937.
5. Rapsomaniki E, Timmis A, George J, Pujades-Rodriguez M, Shah AD, Denaxas S, et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1•25 million people. Lancet 2014; 383: 1899–1911.
6. Bauer UE, Briss PA, Goodman RA, et al. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. Lancet 2014; 384:45–52.
7. Jovanović J, Jovanović M, Batanjac J. Arterial hypertension at industrial workers, Vojnosanit Pregl 1999; 56(3):275–282.
8. Jong-Uk W, Inah K. Compensation for Work-Related Cerebrocardiovascular Diseases. J Korean Med Sci 2014; 29 (Suppl):S12–S17.
9. Lazaridis K, Jovanović J, Jovanović JJ, Šarac I, Jovanović S. The impact of occupational stress factors on temporary work disability related to arterial hypertension and its complications. Int J Occup Saf Ergon 2017; 23(2):259–266.
10. Belkić K. The Occupational Stress Index: An Approach Derived from Cognitive Ergonomics and Brain Research for Clinical Practice, Cambridge International Science Publishing, Cambridge, 2003, ISBN: 1-898326-02-9.
11. Poon S, Rowlinson S, Koh T, Deng Y. Job Burnout and Safety Performance in the Hong Kong Construction Industry. International Journal of Construction Management 2013; 13(1):69–78.
12. Jovanović J, Jovanović M, Đorđević D. Estimation of working ability at workers with arterial hypertension, Vojnosanit Pregl 2001; 58(6):631–639.
13. Prabhakaran D Shah P, Chaturvedi V, Ramakrishnan L, Manhapra A, Reddy KS. Cardiovascular risk factor prevalence among men in a large industry of northern India.Natl Med J India 2005; 18(2):59–65.
14. Sze PT, Wendy WTL, Sungwon Y, Zhang N, Nan X, Weiwei Z, Ke M et al. Workplace Health Promotion: Assessing the Cardiopulmonary Risks of the Construction Workforce in Hong Kong. PLoS One 2016; 11(1):e0146286. doi: 10.1371/journal. pone.0146286, PMCID: PMC4723250
15. Jayakumar D. Occupational Stress and Hypertension among Railway Loco Pilots and Section Controllers.Indian J Occup Environ Med 2017; 21(1):23–28.
16. Jovanović J, Jovanović M. Occupational stress and arterial hypertension. Med Pregl 2004; 57(3–4):153–158.
17. Liu MY, Li N, Li WA, Khan H. Association between psychosocial stress and hypertension: a systematic review and meta-analysis. Neurol Res 2017; 39(6):573–580.
18. Peters ML, Godaert GL, Ballieux RE, van Vliet M, Williemsen JJ, Sweep FC. Cardiovascular and endocrine response to experimental stress: effects of mental effort and controllability. Psichoneuroendocrinology 1998; 23:1–17.
19. Gilbert-Ouimet M, Trudel X, Brisson C, Milot A, Vézina M. Adverse effects of psychosocial work factors on blood pressure: systematic review of studies on demand-control-support and effort-reward imbalance models. Scand J Work Environ Health 2014; 40(2):109–132.
20. Nieuwenhuijsen K, Bruinvels D, Frings-Dresen M. Psychosocial work environment and stress-related disorders, a systematic review. Occup Med (Lond) 2010; 60(4):277–286.
21. Netterstrom B, Nielsen FE, Kristensen TS, Bach E, Moller L. Relation between job strain and myocardial infarction: a case control study. Occup Environ Med 1999; 56(5):339–342.
22. Djindjic N, Jovanovic J, Djindjic B, Jovanovic M, Jovanovic JJ. Associations between the occupational stress index and hypertension, type 2 diabetes mellitus, and lipid disorders in middle-aged men and women. Ann Occup Hyg 2012; 56(9): 1051–1562.
23. Spurgeon A, Harrington JM, Cooper CL. Health and safety problems associated with long working hours. A review of the current position. Occup Environ Med 1997; 54:367–375.
24. Hino A, Inoue A, Kawakami N, Tsuno K, Tomioka K, Nakanishi M, Mafune K, Hiro H. Buffering effects of job resources on the association of overtime work hours with psychological distress in Japanese white-collar workers.Int Arch Occup Environ Health 2015; 88(5):631–640.
25. Yang H, Schnall PL, Jauregui M, Su TC, Baker D. Work hours and self-reported hypertension among working people in California. Hypertension 2006; 48(4):744–750.
26. Kivimäki M, Solja T. Nyberg, G. David Batty, Kawachi I, Markus J, Lars A et al. Long working hours as a risk factor for atrial fibrillation: a multi-cohort study. Eur Heart J 2017; 38(34):2621–2628.
27. Kahn RL. Conflict, ambiguity and overload: three elements in job stress. In: McLean A. Ocupational Stress. Springfield: Charles C Thomas, 1974: 47–61.
28. Panter-Brick C, Eggerman M, Mojadidi A, McDade TW. Social stressors, mental health, and physiological stress in an urban elite of young Afghans in Kabul. Am J Hum Biol 2008; 20(6):627–641.
29. Karlsbeek JWH. Standards of acceptable load in ATC tasks. Ergonomics 1971; 14:641–650.
30. Verbeek JH, Dijk FJ, Vries FF. Non auditory effects of noise in industry. Int Arch Occup Environ Health 1987; 59(1):51–54.
31. Jovanović J, Batanjc J, Jovanović M. Circulatory effects of industrial noise and vibrations. Annals of the Institute of Public Health, Timisoara, Romania, XXII 1997; 5 (14): 99–101.
32. Münzel T, Schmidt FP, Steven S, Herzog J, Daiber A, Sørensen M. Environmental Noise and the Cardiovascular System. J Am Coll Cardiol 2018; 71(6):688–697.
33. Jovanović J, Popović V, Milošević Z, Jovanović M. Cumulative effects of communal and industrial noise on cardiovascular system. Facta Universitatis Series Medicine and Biology 1997; 4(1):57–61.
34. Paunović K, Jakovljević B, Stojanov V. The timeline of blood pressure changes and hemodynamic responses during an experimental noise exposure. Environ Res 2018; 163:249–262.
35. Vacheron A. Cardiovascular effects of noise. Bull Acad Natl Med 1992; 176(3):387–392. [In French]
36. Tzvetkov D, Boev M, Baykoushev B. Vibrations-discriminant models and possibilities for prognosticating specific and non specific effects on the organism. Ann Occup Hyg 1992; 36(3): 253–264.
37. Xu LJ, Li DG, Holbrrook NJ, Udelsman R.. Acute hypertension induces heat shock protein 70 gene expression in rat aorta. Circulation 1995; 92(5):1223–1229.
38. Brook RD. The Environment and Blood Pressure. Cardiol Clin 2017; 35(2):213–221.
39. Valutsina VM, Tkachenk, LN. Age related functional characteristics of miners exposed to heat microclime. Med Tr Prom Ekol 1994; 1,8: 10–15. [In Russian]
40. Blake, MJ, Klevay LM, Halas ES, BodeAM. Blood pressure and heat shock protein expression in response to acute and chronic stress. Hypertension 1995; 25(4):539–544.
41. Havenith G, Inoue Y, Luttikholt V, Kenney NJL. Age predicts cardiovascular , but not thermoregulatory, responses to humid heat stress. Eur J Appl Physiol 1995; 70(1):88–96.
42. Li X, Tokura H, Midorikanja T. The effects of two types of clothing on seasonal cold tolerance. Eur JAppl Physiol 1994; 69(6):498–501.
43. Inoue Y, Nakao M, Ueda H, Araki T. Seasonal variation in physiological responses to mild cold air in young and older men. Int J Biometeorol 1995; 38(3):131–136.

44. Scansetti G. Arterial hypertension and work activities. Med Lav 1991; 82(3):233–237. [In Italian]
45. Melamed S , Ben Avi I, Lua J, Green MS. Repetitive work ,work underload and coronary heart disease risk factors among blue collar workers, The CORDIS Study. J Psyshosom Res 1995; 39(1):19–29.
46. Neuberger M. Smoking and prevention at the work site. Wien Med Wochenschr 1995; 145(4):90–94..
47. Ohlander J, Keskin MC, Stork J, Radon K. Shift work and hypertension: Prevalence and analysis of disease pathways in a German car manufacturing company. Am J Ind Med 2015; 58(5): 549–560.
48. Hirose T, Ohtake Y, Machida M. The effect of fixed night work on blood pressure of male bakery workers in a processing. Sangyo Eiseigaki Zasshi 1995; 37(1):43–46. [Article in Japanese]
49. Frese M, Semmer N. Shift work, stress and psychosomatic complaints: a comparison between workers in different shift work schedules, non shift workers and former shift workers. Ergonomics 1986; 29:98–114.
50. Akerstedt T, Knutsson A, Alfredson L, Theorell T. Shift work and cardiovascular disease. Scand J Work Environ Healt 1984; 10: 409–414.
51. Knutsson A. Do shift workers face higher risk of coronary artery disease. In: Oginski A, Pokorski J, Rutenfranz J. Contemporary Advances in Shift Work Research. Krakow, Medical Academy 1987; 305–512.
52. Cervinka R, Kundi M, Koller M, Heider M, Arnof J. Shift related nutrition problems. In: Wedderburn A, Smith P. Psychological approaches to night and Shift work. Edinburgh, Herriot Watt University, 1984; 14:1–18.
53. Yang WY, Efremov L, Mujaj B, Zhang ZY, Wei FF, Huang QF, Thijs L, Vanssche T, Nawrot TS, Staessen JA. Association of office and ambulatory blood pressure with blood lead in workers prior to occupational exposure. J Am Soc Hypertens 2018; 12:14–24.
54. Solomenchuk, TM. The effect of lead compounds on the development of hypertension in workers in the radioelectronics industry. Vrach Delo 1995; 1–2:94–97.
55. Weddeen PR, Mallik KD, Batuman V. Detection and treatment of occupational lead nepropathy. Arch Intern Med 1979; 139:53–61.
56. Kuneva, T. The incidence of paraprofessional diseases in employees of the Research Institute of Ferros Metallurgy in Kremikovtsi. Probl Khig 1992; 17:218–227.
57. Mohammadi S, Golabadi M, Labbafinejad Y, Pishgahhadian F, Attarchi M. Effects of exposure to mixed organic solvents on blood pressure in non-smoking women working in a pharmaceutical company. Arh Hig Rada Toksikol 2012; 63(2):161–169.
58. Chrostek MJ, Czeczotko B. The evaluation of the health state of the workers occupationally exposed to low concentration of carbon disulphide (CS2). Part one: General medical examination and laboratory tests. Przegl Lek 1995; 52(5):249–251.
59. Stanosz S, Kuligonjski D, Pieleszek A, Zuk E, Rzechula D, Chlubek D. Concentration of dopamine in plasma, activity of dopamine beta-hydroxylase in serum and urinary excretion of free catecholamines and vanillylmandelic acid in women chronically exposed to carbon disulphide. Int J Occup Med Environ Health 1994; 7(3):257–261.
60. Misgeld V, Stolpmann HJ, Schultes S. Intoxication by vinil chloride polymers and /or their additives. Z Haut Geschlechtskrank 1973; 48:425–431.
61. Tania C, Misty JH, Kevin WH, Susan MV, Avima MR. Coronary Artery Disease and Cancer Mortality in a Cohort of Workers Exposed to Vinyl Chloride, Carbon Disulfide, Rotating Shift Work, and o-Toluidine at a Chemical Manufacturing Plant. Am J Ind Med 2014; 57(4):398–411.
62. Mariclj HR, Darke CS, Archibald R, Leroy EC. In vivo observations of skin capillaries in workers exposed to vinyl chloride, An English-American Comparison. Brit J Ind Med 1978; 35:1–12.
63. Anbazhagan S, Ramesh N, Surekha A, Fathima FN, Melina A. Estimation of work capacity and work ability among plantation workers in South India. Indian J Occup Environ Med 2016; 20(2):79–83.
64. de Croon EM, Blonk RW, de Zwart BC, Frings-Dresen MH, Broersen JP. Job stress, fatigue, and job dissatisfaction in Dutch lorry drivers: towards an occupation specific model of job demands and control. Occup Environ Med 2002; 59(6):355–362.
65. Delp L, Wallace SP, Geiger-Brown J, Muntaner C. Job stress and job satisfaction: home care workers in a consumer-directed model of care. Health Serv Res 2010; 45(4):922–940.
66. Chandola T, Brunner E, Michael M. Chronic stress at work and the metabolic syndrome: prospective study. BMJ 2006; 332(7540): 521–525.
67. Momen A, Mascarenhas V, Gahremanpour A, Zhaohui G, Moradkhan R, Kunselman A, Boehmer JP, Sinoway LI., Leuenberger UA. Coronary blood flow responses to physiological stress in humans. Am J Physiol Heart Circ Physiol 2009; 296(3): H854–H861.
68. Kubzansky LD, Thurston RC. Emotional vitality and incident coronary heart disease: benefits of healthy psychological functioning. Arch Gen Psychiatry 2007; 64(12):1393–1401.
69. Surtees PG, Wainwright NW, Luben RN, Wareham NJ, Bingham SA, Khaw KT. Psychological distress, major depressive disorder, and risk of stroke. Neurology 2008; 70:788–794.
70. Kotlęga D, Gołąb-Janowska M, Masztalewicz M, Ciećwież S, Nowacki P. The emotional stress and risk of ischemic stroke. Neurol Neurochir Pol 2016; 50(4):265-270.
71. Costa G. Cardiopathy and stress-inducing factors. Med Lav 2004; 95(2):133–139.
72. Chumaeva N, Hintsanen M, Ravaja N, Juonala M, Raitakari OT, Keltikangas-Järvinen L. Chronic stress and the development of early atherosclerosis: moderating effect of endothelial dysfunction and impaired arterial elasticity. Int J Environ Res Public Health 2009; 6(12):2934–2949.
73. Greenlund KJ, Kiefe CI, Giles WH, Liu K. Associations of job strain and occupation with subclinical atherosclerosis: The CARDIA Study. Ann Epidemiol 2010; 20(5):323–331.
74. Kiarri NK, Abbi D, Mercedes RC, Hilary AT, Kiang L. Chronic Stress and Endothelial Dysfunction: The Multi-Ethnic Study of Atherosclerosis (MESA). Am J Hypertens 2017; 30(1):75–80.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 47–54

UDC 576.3:612.112:616-053.2/.8
https://doi.org/10.22190/FUMB180102008H

Original Article

STRUCTURE OF RELATIONS BETWEEN THE FREQUENCY OF MICRONUCLEI IN PERIPHERAL BLOOD LYMPHOCYTES AND AGE, GENDER, SMOKING HABITS AND SOCIO-DEMOGRAPHIC FACTORS IN SOUTH-EAST REGION OF SERBIA

Andjelka Hedrih[1], Stevo Najman[2], Vladimir Hedrih[3], Olivera Milošević-Djordjević[4]

[1]Department of Mechanics, Mathematical Institute of Serbian Academy of Sciences and Arts, Belgrade, Serbia
[2]Department of Biology and Human Genetics, Faculty of Medicine, University of Niš, Niš, Serbia
[3]Department of Psychology, Faculty of Philosophy, University of Niš, Niš, Serbia
[4]Department of Genetics, Faculty of Science, University of Kragujevac, Serbia, Kragujevac, Serbia

Abstract. *Frequency of micronuclei (MN) in peripheral blood lymphocytes is a measure of genotoxicity and spontaneous chromosomal instability with many modifying factors. The aim of this work was to examine the relation between results of micronucleus test and set of socio-demographic parameters in healthy population in south-east Serbia using structure equation approach. Cytokinesis–block (CB) technique was used for analysis of MN frequency in peripheral blood lymphocytes on 133 healthy volunteers of various ages. Socio-demographic data was collected through a questionnaire. The mean frequency of MN gradually rose with age from 0.56±0.71 in new-borns to 5.48 ±3.65 in the 61-80 years age group (AG), with a decrease in the 81-92 years AG. MN frequency was positively correlated with age, altitude of birth place, altitude of place of residence, nuclear buds and nucleoplasmatic bridges, and negatively with education level and smoking habits. Linear structural model revealed age to be related to all of the examined variables, and indicates probable existence of another factor, independent of age, influencing all of these except nuclear buds. It can be concluded that the frequency of micronuclei is influenced by age and factor/s resembling socioeconomic status or lifestyle and this influence is independent of age.*

Key words: *micronuclei, age, gender, education level, socio-demographic factors.*

Introduction

Micronuclei (MN) are chromatin particles that are created as a result of chromosomal break or elimination of whole chromosomes as a consequence of mitotic spindle damage and a biomarker of genotoxic events [1–3] (and chromosomal instability [4]. Frequency of MN in peripheral blood lymphocytes of healthy subjects is environment specific [5] and increases with chronological age [6–13]. Specific increase of MN frequency with age was found in mice [14]. The origins of MN formation are reviewed recently [4,15].

According to [15] factors that affect MN frequency in healthy subjects are: type of cell line, gender, age, genotype, smoking, species and diet. For gender, results are conflicting: some studies report higher MN in women than in men [8,10,12,16–19] but some studies report no gender effect on MN frequency [13]. MN frequency increases with age. Mayer et al. (1989) report age and gender differences in DNA repairing mechanisms: "Decline in double-strand DNA break damage induction occurs more rapidly (by a factor of *ca.* 2) in women than in men" [20].

Results on the effects of smoking are also conflicting with some studies reporting no effect of smoking on MN frequency [10,13]. Kažimirova et al. (2006) did not find significant differences in micronucleus frequency between vegetarians and non–vegetarians across different age groups [17]. Regarding the relation between occupational exposure to electromagnetic fields and incidence of micronuclei, Lakshmi et al. (2010) point out that overall DNA damage and incidence of micronuclei showed no significant differences between the subjects with occupational exposure to electromagnetic fields emitted from video display terminals and control group. Only long-term users of video display terminals (more than 10 years) showed higher induction of DNA damage and increased frequency of micronuclei and micro nucleated cells [21].

In studies where no correlation was found between MN and gender, one explanation could be that although statistics show that women live longer than men, women's health conditions after the age of 80 are worse than those of men of equal age [22,23].

Many studies explore different social, biochemical, lifestyle and genetic factors that may influence health

Correspondence to: Andjelka Hedrih, Ph.D.
Department of Mechanics, Mathematical Institute of Serbian Academy of Sciences and Arts, Belgrade, Serbia, 36 Kneza Mihaila, 11000 Belgrade, Serbia
Phone: +381 64 80 13 320
E-mail: handjelka@turing.mi.sanu.ac.rs
Received January 2nd, 2018, Accepted June 28th, 2018

status especially in people over 70 years of age [22,24–28]. A decline in T cell function during aging is thought to contribute to reduced response to infection and vaccination and an increase in autoimmunity [29].

Steves et al. (2012) discussed why DNA sequence-identical twins might age differently [27]. Discussing epigenetic and early developmental hypotheses of ageing, Steves et al. (2012) suggest that longevity and lifespan seem not to be so heritable [27]. Some environmental factors like exercise, smoking and diet can strongly influence aging [27]. Other authors [30] found that long-lived parents tend to have children with longer life than their age peers.

Different organs age at different speeds [27,31] probably due to different antioxidant response and DNA repair [32,33)]. Liochev (2013) suggests that reactive oxidative species such as hydrogen peroxide and superoxide are not just causative agents of aging but may also be agents that in small concentrations increase the life span by increasing the upregulation of proteins that act as protective factors in oxidative stress thus providing adaptation (33].

The goal of the current study was to examine the relation between results of micronucleus test and set of socio-demographic parameters: personal factors such as gender, education level and microclimate factors of the environment a person lives in indicated by the altitude of birthplace and of current place of residence using structure equation approach. A working hypothesis was that people who lived in places with higher altitude, non-smokers and people with higher education will have lower frequency of MN due to life style and reduced risk factors for increased MN frequency.

Materials and Methods

Sample

One hundred thirty three healthy volunteers of various age (from new-borns – 0 years of age to 92 years) participated in the study. Participants were divided into 5 groups as follows: age group 1 – new-borns (29 participants of which 13 were females (f) and 16 were males (m); mean age was 0 years, second age group from 21-40 years (30 participants- of which 16 were f and 14 were m, mean age 25.1±4.56); third age group from 41-60 years (29 participants of which 16 were f and 13 were m, mean age 47.47±5.02); fourth age group from 61-80 years (26 participants of which 18 were f and 8 were m; mean age 71.88±4.55) and fifth age group -81 years and above (19 participants-of which 13 were f and 6 were m; mean age 85.18±3.33). Guided by the experience from previous studies (9) we have taken 5 different age groups (for population from 0-100 years) were each age group covers the period of 20 years.

Sampling procedure

From each participant 2 ml of blood was taken from elbow vein (v. cubitalis). From new-borns, blood was taken from umbilical cord after birth at the Clinic for Gynaecology and Obstetrics, Clinical Centre Nis, Serbia. New-borns from healthy mothers that fulfilled the above-mentioned criteria, without gestational diabetes [34], natural conception, regular pregnancy, without infections during pregnancy or consumption of antibiotics during pregnancy, term labour with regular duration, vaginal delivery, and Apgar score 9 or 10, were included in the study. New-borns whose mothers had prolonged labour or caesarean section were excluded from the study. New-borns that had elevated levels of bilirubin or C reactive protein within three days after birth were excluded from the study.

Variables and participants

Spontaneous chromosomal aberration was assessed by using the micronucleus test. Volunteers were recruited during regular control examinations in: the Health Centre Niš, Student Policlinic Nis, Gerontological Centre Niš, Clinic for Gynaecology and Obstetrics, Clinical Centre Nis, Brestovac medical station, and Health centre Bela Palanka, Serbia.

The study was approved by the Ethical committee of the Faculty of Medicine, University of Nis, Serbia (01-9068-7) and the Ethical committee of Clinical Centre Niš, Serbia (2280/11). All participants gave informed consent.

Participants were screened for health status that was assessed on the basis of biochemical (general blood count, sedimentation, blood sugar) and anamnesis data in order to exclude participants with health conditions known to influence the frequency of micronuclei. Exclusion criteria used were absence of diabetes, chemo-or radiotherapy at the moment and in the past, tumour/s, operations of tumour of any kind in the past, exposure to chemical and physical mutagens, alcohol and/or drugs abuse (absolute alcohol non-users, and occasional alcohol use, absolute narcotics and related substances non-users), HCV (Hepatitis C virus) and/or HBV (Hepatitis B virus) infection, dementia, heart attack survivors. Participants who passed exclusion criteria were considered healthy and included in the study. For collecting demographic and anamnestic data of interest (range of altitude of birthplace and place of residence, smoking habits, education level) a short questionnaire was used. Range of altitude of birthplace (300±184.14m above sea level, min 77m, max 1310m above sea level) and current place of residence (237.59±90.34m min 194m, max 574m above sea level) were used as a holistic indicator of the environment a person lives in. In the part of Serbia where the study was conducted, places of higher altitude (above 255m above sea level) tend to have colder climate with more snow and to be more sparsely populated and less developed compared to

places of lower altitude (city in the valley at 194m above sea level). Also, a number of studies have indicated that biological processes can be affected by altitude [35–38] (e.g. haemoglobin affinity for oxygen). Altitude of birthplace and of current place of residence were derived by looking up the data for the place participants indicated living or being born in and expressed in meters above sea level.

Analysis of MN frequency in peripherals blood lymphocytes was conducted by cytokinesis-block technique-modification according to the Fenech and Morley [4,5,9].

NDI was calculated according to the method of Eastmond and Tucker using http://www.nature.com/nprot/journal/v2/n5/full/nprot.2007.77.html - B107 the formula:

$$NDI = \frac{M_1 + 2M_2 + 3M_3 + 4M_4}{500} \text{ [39]}$$

where M1–M4 represents the number of cells with 1–4 nuclei.

Anamnestic/sociodemographic data

Smoking status was determined by asking participants if they were smokers (3), former smokers (2) or non-smokers (1). They were also asked to specify the number of cigarettes they smoke daily (0 for former and non-smokers). Age was registered as number of years since birth at the time the study was conducted (0 for new-borns). Education level was registered as the highest completed level of education (education level of mothers was registered for the new-borns).

Cytokinesis block micronucleus test (MN) protocol

The culture of peripheral blood lymphocytes was set up according to the modified Fenech and Morley method [9]. After 44 h of incubation at 37˚C (Celsius degree) in fully-supplemented medium for culture of peripheral blood lymphocytes for cytogenetic studies (PB-MAX karyotyping medium, Life Technologies 12557), cytochalasin B in final concentration of 4µg/ml was added. After that, cell cultures were incubated for another 24 hrs. Preparation was done in 0.56% KCl hypotonic solution. Fixation of material was conducted by fixative that consisted of glacial acetic acid and methanol (1:3v/v), according to the protocol: fixative was mixed with prepared sample and incubated for 15 min. After that, sample was centrifuged for 12 min on 1700r/min, than supernatant was discarded. This fixation procedure was repeated 3 times. Preparations were stained with 2% Giemsa solution. The analysis of MN as well as nucleoplasmatic bridges (NPB), nuclear buds were done on 1000 binucleated cells per each of 133 tested individuals. Nuclear division index (NDI) was calculated for each individual. The culture of peripheral blood lymphocytes from participants over 80 years of age was set up in double culture.

Statistics

Average value and standard deviation of micronuclei frequency per 1000 binuclear cells were calculated for each age group as well as correlations between the results of MN test and other variables included in the study. To further examine the nature of relationship between the examined variables a linear regression analysis was conducted. Finally, a linear structural model of the examined relationships was created using asymptotically distribution-free discrepancy estimation method as implemented by AMOS software.

Results

Average value and SD of MN and NDI for each age group are presented in Table 1. Distributions of NDI, BN cells with 1, 2 and 3 MN across age groups are shown in Figure 1. MN1 and MN2 increased significantly with age (Spearman's correlation $r = 0.650$, $p < 0.0001$; $r = 0.289$, $p < 0.001$ respectively). NDI did not show significant differences across age groups. Correlations between the results of micronucleus test and other variables included in the study were calculated. Results are shown in Table 2. To test if there are gender differences in NDI, BN cells with 1, 2 and 3 MN in the same age group the analysis of variance was performed. There were no significant differences between genders in tested variables, and this finding persisted throughout the age groups as can be seen in Table 3. Significant negative correlation was found between NDI and nucleoplasmatic bridges (NPB) ($r = –0.183$, $p < 0.05$)

Table 1 Mean micronuclei frequencies and nuclear division indices in studied age groups.

	Age range in years	n	MN (mean±SD)	NDI (mean±SD)
1	0	29	0.56 ± 0.71	1.52 ± 0.30
2	21-40	30	0.82 ± 0.78	1.72 ± 0.26
3	41-60	29	1.26 ± 1.48	1.68 ± 0.35
4	61-80	26	5.48 ± 3.65	1.55 ± 0.24
5	81-92	19	4.48 ± 2.69	1.62 ± 0.34
	Total mean ± S.D.	133	2.36 ± 2.92	1.62 ± 0.30

Legend: n – number of participants in a group. MN – micronuclei frequency per 1000 binuclear cells. NDI – nuclear division index. Means and standard deviations for each of the five age groups and for the total sample are presented.

Correlations between gender and micronuclei frequency were not statistically significant. In the current sample 66.9% were non-smokers, 40.6% of which females, 7.5% former smokers, 25.6% smokers, 15% of which females. Mean value of number of cigarettes/day and time period of smoking in years rise in the third age group (Fig. 2). New-borns were excluded from analysis of smoking status. Distributions of smoking status across age groups for females and males are presented in Figure 3.

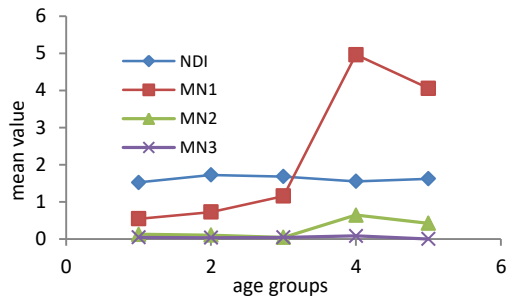


Fig. 1 Distribution of NDI, MN1, MN2, MN3 across age groups. Age groups (1-5) are presented on the horizontal axis. On vertical axis are mean values of NDI, MN1, MN2 and MN3 in each age group.

Table 2 Correlations of micronuclei frequency and studied socio-demographic factors

tested variables	Spearman correlation coefficient	p
Age (in years)	0.61	<0.001
Altitude of birth place (in meters above sea level)	0.34	<0.001
Altitude of current place of residence (in meters above sea level)	0.45	<0.001
Nuclear buds (number of cells with nuclear buds per 1000 binuclear cells)	0.34	<0.001
Nucleoplasmatic bridges (number of cells with nucleoplasmatic bridges per 1000 binuclear cells)	0.18	< 0.05
Education level	-0.39	<0.001
Smoking (1-Non-smoker, 2-former smoker, 3-smoker)	-0.28	<0.05
Number of smoked cigarettes per day	-0.25	<0.05

Table 3 Effect of gender and age interaction with NDI, MN1, MN2 and MN3

Source	Dependent Variable	df	F	Sig.
Gender X Age				
	NDI	4	0.32	0.87
	MN1	4	0.50	0.74
	MN2	4	0.37	0.83
	MN3	4	1.26	0.29

Legend: NDI – nuclear division index. MN1 – number of cells with one micronucleus per 1000 binuclear cells. MN2 – number of cells with two micronuclei per 1000 binuclear cells. MN3 – number of cells with three micronuclei per 1000 binuclear cells.

Older participants from the current sample tended to be born and live at higher altitude (10.5% of participants in the fifth age group live at 574m 36.8% at 395m and 47.4% at 194m above sea level; 53.8% of participants in the fourth age group live at altitude of 395m, 26.9% at altitude of 194m above sea level, 58.6% of participants in the third age group live at altitude of 200m 82.8% of participants in the first age group and 100% of participants in the second age group live at 194m above sea level), to be less educated (18.9% of participants did not finished primary school of which 6.8% and 6.1% belong to the fourth and fifth age group respectively; 12.9% finished primary school of which 5.3% and 3.8% belong to the fourth and fifth age group respectively; 23.5% have secondary school of which 9.1% belong to the third age group; 3.8% have college of which 1.5% belong to the fourth age group; 39.4% have faculty of which 22.7% belong to the second age group; 1.5% have PhD and they all belong to third age group), non-smoking and had a larger percentage of nuclear buds (NB) and nucleoplasmatic bridges, as can be seen in Fig. 4. All these correlations were statistically significant, and are presented in Table 2.

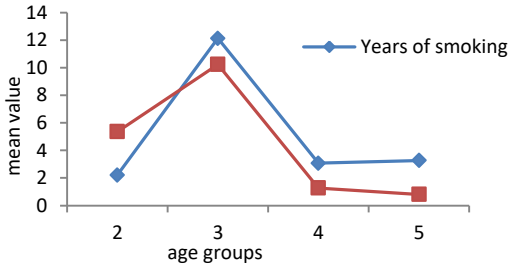


Fig. 2 Mean value of number of cigarettes /day and time period of smoking in years across different age groups. Age group 1, new-borns; Age group 2, 21–40 years; Age group 3, 41–60 years; Age group 4, 61–80 years; Age group 5, 81 years and above.

Taking into account the obtained results, further analyses were conducted in order to examine possible relationship between predictors that would shed additional light on nature of the obtained results. These investigations demonstrated a systematic relationship between age and the number of other included variables. To further examine the nature of relationship between the examined variables a linear regression analysis was conducted. Results show that, when age is included in the model, direct effects of a number of other predictors become statistically insignificant. Finally, a linear structural model of the examined relationships was created using asymptotically distribution-free discrepancy estimation method as implemented by AMOS software.

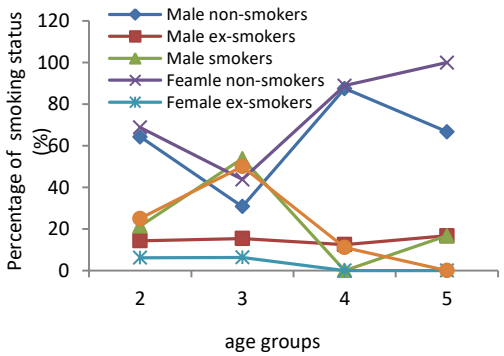


Fig. 3 Distributions of smoking status across age groups for females and males Age group 1, new-borns; Age group 2, 21–40 years; Age group 3, 41–60 years; Age group 4, 61–80 years; Age group 5, 81 years and above.

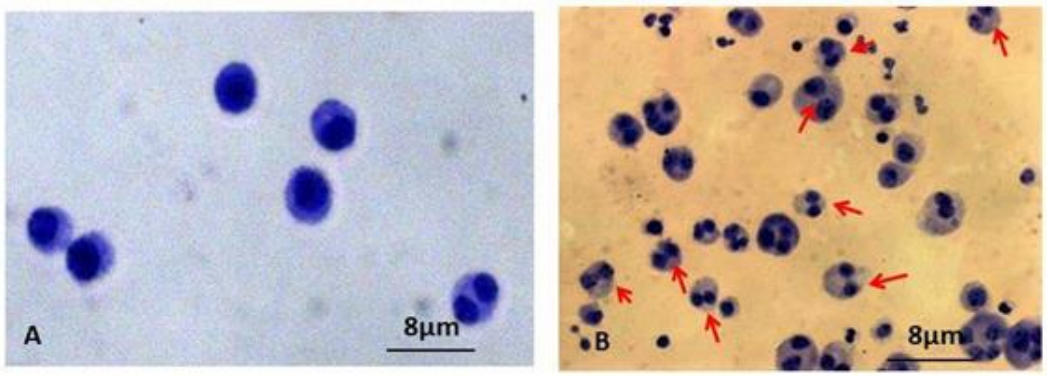


Fig. 4 MN of peripheral blood lymphocytes: A. from new-born baby-boy. B. from man of 90 years of age. NC bridges are visible on B.

This model is presented in Figure 5 and Table 4. The model has good fit parameters: GFI=0.988, AGFI= 0.972, CFI=0.985, NFI=0.899, RMSEA= 0.034, Chi-Square Statistic=17.129, Chi-Square p=0.311), Degrees of freedom=15. The model depicts age influencing MN, altitude of birth and living place, smoking habits, nucleoplasmatic bridges, and nuclear buds. It also shows that correlations of altitude of birthplace and of current place of residence, smoking habits, education level, nucleoplasmatic bridges, nuclear buds with results of micronucleus test, are in fact due to all of them being influenced by age. Results suggest the existence of another factor, independent of age, that influences all these variables expect nuclear buds. For the purposes of proposing and testing a structural model, regression weight of the proposed unknown factor with education level has been constrained to 1.

Table 4 Structural model parameter estimates

To	From	Regression estimate	Standard Error	Statistical significance
Micronuclei	Age	0.505	0.049	p<0.001
Birthplace altitude	Age	0.436	0.070	p<0.001
Altitude of the place person lives in	Age	0.418	0.075	p<0.001
Education	Age	-0.374	0.079	p<0..001
Micronuclei	X	-0.845	0.283	0.003
Education	X	1.000		
Smoking	X	1.122	0.340	p<0.001
Nucleoplasmic bridges	X	-.0992	0.420	0.018
Smoking	Age	-0.204	0.078	0.009
Nucleoplasmic bridges	Age	0.130	0.057	0.022
Elevation of the place person lives in	X	-1.916	0.461	p<0.001
Birthplace elevation	X	-2.028	0.474	p<0.001
Nuclear buds	Age	0.249	.0066	p<0.001

Discussion

The results presented here showed that mean MN frequency does not increase linearly with age; in the 5th age group (participants over 80 years of age) it decreases, compared to the 4th age group. A probable explanation for this finding is that 80+ year olds that also pass the exclusion criteria to be included in the study are a group of individuals with much better health compared to an average person of this age. As the age of 80 is well above the mean life expectancy for the population of Serbia (which is around 75), it can be argued that 80+ year olds that lived to this age have unique genetic background that allowed them to live and stay healthy up to the age they have. The MN frequency is just one of the indicators of this. It is possible that in this age group, spontaneous chromosomal instability is stabilised at the lower level. In support of this hypothesis are results of the study [40] which reported no correlation between telomeres shortening and age in 79 years old subjects. Harrisa et al. (2012) found that telomeres shortening in people of 70 years of age did not correlate with their socio-economic status [26]. Telomere shortening is connected with oxidative stress and it is possible that the level of oxidative stress is lower in this age group [40]. Beside long-life experience and adaptation to stressful situations [41], lower level of stress in this age group could be explained by reduced caloric intake and gradual decrease in proliferative cell capacities [9,43] suggesting that mechanisms leading to the formation of micronuclei containing sex chromosomes differ with age. They also report the nonlinear increase of MN frequency with age-after 70 years of age it decreases [42].

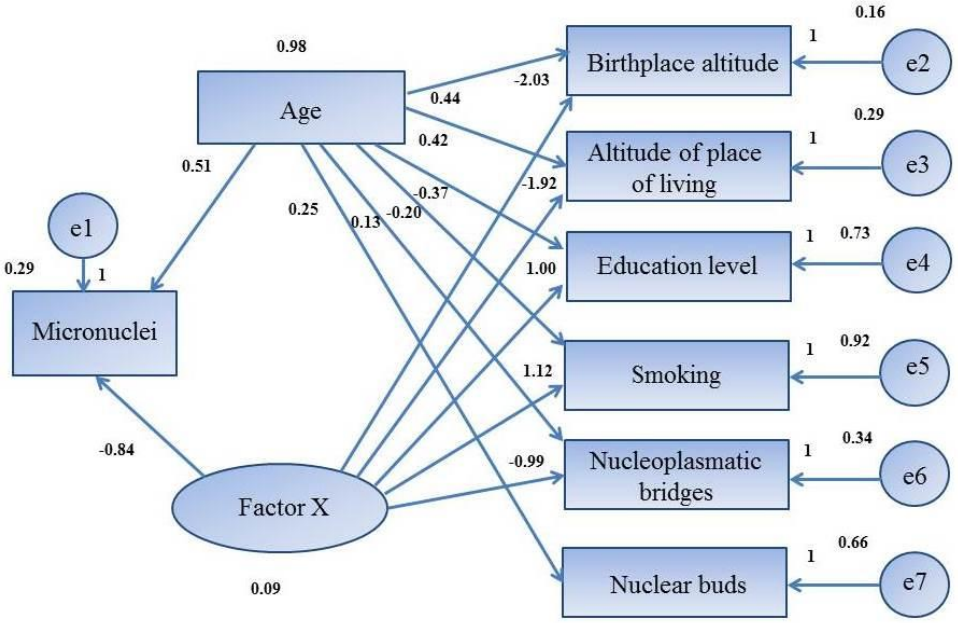


Fig. 5 Structure linear model relationship between age and unknown Factor X and altitude of birth and living place, education level, smoking habits, nucleoplasmatic bridges, nuclear buds and micronuclei. Numbers represent structural coefficients for each relation.

Cigarette smoking is an important and established risk factor in many age-related diseases, and is associated with increased cumulative and systemic oxidative stress and inflammation like increased circulating blood leucocytes [43] and have been linked to poorer health outcomes and associated with shorter telomeres (Valdes et al. by [26]. Negative correlations between MN frequency and number of smoked cigarettes/day were obtained. Some recent studies reported smoking having no effect on MN frequency [10,13], while other reported positive correlations between MN frequency and smoking [11,12]. Smoking more than 30 cigarettes/day increased the MN frequency [8]. Our results showed negative correlation between education level and MN frequency which is in accordance with results of study in Poland (Szklarska and Rogucka 2001), they found relation between education level and biological age: better-educated men were biologically younger than their poorer educated peers [44]. Linear structural model shows that age is the factor explaining all of the above mentioned variables, although we know that the linear relationship does not hold with the oldest age group (if it did, the relationship with age, described by using linear statistics, would be even stronger). The explanation for this could be in social changes that occurred during the second half of the 20th century, like migration to cities, increasing education levels and changes in smoking habits.

Tobacco is a habit originating in cities [45,46] and so elder participants in the current sample who live in villages at high elevation tend to be non-smokers. Chromosomal changes that can cause NPB and NB become more frequent with age [4]. In the current sample there were no gender differences in MN frequency across different age groups.

In the study of 50 healthy children from urban areas in Croatia, ages from 4–14 years, girls showed higher mean values of the mean frequency of MN, mean frequency of nucleoplasmic bridges (NPBs), nuclear buds and the mean nuclear division index (NDI) compare to boys, but only total number of NPBs was statistically significant [5]. In the current study the mean frequency of MN for all subjects was 2.32±0.28 per 1000 BN cells which is higher value than the level obtained for the second age group. That could be due to differences in environmental factors in urban area [47]. As Regan et al. reported [23], when female-specific health dimensions are considered, females are found to accumulate more deficits than males and speculate that such opposite trends for males and females can result in no gender differences. In the current sample there were no differences in MN frequency between males and females for the same age group. Results of [23] suggest that aging rates approximated by the rates of changes in respective deficit indices (Dis) are not altered by gender but are specific for place of living. Similar results were obtained [28] with Italian siblings of 90 years of age and older from different parts of Italy. Large longitudinal study of aging Danish twins confirmed negative influence of sun exposure, smoking and of low BMI on facial ageing. This study indicated that high social status, low depression score and being married are associated with a younger appearance, but the strength of the associations varied between genders [24]. Basal level of MN frequency in healthy population is different in different

nations and may vary due to characteristics and genetic origin of the sample [4,9,10,11,13].

Relative to the unknown factor that independently of age, influences all these variables expect nuclear buds, we are free to suggest that it could be a lifestyle component that varies across age groups (middle age people tend to live in urban areas and lead more stressful lives, while elderly people from the current sample are 20-30 years in retirement, lead active but non–stressful lives. It is possible that in the 5th age group natural selection was the most pronounced - in childhood they were exposed to more stressful environmental factors than participants in other age groups (they survived Second World War, and scarcity of basic goods in the post-war years), and other large social changes, like massive migrations to cities, occurred during their lifetimes. Studies showed that animals exposed to stress early become more adaptive to stress [7]. Relation between lifestyle and telomere length was discussed by Lin et al. [48] suggesting several inter-related biochemical pathways: stress hormones, inflammation and oxidative stress [48]. It is possible that unique selection factors that were present in generation born in the previous century as well as cultural differences contributes to results we obtained, but more detailed study is required to test these hypotheses.

Conclusion

The study has demonstrated a clear relation between the frequency of micronuclei and age. Age can also be taken to explain the relations of several other studied variables with the frequency of micronuclei. Another factor that independently of age influences all examined variables except nuclear buds was proposed based on the data. According to our results MN frequency is related to age and unknown factor, which seems to be some aspect of socio-economic/demographic status. Further analyses are needed to determine this factor. Determining the nature of this factor could contribute to better understanding the process of aging and contributions of non-genetic factors on process of aging.

Acknowledgements: *We thank all examinees that participated in the study. Special thanks to Gerontology Center Niš for selecting the participants, to Dr. Ivana Stankovic and Health centre Bela Palanka for selecting the examinees above 80 years of age, Dr. Olga Stojanovic from Clinic Brestovac and Student Policlinic Nis for excellent cooperation. Parts of this research were supported by the Ministry of Education, sciences and technology of the Republic of Serbia through Mathematical Institute SASA, Belgrade, Grant ON 174001 and Grant III41010 through Faculty of Science University of Kragujevac.*

References

1. Martino-Roth MG, Viégas J, Amaral M, Oliveira L, Ferreira FLS and Erdtmann B. Evaluation of genotoxicity through micronuclei test in workers of car and battery repair garages. Genet Mol Biol 2002; 25(4):495–500.
2. Silva-Barbosa I, Pereira-Magnata S, Amaral A, Sotero G, Homero Melo C. Dose assessment by quantification of chromosome aberrations and micronuclei in peripheral blood lymphocytes from patients exposed to gamma radiation. Genet Mol Biol 2005; 28(3):452–457.
3. Diler SB, and Ergene S. Nuclear anomalies in the buccal cells of calcite factory workers. Genet Mol Biol 2010; 33(2):374-–378.
4. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 2011; 26(1):125–132.
5. Gajski G, Gerica M, Oreljanin V, Garaj-Vrhova V. Cytogenetic status of healthy children assessed with the alkaline comet assay and the cytokinesis-block micronucleus cytome assay. Mutat Res 2013; 750(1–2):55–62.
6. Dass SB, Ali SF, Heflich RH, Casciano DA. Frequency of spontaneous and induced micronuclei in the peripheral blood of aging mice. Mutat Res 1997; 381(1):105–110.
7. Bolognesi C, Lando C, Forni A, Landini E, Scarpato R, Migliore L, Bonassi S. Chromosomal damage and ageing: effect on micronuclei frequency in peripheral blood lymphocytes. Age Ageing 1999; 28(4):393–397.
8. Bonassi S, Fenech M, Lando C, Lin YP, Ceppi M, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, et al. Human micronucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. Environ Mol Mutagen 2001; 37(1):31–45.
9. Milosevic-Djordjevic O, Grujicic D, Novakovic T, Arsenijevic S, and Marinkovic D. Micronuclei and Ageing in a Sample of Yugoslavian Population. Genetica 2002; 38(2):264–267.
10. Garaj-Vrhovac V, Đurinec M, Kopjar N, Oreščanin V. A survey on the cytogenetic status of the Croatian general population by use of the cytokinesis-block micronucleus assay. Mutat Res 2008; 649(1–2):91–100.
11. Donmez-Altuntas H, Bitgen N. Evaluation of the genotoxicity and cytotoxicity in the general population in Turkey by use of the cytokinesis-block micronucleus cytome assay. Mutat Res 2012; 748(1–2):1–7.
12. Nefic H, Handzic I. The effect of age, sex, and lifestyle factors on micronucleus frequency in peripheral blood lymphocytes of the Bosnian population. Mutat Res 2013; 753(1):1–11.
13. Coskun M, Akın Cayır, Coskun M, Tok H. Evaluation of background DNA damage in a Turkish populationmeasured by means of the cytokinesis-block micronucleuscytome assay. Mutat Res 2013; 757(1):23–27.
14. Sato S-I, Taketomi M, Nakajima M, Kitazawa M, Shimada H, Itoh S, Igarashi M, Higashikuni N, Sutou S, Sasaki YF. Effect of aging on spontaneous micronucleus frequencies in peripheral blood of nine mouse strains: the results of the 7th collaborative study organized by CSGMT/JEMS MMS. Mutat Res 1995; 338(1–6):51–57.
15. Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Vande Loock K, Decordier I. The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch Toxicol 2011; 85(8):873–899.
16. Fenech M, Neville S, Rinaldi J. Sex is an important variable affecting spontaneous micronucleus frequency in cytokinesis-blocked lymphocytes. Mutat Res 1994; 313(2–3):203–207.
17. Kažimirova M, Barančokova M, Krajčovičova-Kudlačkova M, Volkovová K, Staruchová M, Valachovicová M, Pauková V, Blazícek P, Wsólová L, Dusinská M. The relationship between micronuclei in human lymphocytes and selected micronutrients in vegetarians and non-vegetarians. Mutat Res 2006; 611(1–2):64–70.

18. Dahlström Heuser V, de Andrade VM, Peres A, de Macedo Braga LMG, Chies JAB. Influence of age and sex on the spontaneous DNA damage detected by Micronucleus test and Comet assay in mice peripheral blood cells. Cell Biol Int 2008; 32(10):1223–1229.
19. Bhilwade HN, Jayakumar S, Chaubey RC. Age-dependent changes in spontaneous frequency of micronucleated erythrocytes in bone marrow and DNA damage in peripheral blood of Swiss mice. Mutat Res 2014; 770:80–84.
20. Mayer PJ, Lange CS, Bradley MO, Nichols WW. Gender differences in age-related decline in DNA double-strand break damage and repair in lymphocytes. Ann Hum Biol 1989; 18(5):405–415. Published online: 09 Jul 2009.http://dx.doi.org/10.1080/03014469100001702.
21. Lakshmi NK, Tiwari R, Bhargava SC and Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs) Genet Mol Biol 2010; 33(1):154–158.
22. Martin-Ruiz C, Jagger C, Kingston A, Collerton J, Catt M, Davies K, Dunn M, Hilkens C, Keavney B, Pearce SH, den Elzen WP, Talbot D, Wiley L, Bond J, Mathers JC, Eccles MP, Robinson L, James O, Kirkwood TB, von Zglinicki T. Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85+ study. Mech Ageing Devl 2011; 32(10):496–502.
23. Regan JC, Partridge L. Gender and longevity: Why do men die earlier than women? Comparative and experimental evidence. Best Pract Res Clin Endocrinol Metab 2013; 27(4):467–479.
24. Rexbye H, Petersen I, Johansen M, Klitkou L, Jeune B, Christensen K. Influence of environmental factors on facial ageing. Age Ageing 2006; 35(2):110–115.
25. Johnson TE. Recent results: Biomarkers of aging. Exp Gerontol 2006; 41(12):1243–1246.
26. Harrisa SE, Martin-Ruizc C, von Zglinickic T, Starrd JM, Deary IJ. Telomere length and aging biomarkers in 70-year-olds: the Lothian Birth Cohort 1936. Neurobiol Aging 2012; 33(7):1486.e3–8.
27. Steves CJ, Spector TD, Jackson SHD. Ageing, genes, environment and epigenetics: what twin studies tell us now, and in the future. Age Ageing 2012; 41(5):581–586.
28. Cevenini E, Cotichini R, Stazi MA, Toccaceli V, Scurti M, Mari V, Berardelli M, Passarino G, Jeune B, Franceschi C. How to classify the oldest old according to their health status: A study on 1160 subjects belonging to 552 90+ Italian sib-ships characterized by familial longevity recruited within the GEHA EU Project. Mech Ageing Dev 2013; 134(11–12):560–569.
29. Torrão RC, Bennett SJ, Brown JE, Griffiths HR. Does metabolic reprogramming under pinage-associated changes in T-cell phenotype and function? Free Radic Biol Med 2014; 71:26–35.
30. Klein BEK, Knudtson MD, Lee KE, Klein R. Parents' attained age and biomarkers of aging in their children. Arch Gerontol Geriat 2009; 49(2):284–8.
31. Fyhrquist F, Saijonmaa O. Telomere length and cardiovascular aging. Ann Med 2012; 44 Suppl 1:S138–42.
32. Radak Z, Koltai E, Taylor AW, Higuchi M, Kumagai S, Ohno H, Goto S, Boldogh I. Redox-regulating sirtuins in aging, caloric restriction, and exercise. Free Radic Biol Med 2013; 58:87–97.
33. Liochev SI. Reactive oxygen species and the free radical theory of aging. Free Radic Biol Med 2013; 60:1–4.
34. Witczaka M, Ferenca T, Gulczynskab E, Nowakowskac D, Łopaczynskaja D, Wilczynskic J. Elevated frequencies of micronuclei in pregnant women with type 1diabetes mellitus and in their newborns. Mutat Res 2014; 763:12–17.
35. Moore LG. Human Genetic Adaptation to High Altitude. High Alt Med Biol 2004; 2(2):257–279.
36. Storz FJ. Perspectives. Genes for High Altitudes. Science 2010; 329(5987):40–41.
37. van Patot MCT, and Gassman M. Hypoxia: Adapting to high altitude by mutating EPAS-1, the gene encoding HIF-2α. High Alt Med Biol 2011; 12(2):157–167.
38. Lorenzo RF, Huff C, Myllymäki M, Olenchock B, Swierczek S, Tashi T, Gordeuk V, Wuren T, Ri-Li G, McClain AD, Khan MT, Koul AP, Guchhait P, Salama EM, Xing J, Semenza LG, Liberzon E, Wilson A, Simonson S.T, Jorde B.L, Kaelin Jr GW, Koivunen P, Prchal TJ. A genetic mechanism for Tibetan high-altitude adaptation. Nat Genetics 2014; 46(9):951–956.
39. Fenech M. Cytokinesis-block micronucleus cytome assay. Nat Protoc 2007; 2(5):1084–1104.
40. Starr JM, Shiels PG, Harris SE, Pattie A, Pearce MS, Relton CL, Deary IJ. Oxidative stress, telomere length and biomarkers of physical aging in a cohort aged 79 years from the 1932 Scottish Mental Survey. Mech Ageing Dev 2008; 129(12):745–751.
41. Mohammed AH, Henriksson BG, Soderstrom S, Ebendal T, Olsson T, Seckl JR. Environmental influences on the central nervous system and their implications for the aging rat. Behav Brain Res 1993; 57(2):183–191.
42. Jonesa KH, Yorkb TP, Jackson-Cooka C. Mechanisms leading to the formation of micronuclei containing sex chromosomes differ with age. Mutat Res 2012; 747(2):207–217.
43. Babizhayev MA, Savel'yeva EL, Moskvina SN, Yegorov YE. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther 2011; 18(6):209–226.
44. Szklarska A, and Rogucka E. An assessment of biological age of males in Poland. Ann Hum Biol2001; 28(1):30–37.
45. Liu L, Edland S, Myers MG, Hofstetter CR, Al-Delaimy WK. Smoking prevalence in urban and rural populations: findings from California between 2001 and 2012. Am J Drug Alcohol Abuse 2016; 42(2):152–161.
46. Liu Y, Gao J, Shou J, Xia H, Shen Y, Zhu S, Pan Z. The Prevalence of Cigarette Smoking Among Rural-to-Urban Migrants in China: A Systematic Review and Meta-Analysis. Subst Use Misuse 2016; 51(2):206–215.
47. Pedersen M, Wichmann J, Autrup H, Dang DA, Decordier I, Hvidberg M, Bossi R, Jakobsen J, Loft S, Knudsen LE. Increased micronuclei and bulky DNA adducts in cord blood after maternal exposures to traffic-related air pollution. Environ Res 2009; 109(8):1012–1020.
48. Lin J, Epel E, Blackburn E. Telomeres and lifestyle factors: roles in cellular aging. Mutat Res 2012; 730(1–2):85–89.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 55–60

UDC 616-008.6:616.12-331.1:616.133-004.6
https://doi.org/10.22190/FUMB180424009K

Original Article

PREVALENCE OF METABOLIC SYNDROME IN PATIENTS WITH ARTERIAL HYPERTENSION AND ITS IMPACT ON ASYMPTOMATIC CAROTID ATHEROSCLEROSIS

Svetlana Kostić[1], Ivan Tasić[1,2], Dragan Djordjević[1,2], Viktor Stoičkov[1,2], Nikola Stojanović[1]

[1]*Institute for Therapy and Rehabilitation "Niška Banja", Niška Banja, Serbia*
[2]*Faculty of Medicine, University of Niš, Niš, Serbia*

Abstract. *The importance of metabolic syndrome (MetSy) lies in the fact that its components are proven risk factors for early blood vessel atherosclerosis and thrombosis.Aim of the paper: Our aim was to establish the prevalence of MetSy in patients with arterial hypertension (AH) and its impact on asymptomatic carotid atherosclerosis. The study involved 391 examinees, divided into two groups. The study group consisted of patients with arterial hypertension (n=342; average age, 66.56 ± 09.52; with 51% of female gender). The presence of cardiovascular (CV) risk factors was established for all involved patients, cardiovascular risk score was determined (SCORE risk), laboratory analyses were performed, as well as anthropometric measurements and color Doppler sonography of the great blood vessels of the neck. The patients with AH were divided into two groups according to the presence of MetSy. Metabolic syndrome was confirmed in 198 patients who comprised group I; there were 144 examinees without MetSy and these comprised group II. Those with MetSy had a greater average number of CV risk factors, a higher SCORE risk score, higher body mass index (p<0.0001), and more frequently had diabetes, hyperlipidemia and obesity. The thickness of the intimal medial complex (IMC) of the carotid arteries was significantly greater in the group with MetSy (p<0.0001) – 51% of examinees had IMC thickness ≥0.90. The patients with MetSy more commonly had one or more carotid plaques (p=0.03), a higher average number of plaques (p=0.01) and percentage of stenosis (p=0.01). As the most important factors associated with early carotid atherosclerosis, multivariant regression analysis singled out the following (for the model R=0.512. R2=0.262. adjusted R2=0.255. standard error of the estimate = 0.174; p<0.0001): age (coefficiant β=0.331. p<0.0001), number of MetSy components (coefficient β=0.158. p=0.002), level of serum uric acid (coefficient β=0.284; p<0.0001). Our results demonstrated a significant association of MetSy and its components with early atherosclerotic changes in the carotid arteries.*

Key words: *metabolic syndrome, arterial hypertension, asymptomatic carotid atherosclerosis, carotid intima-media thickness, carotid plaque.*

Introduction

Metabolic syndrome (MetSy) represents a group of cardiovascular risk factors, such as obesity, hypertension, insulin resistance and dyslipidemia. The importance of MetSy lies in the fact that its components are proven risk factors for early blood vessel atherosclerosis and thrombosis [1], and individuals with MetSy have a three times greater risk to develop cardiovascular complications compared to those who do not have the syndrome [1,2]. Further, adult individuals with MetSy are exposed to a five-time greater risk of type 2 diabetes mellitus [3]. There are several definitions of MetSy proposed by various associations, such as World Health Organization (WHO), International Diabetes Federation (IDF), American Heart Association and National Heart, Lung and Blood Institute (AHA/NHLBI), National Cholesterol Education Program, Third Adult Treatment Panel (NECP/ATP III), and European Group for the Study of Insulin Resistance (EGIR). The definition by the expert team of the Third Panel of the National Cholesterol Education Program is based on the presence of three of the following five criteria: abdominal obesity, hypertriglyceridemia, low HDL cholesterol, elevated blood pressure and hyperglycemia [4]. The prevalence of MetSy varies considerably depending on the criteria used to diagnose the condition.

In patients without manifest cardiovascular disease, the thickness of the intimal medial complex (IMC) and ankle-brachial (pressure) index (ABI) represent non-invasive surrogate markers of atherosclerosis associated with an increased risk for cardiovascular morbidity and mortality [5]. The literature data suggest a clear association between cardiovascular risk factors and intimal medial thickness, as well as between intimal medial thickness and presence of clinical manifestations of coronary disease [6–8]. It has been shown that intimal medial thickness of the carotid arteries directly correlates with the involvement of cerebral, peripheral, and coronary vascular bed by the process of atherosclerosis [6].

Correspondence to: Ivan Tasić, M.D., Ph.D.
Faculty of Medicine, 81 Zoran Đinđić Blvd., 18000 Niš, Serbia
Phone: +381 64 86 09 295
E-mail: dr.ivan.tasic@gmail.com
Received April 24th, 2018, Accepted December 18th, 2018

Aim of the paper

Our aim in this paper was to establish the prevalence of metabolic syndrome in patients with arterial hypertension (AH), as well as its impact on asymptomatic carotid atherosclerosis.

Methods

The study involved 391 examinees divided into two groups. The study group consisted of the patients with arterial hypertension (n=342; average age, 66.56 ± 09.52; with 51% of women). In all the patients the presence of cardiovascular risk factors was established, cardiovascular risk score was determined (SCORE risk), laboratory analyses were performed, as well as anthropomentric measurements and color Doppler sonography of the great blood vessels of the neck.

Analysis of the risk factors

Based on the patient history and relevant medical records, the presence of both modifiable and unmodifiable risk factors for cardiovascular diseases was analyzed: AH, hyperlipidemia, smoking, diabetes, obesity, gender and age. Hypertension was defined based on the anamnestic data, medical records, measured systolic blood pressure values of over 140 mmHg and diastolic blood pressure of over 90 mmHg, or use of antihypertensive medications. The examinees with total cholesterol values of over 5.0 mmol/L and/or triglycerides of over 1.7 mmol/L who were taking hypolipemic therapy were considered to be hyperlipidemic. The presence of diabetes was confirmed based on patient history information, available medical records, measured fasting glucose values of over 5.6 mmol/L, or use of oral hypoglycemic medication or insulin therapy. The examinees with body mass index (BMI) ≥30 kg/m² were considered to be obese. An interactive electronic version of the SCORE risk charts from the European Society of Cardiology guidelines on CVD prevention (prepared by the Third Joint Working Group of the European Association of Preventive Cardiology – *Heartscore*) was used to assess the risk of fatal cardiovascular events in our examinees.

Metabolic syndrome was diagnosed in accordance with the NECP/ATP III metabolic syndrome diagnostic criteria (The National Cholesterol Education Program, Adult Treatment Panel).

Laboratory analyses

General laboratory analyses were done in all the examinees. Values of the following laboratory parameters were measured: total cholesterol, HDL and LDL cholesterol fractions, triglycerides, creatinine, serum uric acid (employing standard laboratory methodology and using a Humastar 600 biochemical analyzer). Creatinine clearance was determined using the Cockcroft-Gault formula.

Anthropometric measurements

Anthropometric measurements involving body weight, body height, waist and hip circumference measurements were done in all examinees in order to get an insight into their nutritional status. The measurements were performed with light clothes and without any footwear. Body mass index (BMI) was determined using the following formula: body weight in kg/body height in m^2.

Color Doppler sonography (CDS) of the great blood vessels in the neck

Color Doppler sonography of the great blood vessels in neck was done in all our examinees using the ultrasound scanner system EsaoteBiomedica, My Lab60 Xvision, with a multifrequency linear probe of 4–13 MHz. Intraluminal lesions were determined using B-mode imaging and defined as changes in the form of IMC thickening and appearance of plaques as focal intimal thickenings. IMC thickness was measured in the posterior wall of the common carotid artery 2 cm away from the top of the bifurcation, in the region without any focal changes. Longitudinal images of the common carotid artery and its branches (internal and external carotid artery) were analyzed bilaterally, determining the diameter of stenosis and analyzing plaque properties. Interpretations were based on the combination of B-mode, i.e. real-time tissue imaging, and Doppler spectral analysis.

Statistical data processing

The following statistical parameters were presented in the description: arithmetic mean (Xsr), standard deviation (SD), absolute value and percentage (%) of attributive properties. Comparison of the mean values of numerical properties between the two groups of examinees was done using the Student's t-test. Linear regression analysis (by Spearman) was used to evaluate the association of the factors of interest. The factors demonstrating a considerable impact on dependent variables in univariate analysis were included in multivariate models as independent variables. Statistical data processing was done using the SPSS software, 17.0 version. In all the analyses, the assessment error of 0.05 or 5% was taken as the cut-off of statistical significance.

Results

The patients with hypertension were divided into two groups by the presence of MetSy. Metabolic syndrome was confirmed in 198 patients and these comprised group I, while 144 examinees were without MetSy and these comprised group II. There was no age difference between the examinees with and those without MetSy. Those with MetSy had a greater average number of risk factors for cardiovascular diseases (CVD), a higher CV risk score – SCORE, and higher body mass index

(p<0.0001), and in this group of patients, diabetes, hyperlipidemia and obesity were more common (p <0. 0001), (Table 1).

Table 1 Comparison of characteristics of the groups of examinees with and without metabolic syndrome

Characteristic	I group n=198	II group n=144	p value
Age (in years)	66.80±9.76	66.24±9.17	n.s.
Average number of risk factors	2.69±0.82	1.85±0.58	0.0001
SCORE risk	5.52±3.13	4.42±2.68	0.001
Number of MetSy components	3.47±0.70	1.76±0.45	0.0001
Hyperlipidemia (%)	162 (81.2)	91 (63)	0.0001
Diabetes mellitus (%)	59 (30)	3 (2.1)	0.0001
Smoking (%)	26 (13)	18 (12.5)	n.s.
Obesity (%)	110 (55.5)	14 (9.7)	0.0001
BMI (kg/m^2)	30.31±4.50	25.71±3.03	0.0001

n.s. – no statistically significant difference between the two groups

Examinees from the group I (with MetSy) had higher values of fasting glucose in the serum, higher levels of triglycerides and uric acid (p<0.0001), and lower values of HDL cholesterol fraction (Table 2).

Table 2 Overview of laboratory analyses performed in the studied groups

Characteristic	I group n=198	II group N=144	p value
Glycemia (mmol/L)	6.25±1.98	5.47± 1.24	0.0001
Cholesterol (mmol/L)	5.52±1.33	5.71± 1.15	0.14
LDL-cholesterol (mmol/L)	3.53±1.28	3.65± 0.98	0.32
HDL-cholesterol (mmol/L)	1.12±0.27	1.36± 0.35	0.0001
Triglycerides (mmol/L)	2.35±1.51	1.42± 1.05	0.0001
Uric acid (μmol/L)	348.17±92.16	302.89±80.86	0.0001
Creatinine (μmol/L)	86.86±22.81	82.11±22.01	0.05
Creatinine clearance (ml/min)	76.57±20.55	84.54±28.37	0.0001

Analysis of color Doppler ultrasound of the great blood vessels in the neck

The thickness of the carotid IMC was significantly greater in the group with MetSy compared to those without the condition (p<0.0001), and 51% of examinees had IMC thickness of ≥0.90. Those with MetSy more commonly had one or more carotid plaques (p=0.03), a greater average number of plaques (p=0.01) and a higher percentage of stenosis (p=0.01) compared to group II examinees (Table 3).

Table 3 Color Doppler sonography of the great blood vessels in the neck in the studied groups

Characteristic	I group n=198	II group n=144	p value
Thickness of the carotid IMC (mm)	0.91±0.21	0.81±0.17	<0.0001
Increased IMC thickness, n(%)	101 (51)	47 (32.6)	<0.001
Presence of carotid plaques, n (%)	142 (71)	88 (61)	0.03
Average number of plaques	1.62±1.35	1.27±1.20	0.01
Average percentage of stenosis	41.12±12.02	37.54±9.01	0.01

Analyzing the carotid plaque characteristics, it was found that in the MetSy group plaques were most commonly fibrocalcified (29%), then fibrous (22%) and calcified (19%), and in the group without MetSy fibrous plaques were most prevalent (28%) (Table 4).

Table 4 Carotid plaque characteristics in the studied groups

Plaque characteristics, n (%)	I group - MetSy		II group - without MetSy	
Lipid	0	(0)	0	(0)
Fibrolipid	2	(1)	3	(2.1)
Fibrous	44	(22)	40	(28)
Fibrocalcified	57	(29)	31	(21.5)
Calcified	38	(19)	14	(9.7)

As the most significant factors associated with early carotid atherosclerosis, multivariant regression analysis singled out the following (for the model R=0.512. R^2=0.262. adjusted R^2=0.255. standard error of the estimate = 0.174; p<0.0001): age (coefficiant β=0.331. p<0.0001), number of MetSy components (coefficient β=0.158. p=0.002), and serum level of uric acid (coefficient β=0.284; p<0.0001).

Discussion

Disorders of lipid and glucose metabolism, obesity and AH are defined as risk factors for cardiovascular diseases, and these are at the same time the components of MetSy [1]. The prevalence of MetSy has been on the rise worldwide, but it is difficult to establish the prevalence more precisely due to different criteria used to define the condition. The INTERHEART study, performed in 52 countries throughout the world, demonstrated the prevalence of MetSy to be about 26% [9]. According to the data of the National Health and Examination Survey, the prevalence of MetSy in the United States of America changes with age; in men below 40 years of age it is 20%, and in women of the same age it is 16%, while the prevalence of the condition in individuals of either gender aged over 60 years even exceeds 50% [10].

In our examinees with AH, the prevalence of MetSy was significantly higher compared to controls, as well as the average number of present MetSy components. Those with AH and MetSy had a greater average number of risk factors for cardiovascular diseases, a higher CV risk score – SCORE, higher BMI, and diabetes, hyperlipidemia and obesity were more common in these individuals (Table 1).

In the PIUMA study in Umbria, Italy, 1.742 examinees without any manifest CV disease were prospectively observed for 10.5 years on the average; MetSy was established in 34% of them. Those with MetSy were older, and with longer history of AH and higher systolic blood pressure values. It was shown that MetSy was an independent predictor of future cardiovascular diseases in individuals of either gender with essential AH, and this prognostic effect of MetSy was not dependent on the traditional cardiovascular factors of risk, including left ventricular hypertrophy and ambulatory blood pressure measurement [11]. In the PIUMA study, the prevalence of diabetes was 14% [11], while in our examinees with MetSy the prevalence of diabetes was 30%, while in those without MetSy it was 2.1% (Table 1). The study by Marjani et al. showed a rather high prevalence of MetSy in patients with type 2 diabetes, with the percentage being higher in women (53.27%) than in men (48.71%) [12].

The prevalence of hyperlipidemia in our examinees was rather high – 81.2% in the first, and 63% in the second group. Laboratory analyses showed higher values of fasting glucose in the serum in patients with MetSy, higher triglyceride and uric acid levels, and lower values of HDL cholesterol fraction. There were no differences in the values of total and LDL cholesterol, probably due to the fact that the examinees with previously diagnosed hypercholesterolemia were treated with hypolipemic agents and/or employed non-pharmacological treatment measures. In individuals with MetSy, the so called atherogenic dyslipidemia predominates, characterized by increased triglycerides and decreased HDL cholesterol, while LDL may be normal or only slightly elevated. Triglycerides play a significant role in the processes of atherogenesis and thrombogenesis; in a meta-analysis involving 101 prospective studies with 302.430 examinees, it has been confirmed that elevated triglycerides are an important factor associated with the development of coronary heart disease [13].

The studies undertaken in Greece and the USA have shown that the prevalence of metabolic syndrome is similar in both genders [14]. On the other hand, there have been studies indicating a higher prevalence of MetSy in women or in men [15]. In our study, women more commonly had MetSy (59.6%). Deibert et al. demonstrated a prevalence of MetSy in postmenopausal women of 36.1% [16]. Furthermore, there have been studies suggesting that the differences in MetSy prevalence may be influenced by genetic factors and ethnicity as well [17].

Cardiovascular risk in individuals with MetSy is the product of different combinations of the components of the syndrome, which are not at all uniform. Each of the components is an independent risk factor for CVD and their synergistic action additionally increases the risk. Cardiovascular mortality is increased with an increasing number of MetSy components [18]. The results of multivariate regression analysis in our study singled out the number of MetSy components as one of the most important factors associated with atherosclerosis of the carotid arteries.

Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) observed for 11.6 years on the average 1.209 seemingly healthy men aged 42 to 60 years, where it was shown that CVD fatalities were 2.9–4.2 times more common in the presence of MetSy [19]. Among the patients of more advanced age (70–79 years), the presence of MetSy was associated with a higher prevalence of coronary events, myocardial infarction, heart failure and hospitalizations regardless of the cause in the Health, Aging and Body Composition Study (HealthABC study) [20]. In the Medicine, Angioplasty or Surgery Study (MASS II), the patients with stable coronary disease were followed up for two years; those with MetSy had poorer CV outcomes and 2.5 times higher mortality independent of the factors of age, gender, smoking, LDL cholesterol and number of involved coronary blood vessels. The mortality of patients with MetSy was 10.6% after two years, compared to 5.2% in those without MetSy [21].

The current European guidelines for CVD prevention in clinical practice, similarly to their predecessors, recommend the use of the SCORE system to assess the risk in asymptomatic patients since it has been based on a large representative European cohort [22]. In all the patients involved in our study SCORE was determined, and the obtained results demonstrated statistically significantly higher SCORE values in those with AH and MetSy (Table 1). The results such as these could be in part expected, since the SCORE system was based on the presence or absence of some of the MetSy components. Non-invasive imaging methods are able to identify asymptomatic atherosclerosis in various arterial areas and therefore supplement the assessment of risk. A special significance of these approaches lies in the detection of changes in their subclinical phase in individuals at high risk for the development of cardiovascular diseases, so that the measures of prevention and appropriate treatments could be timely introduced.

IMC thickness in the common carotid arteries represents a solid indicator of early atherosclerosis [23], and a number of studies, including the Multi-Ethnic Study of Atherosclerosis (MESA) in asymptomatic patients, have shown that the IMC thickness exceeding 75 percentiles for age, gender and race is associated with the risk for myocardial infarction, stroke and cardiovascular mortality independently of other conventional risk factors [24]. The association of MetSy with subclinical atherosclerosis was the subject of a number of studies employing non-invasive imaging techniques. In the study by Holewijn et al., involving 1.517 individuals

with MetSy aged 50 to 70 years, a significant association was found between asymptomatic atherosclerosis and MetSy, regardless of the employed imaging method [25].

IMC thickness of the carotid arteries in our examinees was significantly greater in the group with MetSy compared to those without MetSy (Table 3), where increased IMC thickness (≥ 0.90) was found in 51% of examinees with MetSy. Those with MetSy more commonly had one or more carotid plaques, a greater average number of plaques and percentage of stenosis (Table 3). Similar results were published by Olijhoek et al. in the Netherlands, examining the association of MetSy with changes in the coronary, cerebral and peripheral arteries. The prevalence of MetSy in that study was 45%; their patients had greater IMC thickness, a lower ankle-brachial index, and more prevalent microalbuminemia compared to those without MetSy. Furthermore, they demonstrated that with an increasing number of MetSy components, IMC thickness increased as well [26].

In our analysis of the characteristics of carotid plaques, we found that in our examinees with MetSy plaques were most commonly fibrocalcified, then fibrous and lastly calcified, while in those without MetSy fibrous plaques predominated (Table 4). However, it was interesting to note that lipid plaques were not present in any of the 342 patients with AH, with or without MetSy. The presence of soft lipid and fibrolipid plaques has been usually associated with the risk for neurological complications (transitory ischemic attacks, reversible ischemic deficits or cerebrovascular insults) due to an increased embolic potential of these plaques [27]. On the other hand, the presence of carotid plaques suggests that plaques in the coronary arteries may be present as well. The presence of less echo-lucent plaques in stable coronary patients has been able to predict acute coronary syndrome in the future, regardless of the presence of other factors of risk [28]. Khoury et al. have demonstrated that patients with multi-vessel coronary disease had a higher carotid plaque score compared to those with single-vessel coronary disease or normal coronary blood vessels [29]. Recent analyses have shown that the presence plaques in the carotid arteries has a greater predictive significance regarding future CV events, compared to IMC thickness [30].

Conclusion

Arterial hypertension is commonly associated with metabolic disorders, especially with insulin resistance and MetSy. In our study, we demonstrated that in patients with AH the prevalence of MetSy was significantly higher than in healthy individuals. Patients with AH and MetSy had a greater average number of risk factors for cardiovascular diseases, higher score of CV risk – SCORE, higher body mass index, diabetes was more often present, as well as hyperlipidemia and obesity. The results indicated the presence of a significant association between MetSy and its components and atherosclerotic changes in the carotid arteries.

References

1. Isomaa B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24:683–689.
2. Eckel RH, Grundy SM, Zimmet PZ. The Metabolic Syndrome. Lancet 2005; 365:1415–1428.
3. Stern MP, Williams K, Gonzalez-Villalpando C, Hunt KJ, Haffner SM. Does the metabolic syndrome improve identification of individuals at risk of type 2 diabetes and/or cardiovascular disease? Diabetes Care 2004; 27:2676–2681.
4. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult. Treatment Panel III). JAMA 2001; 285:2486–2497.
5. Holewijn S, den Heijer M. et al. The Metabolic Syndrome and Its Traits as Risk Factors for Subclinical Atherosclerosis. J Clin Endocrinol Metab 2009; 94 (8):2893–2899.
6. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G, for the ARIC Study Group. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. Stroke 1995; 26:386–391.
7. Mannami T, Baba S, Ogata J. Strong and significant relationships between aggregation of major coronary risk factors and the acceleration of carotid atherosclerosis in the general population of a Japanese city. Arch Intern Med 2000; 160:2297–2303.
8. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med 1998; 128:262–269.
9. Jusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC,Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Vangai PJr, Razak F, Sharma AM, Anand SS; INTERHEART Study Investigationi. Obesity and the risk of myocardial infarction in 27.000 participans from 52 countries: a case-control study. Lancet 2005; 366 (9497):l640–1649.
10. Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003-2006. Natl Health Stat Report 2009; 13:1–7.
11. Giuseppe Schillaci, Matteo Pirro, Gaetano Vaudo et al. Prognostic Value of the Metabolic Syndrome in Essential Hypertension. J Am Coll Cardiol 2004; 43:1817–1822.
12. Marjani A, Mojerloo M. The metabolic syndrome in type 2 diabetic subjects in Gorgan, Iran. J Pak Med Assoc. 2011; 61(5):458–461.
13. Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration, Sarwar N, Sandhu MS, Ricketts SL, Butterworth AS, et al. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. Lancet 2010; 375:1634–1639.
14. Athyros VG, Ganotakis ES, Bathianaki M, Monedas I, Goudevenos IA, Papageorgiou AA, Papathanasiou A, et al. Awareness, treatment and control of the metabolic syndrome and its components: a multicentre Greek study. Hellenic J Cardiol 2005; 46(6):380–386.
15. Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: prevalence in worldwide populations. Endocrinol Metab Clin North Am. 2004; 33(2):351–375.
16. Deibert P, Konig D, Vitolins MZ, Landmann U, Frey I, Zahradnik HP, Berg A. Effect of a weight loss intervention on anthropometric measures and metabolic risk factors in pre - versus postmenopausal women. Nutr J 2007; 6:31.
17. Marjani A. A Review on Metabolic Syndrome. J Endocrinol Metab 2012; 2(4–5):166-170.

18. Hu G, Qiao Q, Tuomilehto J et al for the DECODE Study Group. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. Arch Intern Med 2004;164:1066–1076.
19. Lakka HM, Laaksonen DE, Lakk TA, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002; 288:2709–2716.
20. Butler J, Rodondi N, Zhu Yet. al. The Health, Aging, and Body omposition Study (Health ABC study). Metabolic Syndrome and the Risk of Cardiovascular Disease in Older Adults. J Am Coll Cardiol 2006; 47:1595–1602.
21. Lopes NH, Paulitsch FS, Pereira AC, et al. Impact of metabolic syndrome on the outcome of patients with stable coronary artery disease: 2-year follow-up of the MASS II study. Coron Artery Dis 2008; 19:383.
22. Piepoli M, Hoes A, Agewall S et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts). Eur Heart J 2016; 37:2315–2381.
23. Petrović B. Bolesti arterija vrata. Akademska misao. Beograd. 2000; 18–23.
24. Folsom AR, Kronmal RA, Detrano RC, et al. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). Arch Intern Med. 2008; 168:1333–1339.
25. Holewijn S, den Heijer M, Swinkels DW, et al. The metabolic syndrome and its traits as risk factors for subclinical atherosclerosis. J Clin Endocrinol Metab 2009;94:2893–2899.
26. Jobien K. Olijhoeka, Yolanda van der Graaf, Jan-Dirk Banga, Ale Algra et al., for the SMART Study Group. The Metabolic Syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm. Eur Heart J 2004; 25:342–348.
27. Petrović B. Bolesti arterija vrata. Akademska misao. Beograd. 2000; 56–64.
28. Honda O, Sugiyama S, Kugiyama K, Fukushima H, Nakamura S, Koide S, Kojima S, Hirai N, Kawano H, Soejima H, Sakamoto T, Yoshimura M, Ogawa H. J Am Coll Cardiol. Echolucent carotid plaques predict future coronary events in patients with coronary artery disease. J Am Coll Cardiol 2004; 43(7):1177–1184.
29. Khoury Z, Schwartz R, Gottlieb S, Chenzbraun A, Stern S and Keren A. Relation of Coronary Artery Disease to Atherosclerotic Disease in the Aorta, Carotid, and Femoral Arteries Evaluated by Ultrasound. Am J Cardiol 1997; 80: 1429–1433.
30. Yoichi Inaba, Jennifer A. Chen, Steven R. Bergmann. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: A meta-analysis. Atherosclerosis 2012; 220:128–133.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 61–64

UDC 616.248-02-053.2-085: 613.84-055.26
https://doi.org/10.22190/FUMB180201011J

Original Article

INCIDENCE OF PRENATAL AND POSTNATAL RISK FACTORS FOR CHILDHOOD ASTHMA

Milica Jakovljević[1], Dragana Mitrović[2], Snežana Živanović[1,3]

[1]Faculty of Medicine, University of Niš, Niš, Serbia
[2]Health Center Knjaževac, Serbia
[3]Pediatric Clinic, Clinical Center Niš, Serbia

Abstract. *Asthma is one of the most frequent chronic diseases of childhood. Its etiology is not fully understood and risk factors for its development are numerous. The aim of this study was to determine the incidence of prenatal and postnatal risk factors in children suffering from asthma. The study was conducted on a sample of 100 patients with a diagnosis of asthma according to Global Initiative for Asthma guidelines. The data was collected using a questionnaire. We have considered prenatal (smoking during pregnancy, hormone and antibiotic therapy, the use of acetaminophen and dietary supplements, maternal blood sugar level, the term of delivery and the method of childbirth) and postnatal risk factors (oxygen and antibiotic therapy during the first year of life, immunization status during the first 6 months of life and presence of the older sibling in the family). In our study group, the most common prenatal risk factors related to asthma were: smoking during pregnancy, hormone therapy, the use of the dietary supplements and delivery via Caesarean section, while the most frequently found postnatal risk factors were: antibiotic therapy during the first year of life and the presence of older child in the family.*

Key words: *asthma, prenatal risk factors, postnatal risk factors.*

Introduction

Asthma is one of the most frequent chronic diseases of childhood. According to GINA (Global Initiative for Asthma) guidelines, it affects around 300 million individuals [1]. The prevalence of asthma in children aged 13–14 years is 1.5–15.6%, thus making it a global health problem [1]. It is believed that chronic inflammation and structural changes in respiratory tract lead to reversible airflow obstruction and bronchial hyper-reactivity [2]. Its etiology is not fully understood. It is believed that both genetics and environmental factors play a role in its etiology [3]. According to literature data, the most important prenatal risk factors for childhood asthma are: smoking during pregnancy, maternal diet, low birth weight, use of antibiotics during pregnancy and delivery via cesarean section, while the most important postnatal risk factors are allergic sensitization, environmental tobacco smoke exposure, exposure to animals, breastfeeding, decreased lung function during infancy, family size and structure, socio-economic status, antibiotics use and gender [3,4]. Studies performed among twins showed that genetic factors have a significant role in the development of asthma [5].

Correspondence to: Milica Jakovljević, M.D.
Faculty of Medicine, 81 Zoran Đinđić Blvd., 18000 Niš, Serbia
Phone: +381 63 87 27 136
E-mail: milicajakovljevic@mail.com
Received February 1st, 2018, Accepted December 20th, 2018

Objective

The aim of this investigation was to determine the frequency of prenatal and postnatal risk factors in children with asthma.

Material and Methods

The study was conducted at the Pulmology and Allergology Department of Nis Clinical Center Pediatric Clinic, from November 2016 to March 2017, on a sample of 100 patients with a diagnosis of asthma according to GINA (Global Initiative for Asthma) guidelines. In our study group, male to female ratio was 61:39 (n = 61; n = 39). The youngest patient included in the study was 19 months old and the eldest 17 years and 6 months. In 41 children (41%), the first symptoms of asthma occurred before 3 years of age. The data was collected from children's parents by using a questionnaire. Prenatal risk factors included: smoking during pregnancy with the number of cigarettes smoked per day, hormone and antibiotic therapy as well as the use of acetaminophen and dietary supplements, high maternal blood sugar level. The data regarding the delivery term and method have been also included in the questionnaire. Postnatal risk factors were: oxygen and antibiotic therapy during the first year of life, incomplete immunization in the first 6 months of life and the presence of the older sibling in the family.

Results

An analysis of prenatal risk factors showed that mothers were active smokers during pregnancy in 18% of all cases (number of cigarettes ≤ 5 per day in 12% and 6%> 5 cigarettes). Hormone therapy was used in 26% of the pregnancies (n = 26), especially during the second and third trimester. Progesterone medications were most commonly used (n = 10; 38%). Eleven mothers (11%) received antibiotics during pregnancy. β-lactam antibiotics were most commonly used (n = 6; 55%), followed by cephalosporins (n = 2; 18%) and macrolides (n = 1; 8%). Antibiotics were used during different periods of pregnancy. Acetaminophen was used during 3% of pregnancies, especially during the last trimester. Dietary supplements were used in 57% of cases. Vitamin complex (n = 24; 42.1%) and folic acid (n = 14; 24.5%) were most frequent. In 7% of cases (n = 7) glucose intolerance or gestational diabetes was found. 95% (n = 95) had full term delivery. Caesarean section was found in 23% of all cases (n = 23). An analysis of postnatal risk factors showed that 26% of children (n = 26) had oxygen and 61 children (61%) antibiotic therapy during the first year of life. Immunization was completed according to immunization schedule in 87% of cases (n = 87). Forty five percent of children (n = 45) had older sibling.

The results are shown in Tables 1, 2 and 3.

Table 1 Prenatal risk factors in asthmatic children

	Present (%)	Not present (%)
Mother as active smoker	18	82
Glucose intolerance or gestational diabetes	7	93
Antibiotic therapy	11	89
Hormone therapy	26	74
Acetaminophen	3	97
Dietary supplements	57	43
Full-term delivery	95	5
Caesarean section	23	77
The number of cigarettes per day	≤ 5	> 5
	12	6

Discussion

In the study group, 18% of the mothers were active smokers during the pregnancy. In the literature, the incidence of mothers who smoked during pregnancy is up to 40% [6]. Six mothers (30%) smoked more than 5 cigarettes a day. Studies have shown that nicotine passes through the placenta and affects the development of fetal lungs [7,8], thus increasing the risk of asthma onset in childhood [4,9]. Smoking during pregnancy was associated with an increased risk for wheezing before the second year by 40% and between 5 and 18 years of age by 52%, while the children of mothers who smoked more than 10 cigarettes during pregnancy had a 35% increased risk of asthma before age of seven [10,11]. Eleven mothers received antibiotic therapy during pregnancy.

According to the data from literature, 35% of mothers whose children had asthma used antibiotic therapy during pregnancy [12]. The connection between antibiotics use during pregnancy and the increased risk of asthma onset is controversial. Some studies have shown increased risk of asthma, while in other, authors have come to the conclusion that the use of antibiotics is in fact an indicator of the mother's tendency towards inflammatory reactions and infections and not a risk factor by itself [13–17]. According to the "hygiene hypothesis" reduced exposure to microorganisms can lead to a change in immune system and predispose the individual to atopic reactions [18]. The use of antibiotics in pregnancy may cause a change in the mother's microbiota and predisposes the child to atopic reactions, particularly by stimulating the Th2 cell response [19,20]. In pregnant mothers β-lactam antibiotics were most commonly used (n = 6; 55%), which is in accordance to literature data. The use of penicillin, especially during the first trimester of pregnancy, significantly increases the risk of asthma in childhood [21]. In our study, antibiotics have been administered during all trimesters of pregnancy. The use of antibiotics during the second and third trimesters proved to be a risk factor for the onset of asthma in childhood [22].

Table 2 Representation of prenatal risk factors during gestation period in asthmatic children (%)

	First trimester	Second trimester	Third trimester	First and second trimester	Second and third trimester	All 3 trimesters
Antibiotic therapy	3	4	3			
Hormone therapy	3		2	2	11	8
Acetaminophen			3			

Table 3 Postnatal risk factors in asthmatic children

	Present (%)	Not present (%)
Antibiotic therapy	61	39
Oxygen therapy	26	74
Immunization completed	87	13
The presence of older sibling in family	45	55

In our study group, Acetaminophen was used in only 3% of all cases. Numerous studies have linked the prenatal use of Acetaminophen with an increased risk of childhood asthma, but it was not possible to exclude that these were just confounding factors [23,24].

The use of dietary supplements, especially folic acid, which reduces the risk of neural tube defects, has been reported in more than half of all cases. The data showing that the use of folate increases the possibility of

developing asthma in childhood are controversial - studies are few and some of them show that there is no link between prenatal use of folate and asthma while others show that folate increases the risk of asthma [25,26].

In 7% of cases (n = 7) the glucose intolerance or gestational diabetes were present. The incidence of these maternal risk factors in the literature is 2% and children whose mothers were suffering from diabetes and asthma both had a 11.3 times greater risk for developing asthma [6, 27].

The full-term delivery was present in 95% (n = 95) and Caesarean section in 23% of all cases (n = 23). According to data from literature, Caesarean section is considered a risk factor for the onset of asthma in childhood [3,4].

An analysis of postnatal risk factors found that 26% of children (n = 26) had oxygen and 61 children (61%) antibiotic therapy during first year of life. It is believed that the use of mechanical ventilation and oxygen causes barotrauma and exposes the lungs to oxygen free radicals and contributes to the development of bronchopulmonary dysplasia in premature babies thus increasing the incidence of asthma and bronchial hyperreactivity [28]. Broad spectrum antibiotics, especially when administered to children with no genetic predisposition for asthma, increase the risk of developing childhood asthma [29–31]. Immunization was completed according to immunization schedule in 87% of cases (n = 87). The KOALA study (Child, parents and health: lifestyle and genetic constitution study) showed that there was no statistically significant difference in the incidence of asthma between immunized and non-immunized children [31]. Forty five percent of children (n = 45) had older sibling, which is in accordance with the literature data [4].

The limitation of our study is a relatively small and heterogeneous sample in terms of age (the age ranging from 19 months to 17 years and 6 months) as well as the fact that the data were obtained from the parents and not from the medical records and as such are susceptible to memory bias. More detailed research is necessary due to possibility of one risk factor influencing another and the possible presence of confounding factors.

Conclusion

In our study group, the most common prenatal risk factors related to asthma were: smoking during pregnancy, hormone therapy, the use of the dietary supplements and delivery via Caesarean section, while the most frequently found postnatal risk factors were: antibiotic therapy during the first year of life and the presence of older child in the family.

Risk factors during pregnancy can affect the occurrence of asthma in children by epigenetic modifications of the genome, whereas postnatal risk factors affect early onset and more severe forms of asthma. Taking into account that the incidence of asthma is rising and that it is an etiologically heterogeneous, chronic disease affecting the health and quality of life, it is important to identify and minimize risk factors as much as possible.

References

1. http://ginasthma.org/2017-online-appendix-global-strategy-for-asthma-management-and-prevention/
2. Britt RD, Faksh A, Vogel E, Martin RJ, Pabelick CM, Prakash YS. Perinatal factors in neonatal and pediatric lung diseases. Expert Rev Resp Med 2013; 7:515–531.
3. Subbarao P, Mandhane PJ, Sears MR. Asthma: epidemiology, etiology and risk factors. CMAJ 2009; 181:E181–E190.
4. Davidson R, Roberts SE, Wotton CJ, Goldcre MJ. Influence of maternal and perinatal factors on subsequent hospitalisation for asthma in children: evidence from the Oxford record linkage study. BMC Pulm Med 2010; 10:14.
5. Willemsen G, van Beijsterveldt TC, van Baal CG, Postma D, Boomsma DI. Heritability of self-reported asthma and allergy: a study in adult Dutch twins, siblings and parents. Twin Res Hum Genet 2008; 11:132–142.
6. Nepomnyaschy L, Reichman NE. Low Birthweight and Asthma Among Young Urban Children. Am J Public Health 2006; 96:1605–1610.
7. Wu FY, Chiu HT, Wu HD, Lin CJ, Lai JS, Kuo HW. Comparison of urinary and plasma cotinine levels during the three trimesters of pregnancy. Paediatr Perinat Epidemiol 2008; 22:296–301.
8. Lødrup Carlsen KC, Jaakkola JJ, Nafstad P, Carlsen KH. In utero exposure to cigarette smoking influences lung function at birth. Eur Respir J 1997; 10:1774–1779.
9. Metsälä J, Kilkkinen A, Kaila M, Tapanainen H, Klaukka T, Gissier M, Virtanen SM: Perinatal factors and the risk of asthma in childhood – A population-based register study in Finland. Am J Epidemiol 2008; 168:170–178.
10. Burke H, Leonardi J, Hashim A, Pine-Abata H, Chen Y, Cook DG, Britton JR, McKeever TM. Prenatal and Passive Smoke Exposure and Incidence of Asthma and Wheeze: Systematic Review and Meta-analysis. Pediatrics 2012; 129:735–744.
11. Jaakkola JJK, Gissler M: Maternal smoking in pregnancy, fetal development, and childhood asthma. Am J Public Health 2004; 94:136–140.
12. Lapin B, Piorkoski J, Ownby D, Freels S, Chavez N, Hernandez E, Wagner-Cassanova C, Pelzel D, Vergara C, Persky V. The Relationship between Prenatal Antibiotic Use and Asthma in At-Risk Children. Ann Allergy Asthma Immunol 2015; 114:203–207.
13. Metsälä J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. Clin Exp Allergy 2015; 45:137–145.
14. Stensballe LG, Simonsen J, Jensen SM, Bønnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. J Pediatr 2013; 162:832–838.
15. Örtqvist AK, Lundholm C, Kieler H, Ludvigsson JF, Fall T, Ye W, et al. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. BMJ 2014;349:g7395.
16. Popovic M, Rusconi F, Zugna D, Galassi C, Merletti F, Migliore E, Trevisan M, Nannelli T, Gagliardi L, Richiardi L. Prenatal exposure to antibiotics and wheezing in infancy: a birth cohort study. Eur Respir J 2016; 47:810–817.
17. Stokholm J, Sevelsted A, Bønnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. Lancet Resp Med. 2014; 2:631–637.

18. Renz H, Blumer N, Virna S, Sel S, Garn H. The immunological basis of the hygiene hypothesis. Chem Immun Allergy 2006; 91:30–48.
19. Lange NE, Celedon JC, Forno E, et al. Maternal intestinal flora and wheeze in early childhood. Clin Exp Allergy 2012; 42:901–908.
20. Stokholm J, Schjørring S, Eskildsen CE, Pedersen L, Bischoff AL, Følsgaard N, et al. Antibiotic use during pregnancy alters the commensal vaginal microbiota. Clin Microbiol Infect 2014; 20:629–635.
21. Chu S, Yu H, Chen Y, Chen Q, Wang B, Zhang J. Periconceptional and Gestational Exposure to Antibiotics and Childhood Asthma. PloS One. 2015; 10:e0140443.
22. Zhao D, Su H, Cheng J, Wang X, Xie M, Li K, Wen L, Yang H. Prenatal antibiotic use and risk of childhood wheeze/asthma: A meta-analysis. Pediatr Allergy Immunol 2015; 26:56–64.
23. Shaheen SO, Newson RB, Sherriff A, et al. Paracetamol use in pregnancy and wheezing in early childhood. Thorax. 2002; 57:958–963.
24. Andersen AB, Farkas DK, Mehnert F, Ehrenstein V, Erichsen R. Use of prescription paracetamol during pregnancy and risk of asthma in children: a population-based Danish cohort study. Clin Epidemiol 2012; 4:33–40.
25. Crider KS, Cordero AM, Qi YP, Mulinare J, Downling NF, Berry RJ. Prenatal folic acid and risk of asthma in children: a systematic review and meta-analysis. Am J Clin Nutr 2013; 98:1272–1281.
26. Veeranki SP, Gebretsadik T, Mitchel EF, Tylavsky FA, Hartert TV, Cooper WO, Dupont WD, Dorris SL, Hartman TJ, Carroll KN. Maternal Folic Acid Supplementation During Pregnancy and Early Childhood Asthma. Epidemiology 2015; 26:934–941.
27. Azad MB Becker AB, Kozyrskyj AL. Association of maternal diabetes and child asthma. Pediatr Pulmol 2013; 48: 545-552.
28. Alshehri MA, Almegamesi TM, Alfrayh AS. Predictors of short-term hospital readmissions of asthmatic children. J Family Community Med 2005; 12:11–17.
29. Risnes KR, Belager K, Murk W, Bracken MB. Antibiotic exposure by 6 months and asthma and allergy at 6 years: Findings in a cohort of 1,401 US children. Am J Epidemiol 2011; 173:310–318.
30. Marra F, Lynd L, Coombes M, et al. Does antibiotic exposure during infancy lead to development of asthma? A systematic review and metaanalysis. Chest 2006; 129:610–618.
31. Kummeling I, Thijs C, Stelma F, Huber M, van den Brandt PA, Dagnelie PC. Diphtheria, pertussis, poliomyelitis, tetanus, and Haemophilus influenzae type b vaccinations and risk of eczema and recurrent wheeze in the first year of life: the KOALA Birth Cohort Study. Pediatrics 2007; 119:e367–373.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 65–73

UDC 616.314-007.21-089
https://doi.org/10.22190/FUMB180307002P

Case Report

CAMOUFLAGE THERAPY OF HYPOPLASIA OF THE MAXILLA. CASE REPORT

Tatjana Perović[1,2], Ilija Aleksić[3], Zorica Blažej[2]

[1]Faculty of Medicine, University of Niš, Niš, Serbia
[2]Dental Clinic Niš, Niš, Serbia
[3]Independet dental technician, Niš, Serbia

Abstract. *Maxillary hypoplasia is one of the forms of class III malocclusion. On average, 60% class III malocclusions are characterized by maxillary underdevelopment in all three directions. Anomalies in position, size and the shape of facial bones, maxilla in particular, usually appear in childhood, become more pronounced in adolescence until the end of the growth period. The aim of the paper is to show the camouflage treatment of the patient in adolescence with maxillary hypoplasia. Using the RME method in the upper jaw, extraction in the lower jaw and fixed orthodontic devices in both jaws, a satisfactory result was achieved.*

Key words: *Maxillary hypoplasia, Camouflage therapy, Rapid Maxillary Expansion (RME).*

Introduction

According to Angle, Class III malocclusion represents a relationship in which the lower first molar is more anteriorly placed related to the upper first molar [1]. It is a symptomatic or phenotypic description of occlusion which uses the relationship of first molars as a criterion and it does not clarify mutual relationship of the maxillary and mandibular skeletal base, as well as their relationship towards the skull base. Regardless of this, this is the most commonly used classification of malocclusion, due to its simplicity. However, many authors criticized its flaws, such as the lack of definition of transversal and vertical dimension [2]. The research carried out to identify anatomic specificities of class III malocclusion showed that deformity is not only limited to occlusion and overdevelopment of the lower jaw (as it was considered until 1970), but it includes the entire craniofacial complex. Most of the individuals with class III malocclusions have a combination of skeletal and dentoalveolar components, such as insufficient length of the frontal skull base, reduced angle of the cranial base, short and retrognathic maxilla, protruded maxillary incisors, retruded mandibular incisors, excessive lower anterior face height and obtuse gonial angle [3].

The etiology of class III malocclusion is multifactorial, with hereditary, ethnical, ecological and habitual components. Factors that contribute to anomaly are complex [4]. It is considered to be very difficult for therapy [5,6].

Correspondence to: Tatjana Perović, Ph.D.
Faculty of Medicine, 81 Zoran Đinđić Blvd., 18000 Niš, Serbia
Phone: +381 63 10 45 363
E-mail: tatjana.tanic@gmail.com
Received March 7th, 2018, Accepted May 8th, 2018

Different ethical groups show various rates of prevalence of class III. It ranges from around 0.8–4% in Caucasians and around 13–14% among the Chinese and Japanese. In Asian population, majority of patients show deficit of the middle third of a face. According to literature, more than 60% of the cases of class III malocclusion occurred due to maxillary skeletal deficit [3,7,8].

A non-esthetic, concave face profile and functional disorders are the most common cause of seeking therapy. However, a concave profile with an altered nasolabial angle is often camouflaged by a natural dentoalveolar compensation. (e.g. with extremely pronounced protruded upper incisors).

This malocclusion can be classified into three types according to anatomic heterogeneity, more precisely, according to mutual relationship of the maxilla and mandible towards the base of the skull [1,3,4,9–12]. Type A has a normal maxilla and overdeveloped mandible. Its name is regular mandibular prognathism, since anterior crossbite or class III malocclusion resulted from the overdeveloped mandible. Type B has an overdeveloped maxilla and mandible, but the mandible has grown more than the maxilla, which results in a reduced nasolabial angle and anteriorly positioned point A. This type is more often found in Asians [2].

A typical characteristic of type C malocclusion is hypoplasia of the maxilla. On average, 60% in class III malocclusion are characterized by maxillary underdevelopment in all three directions [8]. Anomalies in position, size [13] and the shape of facial bones, maxilla in particular, usually appear in childhood, become more pronounced in adolescence until the end of the growth period [14].

In some cases of class III malocclusion, type C, with dentoskeletal mismatch, there are three possible therapeutic possibilities in general: modification of growth;

camouflage through dental compensation; surgical reponing of jaw bases [4,10,15]. For surgical correction, anterior positioning of the maxilla with maxillary osteotomy is necessary for obtaining a normal position of the upper lip and adequate nasolabial angle, and Le Fort I osteotomy are most commonly used [2,16].

As for the modification of growth, it is evident that the basic parameters that need to be considered are age, sex, type of malocclusion and the engagement of the skeleton in existing malocclusion. Age and sex impose precise time limits of each orthopedic treatment, in particular in the treatment of growth modification [17]. Growth modification with a facemask (Delaire) can be used for the correction of this malocclusion during the growth, and the options for treatment are limited to those with mild sagittal discrepancies [18,19].

Currently, there is a rational consensus on two basic questions. First, growth can be positively modified only in certain types of patients, which significantly limits this approach: maxilla and mandible could be stimulated to grow for a few additional millimeters. Therefore, it is not possible to obtain significant transformations. Second, during each orthodontic treatment teeth inevitably move in the direction of the correct occlusal relationship. These movements of teeth, which could be called 'dental compensation of skeletal discrepancy', make the complete orthopedic and skeletal correction difficult and introduce some elements of dental camouflage [20]. Orthodontic camouflage is a therapeutic process which largely uses extractions and orthodontic masking of skeletal discrepancy, instead of correcting them. Consequently, dentoalveolar compensation is performed without the correction of basal discrepancy [21].

The camouflage treatment with selective extractions is usually applied in borderline cases. However, sometimes we use it to treat patients with difficult problems who do not want surgery as a part of treatment. The camouflage treatment should be prescribed for young adults only if there are cephalometric indications before the beginning of the treatment that residual growth will not cause the deterioration of deformity after the therapy. The camouflage therapy also implies that the treatment will have a favorable effect or at least it will be less harmful for face esthetics [21].

Case Report

A male patient, aged 15, came with the complaint of esthetic (crooked teeth which are exposed during smiling and speech) and functional problems (chewing and difficulty breathing through the nose). It was found out from the anamnesis that he has a twin sister who does not have similar problems, that he had frequent respiratory infections in early childhood and the adenoid removed at the age of 3, three years of unsuccessful orthodontic treatment with a removable appliance for the widening of the upper jaw. The extraoral finding points to the presence of a leptoprosopic, adenoid face with paranasal depressions, wide buccal corridors which are exposed during smiling and speech and mostly oral respiration. The concavity of the profile is to a great extent camouflaged with the anteriorly inclined profile and full lips (Fig. 1 A, B, C). Intraorally: the movement of the middle of the upper dental arch to the right side; the lack of the upper right canine in the dental arch with completely closed space for this tooth, narrow and high position of the upper left canine, bilateral crossbite with the lack of occlusal contact on the right side; cutting relationship of central incisors with the antagonists; crowding and retrusion of lower frontal teeth. The analysis of study models showed ½ class III of malocclusion according to the reconstruction followed by big-

A B C

Fig. 1 A B C Pretreatment extraoral photographs (frontal, lateral view and smile)

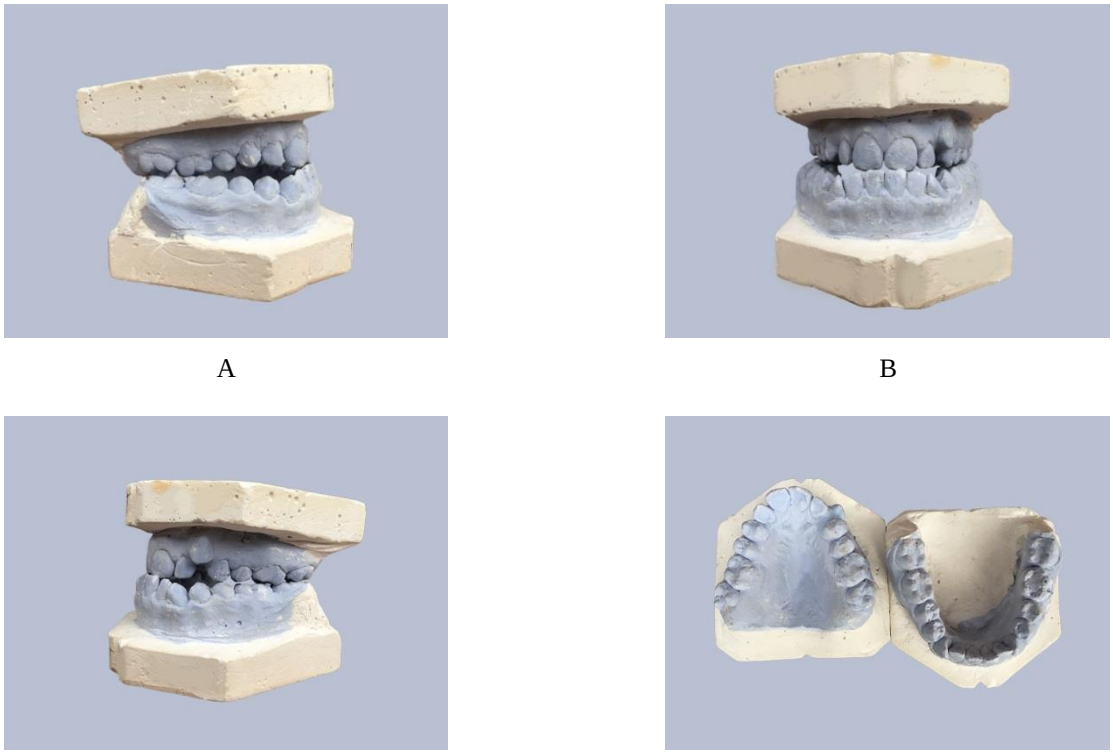


Fig. 2 A B C D Pretreatment models (frontal, left, right and occlusal view)

ger transversal disharmony between the upper and lower dental arch (-6 mm in bimolar distance). The asymmetrical upper dental arch with the complete loss of space for the upper right canine and partly for the left. There is a collapse of upper frontal teeth and the crowding of the lower ones, anterior crossbite on the lateral incisors; an incisal contact only in the area of central incisors in the cutting relationship; the crossbite on the right side and difficult degree non-occlusion - only first molars are in contact. On the left side, the degree of crossing is milder and there is a contact of antagonists (figures of models) (Fig. 2 A, B, C, D).

The analysis of orthopan: Impaction of the upper right canine, the presence of all third molars (Fig. 3).

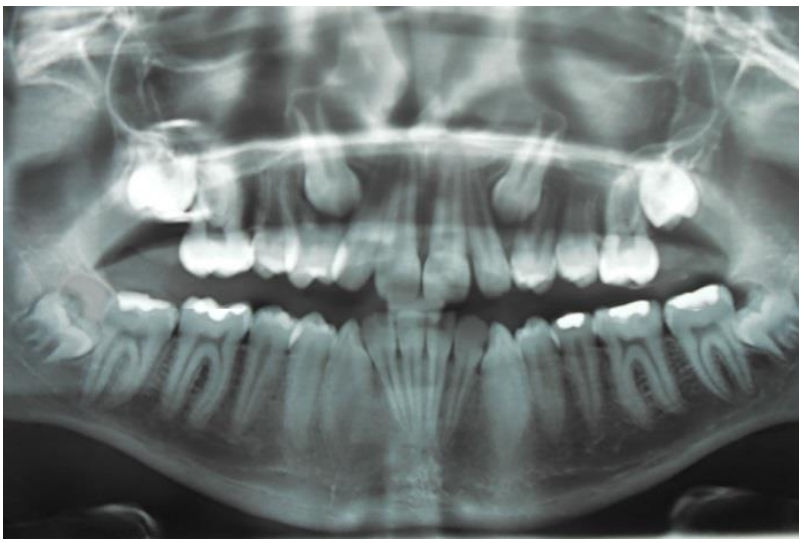
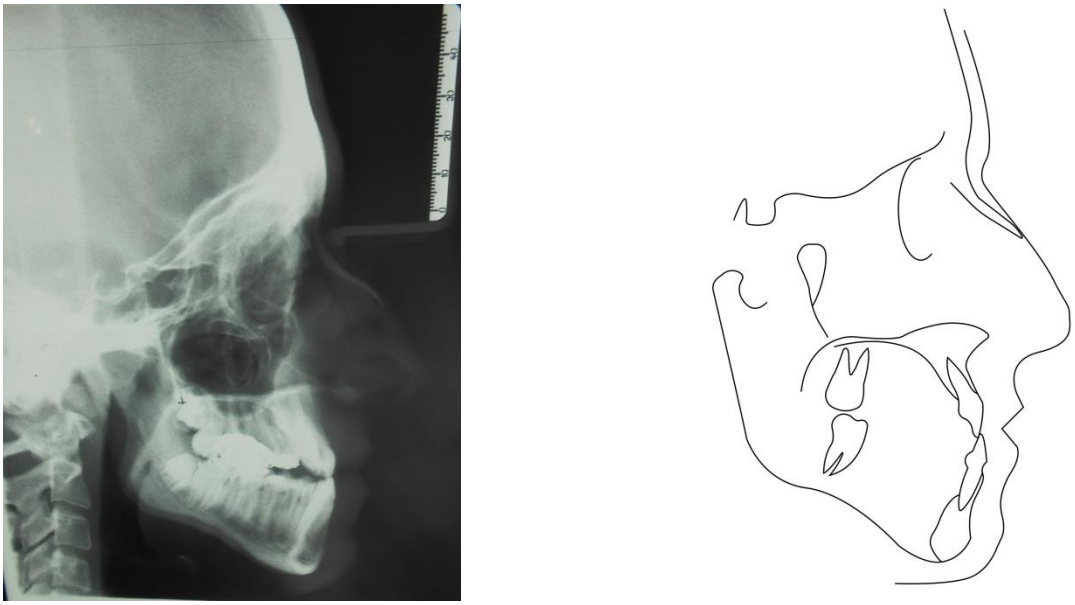


Fig. 3 Panoramic radiograph before therapy



A B

Fig. 4 A B Pretreatment lateral cephalometric radiograph and cephalometric tracings

Profile cephalogram analysis: There was bimaxillary retrognathism whereby retrognathism of the maxilla dominates, as well as mesial gnathic and dentoalveolar relationship. Then, the increased basal angle, sharp incline of the occlusal and mandibular plane, the retrusion of lower incisors, hyperdivergent type of growth (Fig. 4 A, B).

The plan of the treatment

Two options were suggested to the patient:

1. Presurgical orthodontic treatment of rapid palatal expansion, followed by the use of fixed appliance with surgical intervention of releasing the impacted upper canine and the leveling of the upper and lower dental string. In the second phase, the anterior positioning of the maxilla with the maxillary osteotomy was predicted (Le Fort I osteotomy) which would enable optimal reponing and harmonization of sagittal relationship of jaw bases.
2. The other option was camouflage therapy in the second phase, with which the existing disharmony would be regulated on a dental level, by extracting the lower first premolars.

Since the patient rejected the option of surgical treatment of the reposition of the maxilla at first arrival, the other option was then accessed.

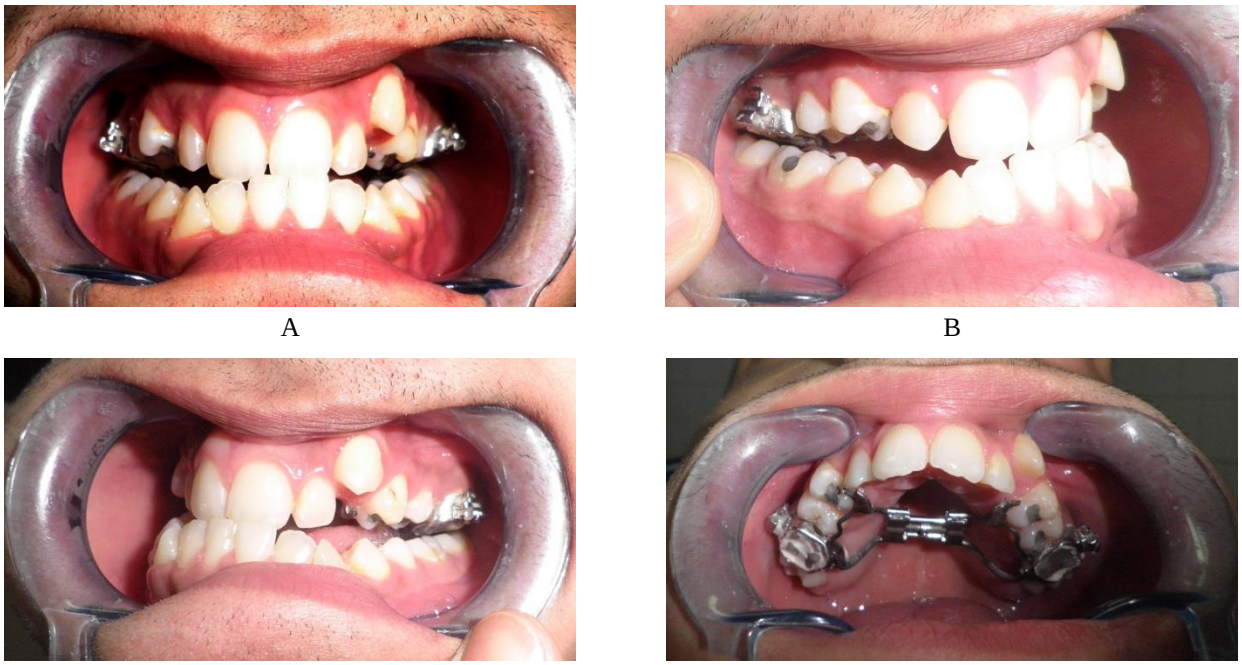
The Objectives of the Treatment

- To transversally develop the maxilla by using the appliance for rapid palatal expansion, with which transversal skeletal discrepancy between the lower and upper jaw and crossbite would be removed, dissipate the existing crowding and create space for canines;
- To extract lower first premolars, and to dissipate the crowding of lower frontal teeth at the expense of postextracted space, as well as to achieve retrusion that would enable improved contact of frontal antagonists.

These therapeutic procedures were expected to enable the improvement of the function of breathing as well as the correction of facial esthetics and the appearance of a smile.

The progress of treatment

In the first phase the appliance for rapid maxillary expansion with the base on the first molars was constructed. Hyrax screw was used in the basis of the construction (manufacturer Leone, stock No A0620-13). The activation of the screw took place once a day. The expansion of the maxilla was achieved in less than a month (Fig. 5 A, B, C, D). Afterwards, both first premolars were extracted. Two months after placing the appliance for rapid palatal expansion, an upper and lower fixed appliance was placed (full arch, prescribed by Roth, slot 0,022") for the leveling in both dental arches. The treatment began with an 0.012" niti archwire on the upper and lower arch and Phase I finished with an upper and lower 0.019" × 0.025" SS archwire (American Orthodontics). It was scheduled to surgically release the impacted upper right canine which had a favorable position for placing in the dental arch and its linking to the system which would enable correct positioning. The lower spaces were closed with a 0.017" × 0.025" SS lower archwire and a short elastomeric chain (American Orthodontics). The therapy lasted 2 years altogether. The appliances were removed, and upper and lower circumferential retainers were placed as retention. The patient was instructed to wear it at night.



A B C D

Fig. 5 A B C D A view shows an adequate space created with the help of the RME appliance

Results

The results were obtained by comparing the initial and final cephalometric values, extraoral and facial photographs and study models.

Extraoral analysis

Post treatment en face photography portrays a harmonious face with balanced facial contours; on the lateral photography an anteriorly slanted profile was noticed, facial harmonious relationship in the lower third, as well as mild increase of the nasolabial angle. In the smile photography a smile with the increased expression of teeth was noticed, by overlapping of middle lines of dental arches and complete elimination of crowding (Fig. 6 A, B, C).

A B C

Fig. 6 A B C Post-treatment extraoral photographs (two years after therapy) (frontal, lateral view and smile).

The analysis of study models

When comparing the initial and final study models the leveling of the upper and lower dental arch is evident; class I in the area of canine teeth and class III in the area of molars, acceptable occlusal intercuspation in the front segment as well as in lateral parts (Fig. 7); reduced overbite and overjet are noticed, symmetrical and oval upper and lower dental arch (Fig. 8 A, B, C, D).

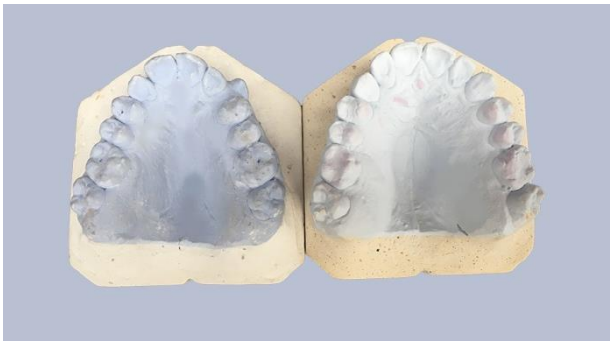
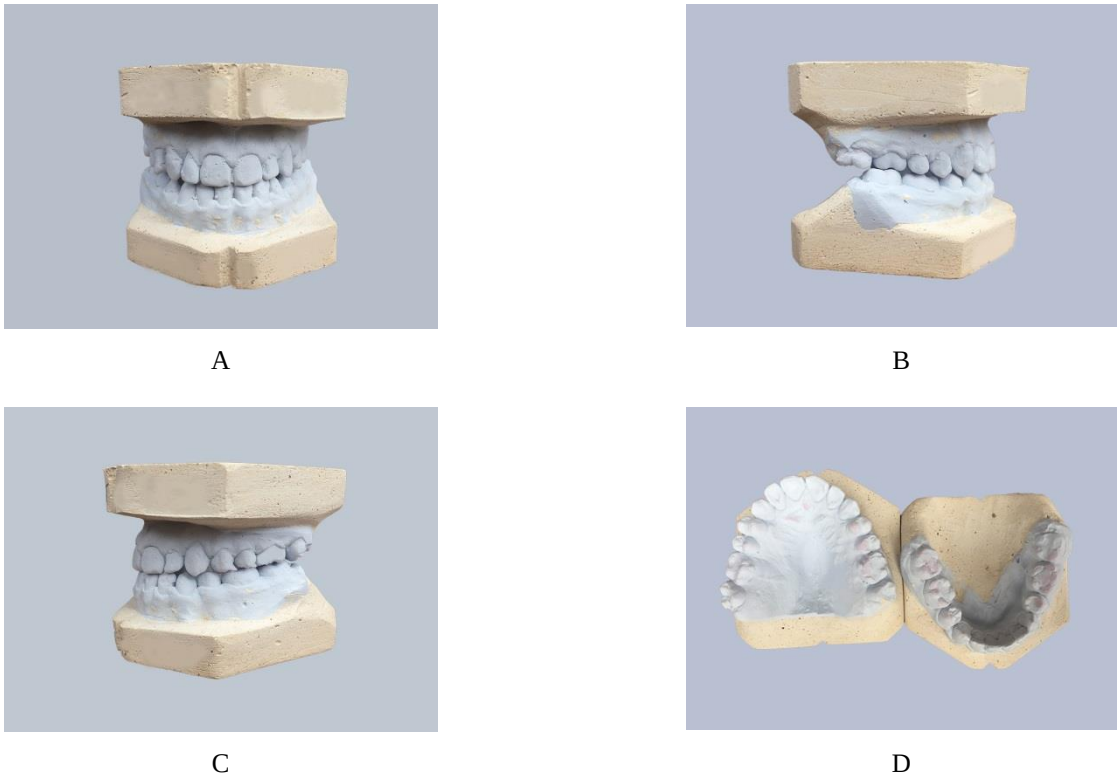


Fig. 7 Pretreatment upper model and upper model after therapy

The analysis of a profile radiography shows the following enhancements: the angle of maxillary prognathism (SNA), the angle of maxillomandibular discrepancy (ANB), the decrease of the basal angle (B), the decreased inclination of occlusal plane (Occl/SN), the improvement in the relationship of the frontal and back height of the face (SGo/NMe), decreased distance of the upper and lower lip from the esthetic line (Table 1, 2). The deterioration of parameters occurred on the dentoalveolar level is the increase in the degree of protrusion of upper incisors and retrusion of lower incisors and the decrease of the interincisor angle (Fig. 9 A, B).

Table 1 Cephalometric evaluation of the patient before and two years after treatment

Measurement	Before treatment	After treatment
SNA (82)	72	75
SNB (80)	75	76
ANB (2-4)	–3	–1
SND (76-77)	70	72
N S Ar (123`)	132	128
S Ar Go (143`)	138	141
Gonial angle (130)	133	132
Y axis (59-+4)	62	59
SN/Occ. plane (14`)	25`	20
SN/Go-Gn (32`)	40	39
N-Me (mm)	116	116
S-Go (mm)	71	72
1/1 (130-150)	139	131
1-NA (4 mm)	4 mm	6 mm
1/NA (22)	19	31
1-NB (4 mm)	3 mm	2 mm
1/NB (25)	26	18
Wits (-1mm)	10 mm	–7 mm
E line-upper lip (3mm)	8	6
E line-lower lip (2mm)	5	4
Gl-Sn-Pg (169±3)	168	168
B (20`)	33	28
AB/SpP (99`)	89	91
PgA/SpP (99`)	89	92



A B C D

Fig. 8 Post-treatment models (frontal, left, right and occlusal view).

Table 2 Occlusal evaluation of the dentoalveolar parameters before and two years after treatment

Features	Pre-treatment	Post-treatment
Incisor relationship	reverse overjet	normal overjet
Overjet (mm)	-1	2
Overbite (mm)	0	2
Midlines	shifted	co-incident
Molar relationship (left/right)	½ class III / ½ class III	class III / class III
Canine relationship (left/right)	½ class III / ½ class III	class I / class I

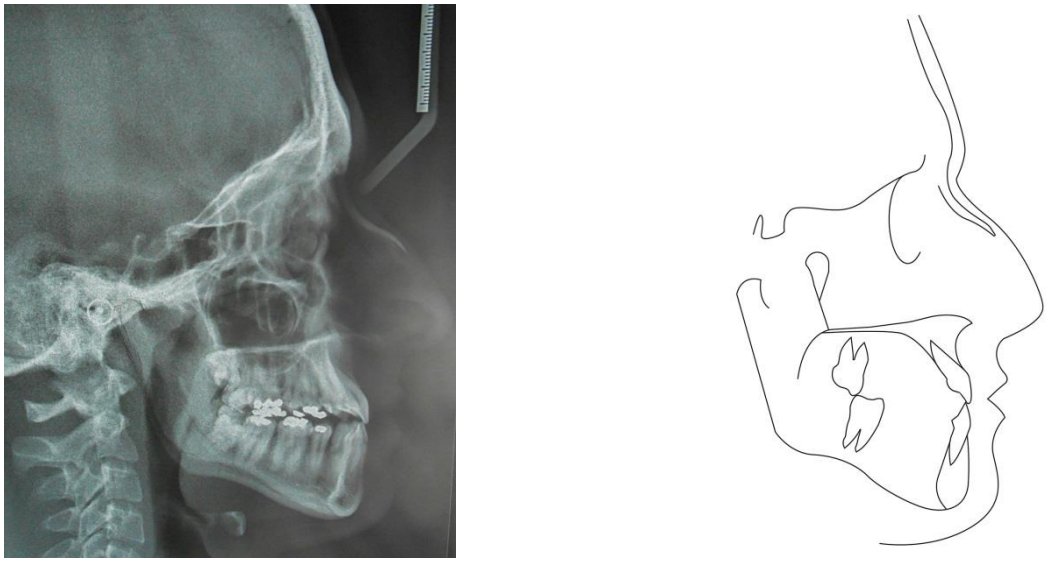


Fig. 9 Post-treatment lateral cephalometric radiograph and cephalometric tracings (two years Figure therapy).

Fig. 10 Panoramic radiograph two years after therapy.

In the panoramic radiography, it can be noticed that all teeth have an adequate parallelism of the root, without the loss of bones or present pathological processes (Fig. 10).

Discussion

The strategy for the treatment of borderline cases with maxillary hypoplasia by camouflage therapy is based on creating dentoalveolar changes that will compensate the disbalance of the skeletal base. The decision on which type of treatment is appropriate is usually based on the degree of anteroposterior and vertical discrepancy of the skeleton, inclination and the position of the incisors and dentofacial appearance [21].

Orthodontic camouflage is a sustainable alternative for treating mild to moderate maxillary hypoplasia with the view to correcting occlusal relationships in patients who reject surgical treatment due to various reasons. The ideal candidate for camouflage treatment should have small residual potential of growth and mild to moderate crowding, so that postextracted space could be used, whereby orthodontic camouflage effect and visual enhancement of dentoskeletal relationships are enabled [19,21].

Type of growth in this kind of malocclusions with camouflage therapy also has great significance. Some retrospective studies have shown that subjects with maxillary retrognathism most commonly have vertical pattern of face growth, which seems to be another mechanism for compensation [22]. The presented patient had precisely that kind of growth pattern. A vertical growth model is an important factor for the successful treatment of maxillary hypoplasia. The reduced lower height of anterior face, increased overbite and passive closing of lips related to Class III malocclusion have a better prognosis, since the rotation of the mandible opposite of clockwise caused by treatment, helps camouflage sagittal discrepancy. When the increased lower height of anterior face is related to this malocclusion, surgical intervention is usually the treatment of choice, since every therapeutic induction of clockwise rotation leads to the increase in vertical dimensions of a face and consequently causes the incompetence of lips [1].

The usual orthodontic camouflage is performed by protrusion of upper and retroclination of lower incisors, which, as mentioned before, results in mandibular rotation downwards and back. Often, extraction is a necessary component of the camouflage method. The most commonly used pattern of extraction is the removal of lower first premolars [9,15,19].

Some authors suggest the extraction of one lower incisor as an alternative to extraction of lower first premolars within camouflage therapy. That kind of extraction is occasionally recommended to patients with frontal crossbite or cutting relationship of the incisors. The decision is determined by factors such as pronounced anterior crowding in the mandibular arch, discrepancy of Bolton's index and the degree of negative overjet and overbite [9]. Other authors think that the extraction of incisors can favorably affect the maintenance of a face profile and the correction of crowding of the lower string of teeth [21,23]. According to the reports of studies which compared the stability of postretention in patients treated with the extraction of lower premolars and those treated with the extraction of one mandibular incisor, these authors found that the more acceptable solution is to level the mandibular arch with the extraction of one incisor [21,24,25].

Sometimes, when the circumstances in the mouth are favorable, the extraction of first lower molars is recommended. These are compromising extractions, which are applied when first molars have extreme cavity, hypoplastic lesions, apical pathologies or great restorations. Other situations in which first molars can be removed are significant crowding in the distal part of the mandibular arch, large angle of the mandibular plane and anterior open bite. The imperfection of first lower molars extraction is a difficulty in closing of the space, due to the fact that lower second molars inclined mesially and lingually, leaving interproximal spaces [15].

Where is the boundary between orthodontic camouflage and orthognathic surgical therapy? Proffit and Ackermanin's concept "3 envelopes of discrepancy" suggested the degree of protrusion of the upper incisors in relation to the retrusion of lower incisors as a critical limitation [26]. Kerr et al. attempted to establish cephalometric criteria to objectivize these boundaries. The most important factors which differentiated operative and camouflage patients in their study were the size of anteroposterior deviation, the incline of mandibular incisors and the appearance of a soft tissue profile [9,21,27]. The presented patient had favorable inclination of upper and lower frontal teeth, whereby these natural compensatory resources were not used, so the biggest part of orthodontic camouflage happened at their expense. Regardless of clear criteria of decision in favor of one or other type of therapy, the choice between camouflage treatment and orthodontic surgery remains the challenge of specialty [9].

In the presented case, the extraction of lower first premolars resulted in the decrease of the angle of maxillomandibular prognathism, basal angle, inclination of occlusal and mandibular plane, as well as the reduce in concavity and obtaining an esthetic profile. What it needs to be taken care of during the retrusion of lower incisors is the fact that these patients have concave profiles, with a thin basal bone over the symphysis. The significant lingual incline or distal movement of incisors after the extraction of mandibular premolars can negatively affect a concave profile in comparison to non-extraction cases and it can even cause unwanted complications such as the expression of the root and the resorption of incisors [21].

The success of sagittal correction of relationship between maxilla and mandible for class III malocclusion depends on coordination of transversal and vertical relationships in combination with the growth potential of every patient [28]. When we talk about transversal underdevelopment of the maxilla, the circumference of required width which is necessary to establish with the expansion, is based on the basic concept that it is necessary to achieve close to 20% of overcorrection of the transversal deficiency [20]. This should be taken into consideration due to the choice of the appliance for transversal expansion since the presented patient had the lack of space of 6mm, the required 7.2mm (with overcorrection) could be obtained only by using the methods of rapid expansion of the maxilla (splitting of the palatal suture). The age of the patient enabled us to apply this method. By choosing this kind of appliance, the space for placing the impacted canine was obtained. What is the durability of the results achieved with this kind of therapy? The presented patient did not show the tendency for relapse for two years after the completion of the therapy, and the literature data point that relapse of class III primarily stems from mandibular growth, and not due to relapses in the maxilla [7].

Conclusion

This case report shows that camouflage orthodontic therapy of skeletal Class III malocclusion with maxillary deficiency in a growing male individual can be successfully managed using the RME procedure followed by extraction therapy with fixed orthodontic treatment. The adequate diagnostic and orthodontic treatment, patient cooperation, and long-term stabilization ensure a treatment result that is successful, stable, and esthetic.

References

1. Yang Z, Ding Y, Feng X. Developing skeletal Class III malocclusion treated nonsurgically with a combination of a protraction facemask and a multiloop edgewise archwire. Am J Orthod Dentofacial Orthop 2011; 140(2):245–255.
2. Park J. U, Baik S H. Classification of Angle Class III malocclusion and its treatment modalities. Int J Adult Orthod Orthognath Surg 2001; 16(1):19–29.
3. Katiyar R, Singh G K., Mehrotra D, Singh A. Surgical–orthodontic treatment of a skeletal class III malocclusion. Natl J Maxillofac Surg 2010; 1(2): 143–149.
4. Furquim BA, de Freitas KM S, Janson G, Simoneti LF, de Freitas MR, de Freitas DS. Class III Malocclusion Surgical-Orthodontic Treatment. Hindawi Publishing Corporation, Case Reports in Dentistry, Volume 2014, Article ID 868390, 9 pages http://dx.doi.org/10.1155/2014/868390
5. Ravi MS, Shetty NK, Prasad RB. Orthodontc-surgic al combination therapy for Class III skeletal malocclusion. Contemp Clin Dent 2012; 3:78–82.
6. Stojanovic Lj, Mileusnic I, Mileusnic B, Cutovic T. Orthodontic-surgical treatment of the skeletal class III malocclusion: a case report. Vojnosanit Pregl 2013; 70(2):215–220.
7. Azamian Z, Shirban F. Treatment Options for Class III Malocclusion in Growing Patients with Emphasis on Maxillary Protraction. Volume 2016 (2016), Article ID 8105163, 9 pages https://www.hindawi.com/journals/scientifica/2016/8105163/
8. Muthukumar K, Vijaykumar NM, Sainath MC. Management of skeletal Class III malocclusion with face mask therapy and comprehensive orthodontic treatment. Contemp Clin Dent 2016;7: 98–102.
9. Ngan P, Moon W. Evolution of Class III treatment in orthodontics.. Am J Orthod Dentofacial Orthop. 2015 Jul;148(1):22–36.
10. Ramírez AE, Valdivia IG, Girón FH, Lugo RR. Surgical-orthodontic treatment in a skeletal class III patient. A case report. Revista Mexicana de Ortodoncia 2016; 4(2):100–108.
11. Tagawa DT, da Cunha Bertoni CLS, Mari MAE, Redivo M.Jr, de Arruda Aidar LA. Orthopedic treatment of Class III malocclusion with rapid maxillary expansion combined with a face mask: a cephalometric assessment of craniofacial growth patterns. Dental Press J Orthod 2012; 17(3) http://dx.doi.org/10.1590/S2176-94512012000300022
12. Bergamo AZ N, Andrucoli MCD, Romano FL, Ferreira JTL, Matsumoto MAN. Orthodontic-Surgical Treatment of Class III Malocclusion with Mandibular Asymmetry. Braz Dent J 2011; 22(2):151–156.
13. Stojanović Z, Milic J, Nikolic P. Radiographic cephalometry assessment of the linear and angular parameters on cranial base in children with skeletal class III. Vojnosanit Pregl 2007; 64(9):604–610. [in Serbian]
14. Meraz GG, Chávez LC. Orthodontic-surgical correction of a class III malocclusion through a surgery-first protocol: case report. Revista Mexicana de Ortodoncia, 2017; 5(1):43–50.
15. Marbán FAN, Arenas AG, Lugo RR, Sánchez N EJ. Orthodontic-surgical correction of a class III malocclussion. Triple orthognathic surgery: case report. Revista Mexicana de Ortodoncia 2015; 3(4) 257–265.
16. Perović T, Pešić Z, Aleksić I, Radojičić J, Blažej Z. Orthodontic-surgical treatment of a class III malocclusion (hypoplasia maxillae) a case report. Facta Universitatis Series Medicine and Biology 2015; 17(2):67–72.
17. Ngan P, Wilmes B, Drescher D, Martin C, Weaver B, Gunel E. Comparison of two maxillary protraction protocols: tooth-borne versus bone-anchored protraction facemask treatment. Prog Orthod 2015; 16:26.
18. Glišić B, Šćepan I, Nikolić Z, Đorđević D. Changes in position and relationship between jaws in children treated with Delair's mask. Stom Glas S 2004; 51:177–182. [in Serbian]
19. Charles A, Subbiah S, Senkutvan RS. Combined Orthodontic and Surgical Management of a Skeletal Class III Malocclusion with Mild Asymmetry–A Non Extraction Approach. Int J Dental Sci Res 2014; 2(1):5–8.
20. Ronchi P, Guariglia A. Surgical Orthodontic Treatment of Class III Malocclusions, Principles in Contemporary Orthodontics. Dr. Silvano Naretto (Ed), ISBN: 978-953-307-687-4, InTech. (2011). Available from: http://www.intechopen.com/books/principles-in-contemporary-orthodontics/surgical- orthodontic-treatment-of-class-iii-malocclusions
21. Bhandari PK, Anbuselvan G J. Nonsurgical management of class III malocclusion: A case report. J Indian Acad Dent Spec Res 2014;1:35–38.
22. Mouakeh, M. Cephalometric evaluation of craniofacial pattern of Syrian children with Class III malocclusion. Am J Orthod Dentofacial Orthop 2001; 119: 640–649.
23. Grob DJ. Extraction of a mandibular incisor in a class I malocclusion. Am J Orthod Dentofacial Orthop 1995;108:533-541.
24. Riedel RA, Little RM, Bui TD. Mandibular incisor extraction: Post-retention evaluation of stability and relapse. Angle Orthod 1992; 62:103–116.
25. Canut JA. Mandibular incisor extraction: Indications and long-term evaluation. Eur J Orthod 1996; 18:485–489.
26. Proffit WR, Ackerman JL. A systematic approach to orthodontic diagnosis\ and treatment planning. In: Graber TM, Swain BF, editors. Current Orthodontic Concepts and Techniques. 3 rd ed. St Louis: C.V. Mosby 1985.
27. Kerr WJ, Miller S, Dawber JE. Class III malocclusion: Surgery or orthodontics? Br J Orthod 1992;19:21–24.
28. Xu Y, Zhu P, Le L, Cai B. Conservative treatment for a growing patient with a severe, developing skeletal Class III malocclusion and open bite. Am J Orthod Dentofacial Orthop 2014; 145(6):807–816.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 20, No 2, 2018, pp. 74–76

UDC 616.314-007.21-089
https://doi.org/10.22190/FUMB181109014Z

Case Report

SURGICAL TREATMENT OF COMPLEX CROUZON SYNDROME CRANIOSYNOSTOSIS

Aleksandra Zdravković, Bojana Živković, Mirko Mićović, Lukas Rasulić

Clinic of Neurosurgery, Clinical Center of Serbia, University of Belgrade, Belgrade, Serbia

Abstract. *Crouzon syndrome is one of the most common syndromic craniosynostosis. We present complex course of treatment in a child with Crouzon syndrome presented with multisuture craniosynostosis and hydrocephalus. A 3-months-old boy was admitted with clinical signs of hydrocephalus and turribrachycephaly. Firstly, the hydrocephalus was addressed. The ventriculoperitoneal shunt with programmable valve was implanted. Secondly, in May 2017, posterior calvarial remodeling was done. Finally, after the child recovered, anterior calvarial remodeling with fronto-orbital advancement was performed. This completed the calvarial remodeling in this patient. Child is doing well, so far completing milestones of child development in time. This case presents an extremely complex case of syndromic multisuture craniosynostosis, in which it was necessary to perform the entire calvarial remodeling to correct the deformity and to provide a chance to the brain to grow and develop without constrictions.*

Key words: *Crouzon syndrome, craniosynostosis, hydrocephalus, surgical treatment.*

Introduction

Crouzon syndrome was described in 1912 by neurologist Louis Crouzon. It is one of the most commonsyndromic craniosynostosis [1]. It is a genetic disorder with autosomal dominant inheritance, including more than 30 mutation of gene FGFR2 (fibroblast growth factor receptor 2) [2]. Clinically, it is presented with craniosynostosis and facial hypoplasia. The craniosynostos is usually involves multiple sutures. The facial appearance is caused by underdeveloped maxilla that is responsible for typical phenotype-hypertelorism, exophthalmos, and short nose. Eye closing can be difficult in prominent exophthalmos while breathing difficulties occur due tomaxilla deformity. Interestingly, children with this disorder are usuallyof normal intelligence [2–4].

We present complex course of treatment in a child with Crouzon syndrome presented with multisuture craniosynostosis and hydrocephalus.

Case presentation

History

He is the second child from third properly administrated pregnancy. There were not earlier congenital anomalies in family. During a routine sonography in the 37th week of pregnancy, dilatation of the cerebral ventricleswas noted with no other fetal disturbances. After birth, turribrachycephalywas apparent, but also large bulging anterior fontaneland palpable calvarial defects. No other anomalies were noted, except high-arched palate.

Operation

At that time, the 3-months-old boy was admitted with clinical signs of hydrocephalus and turribrachycephaly. Computed tomography (CT) showed extremely dilatated cerebral ventricles, all sutures were wide open and the bone was extremely thin due to the raised intracranial pressure. So, during the same hospitalization, in December 2006, ventriculo-peritoneal shunt (VP shunt) with programable valve was implanted to treat the hydrocephalus. The child recovered well after surgery. With addressing the hydrocephalus, time has been given for bone ossification and reduction of the size of bone defects so the remodeling can be done (Figs. 1–2).

In May 2017, posterior calvarial remodeling was done. The bone covering the posterior fossa was completely removed followed by bilateral osteotomies 1cm posterior to the coronal suture. The bone band approximately 2cm wide was removed and repositioned to reconstruct the occiput. Following this, the remaining parietal and occipital bone was divided in two pieces and rotated to create adequate cranial shape. All bone pieces were fixed using 2.0 absorbable surgical sutures. With this, decompression of posterior cranial segment was done as well as remodeling of the calvarium.The child recuperated well after surgery.

In October 2017, final surgical procedure was performed, an anterior calvarial remodeling.Using the same incision of the previous surgery, frontal bone was exposed just below supraorbital ridges bilaterally. After bilateral osteotomy at the coronal suture and 1.5 cm

Correspondence to: Aleksandra Zdravković, M.D.
Clinic of Neurosurgery, Clinical Center of Serbia,
University of Belgrade, Belgrade, Serbia
Phone: +381 64 01 03 013
E-mail: sandrazdr2@gmail.com
Received November 9th, 2018, Accepted December 25th, 2018

Fig. 1 Photographs of the child before first reconstruction surgery

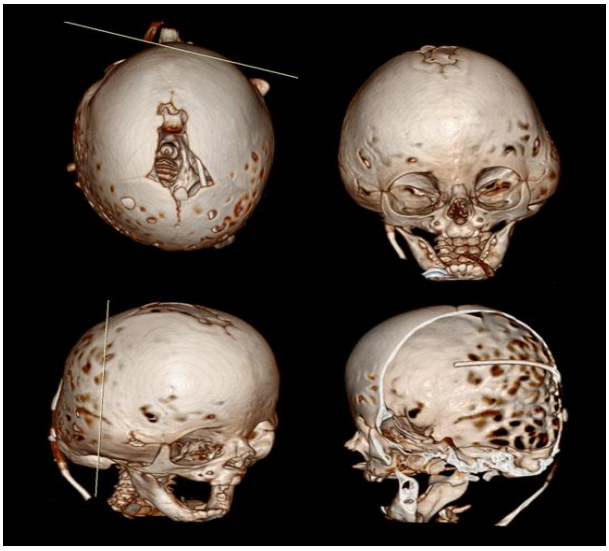


Fig. 2 Preoperative CT three-dimensional bone reconstruction showing complex head deformity and position of the ventricular catheter and valve

above supraorbital margin of the frontal bone, the frontal bone was elevated in one piece. Then, fronto-orbital bandeau was made followed by fronto-orbital advancement. The bone was advanced for approximately 2 cm and fixed using resorbable plates and screws.

Post-operation period

This completed the calvarial remodeling in this patient (Figs. 3–4). Child is doing well so far, completing milestones of child development in time. Facial advancement is due to be done after the child is approximately 6 years old.

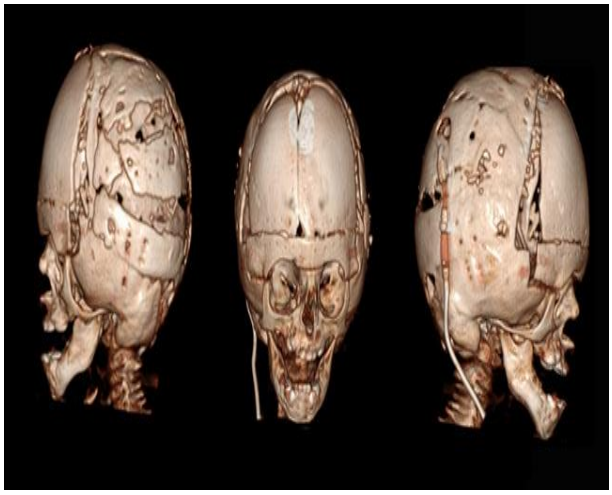


Fig. 3 Postoperative CT three-dimensional bone reconstruction showing entire calvarial remodeling and corrected shape

Fig. 4 Photographs of the child on the second postoperative day, showing immediate excellent results

Discussion

In the literature so far, numerous surgical techniques have been described to treat this complex craniosynostosis. Usually, the patient needs several surgeries to attain good results.

To achieve posterior decompression and remodeling, some authors consider that craniectomy of the posterior fossa is enough [5]. Others, expand entire posterior part of the calvarium using springs or distractors [6]. However, using this technique is creating a need for additional surgery, to remove the distracters and springs, thus additionally complicating the treatment course. Design and size of craniotomy are planned according to wanted gain and shape.

Simultaneous forehead remodeling and fronto-orbital advancement is performed to deal with anterior calvarial deformity [8].

Later in life, facial reconstruction needs to be done since the maxilla, nasal complex, and zygomatic body are hypoplastic and the orbits are shallow. These deformities lead to functional problems that may include airway obstruction (up to 50% of these patients may have sleep apnoea) [8], exorbitism with corneal ulceration, and lid dislocation.

Using the distraction osteogenesisthe mid-face advancement can be achieved. The technique is carried out with monobloc distraction in younger children (less than 2 years of life) and Le Fort III osteotomies and distraction in age 6–10 [9].

Sometimes the treatment course can be simpler. Thompson et al.describeda Crouzon syndrome case, where only occipital cranial vault advancement and decompression was done. Several years later, only the facial correction was necessary [10].

De Jong et al. recommended only fronto-orbital advancement [11]. Jeevanetal. consider a posterior decompression as primary treatment and suggest that the fronto-orbital advancement is recommended for the Crouzon syndrome cases without midface hypoplasia [12].

In our presented case, after dealing with hydrocephalus, we performed firstly posterior decompression and later anterior calvarial remodeling. Decompression was achieved and shape of the skull was satisfactory. Since the patient still has no significant airway obstruction, we decided on the delayed timing for mid-face advancement.

Conclusion

This case presents an extremely complex case of syndromic multisuture craniosynostosis, in which it was necessary to perform the entire calvarial remodeling to correct the deformity and to provide a chance to the brain to grow and develop without constrictions.

References

1. Al-Namnam NM, Hariri F, Thong MK, Rahman ZA. Crouzon syndrome: Genetic and intervention review. J Oral Biol Craniofac Res 2019; 9:37–39.
2. Winn R. Youmans and Winn neurological surgery. 7th ed. 2017, Philadelphia, PA Elsevier.
3. Kalanjiam V, Manoharan GVMG. Crouzon syndrome – A rare case report. Int J Health Sci (Qassim) 2017; 11:74–75.
4. Balyen L, Deniz Balyen LS, Pasa S. Clinical characteristics of Crouzon syndrome. Oman J Ophthalmol 2017; 10:120–122.
5. Cinalli G, Chumas P, Arnaud E, Sainte-Rose C. Renier D. Occipital Remodeling and Suboccipital Decompression in Severe Craniosynostosis Associated with Tonsilar Herniation. Neurosurg 1998; 42:66–73.
6. Ong J, Harshbarger R, Kelley P, George T. Posterior Cranial Vault Distraction Osteogenesis: Evolution of Technique. SeminPlast Surg 2014; 28:163–178.
7. Taylor JA, Bartlett SP. What's New in Syndromic Craniosynostosis Surgery? Plastic and Reconstructive Surg 2017; 140:82e–93e.
8. Pijpers M, Poels PJ, Vaandrager JM, de Hoog M, van den Berg S, HoeveHJ, Joosten KFM. Undiagnosed obstructive sleep apnea syndrome in children with syndromalcraniofacial synostosis. J CraniofacSurg 2004; 15:670–674.
9. Heggie AA, Kumar R, Shand JM. The role of distraction osteogenesis in the management of craniofacial syndromes. Ann Maxillofac Surg 2013; 3:4–10.
10. Thompson D, Jones B, Hayward R, Harkness W. Assessment and treatment of craniosynostosis. Br J Hosp Med 1994; 52:17–24.
11. De Jong T, Bannink N, Bredero-Boelhouwer HH, van Veelen ML, Bartels MC, Hoeve LJ, Hoogeboom AJ, Wolvius EB, Lequin MH, van der Meulen JJ, van Adrichem LN, Vaandrager JM, Ongkosuwito EM, Joosten KF, Mathijssen IM. Long-term functional outcome in 167 patients with syndromic craniosynostosis; defining a syndrome-specific risk profile. J Plast Reconstr Aesthet Surg 2010; 63(10):1635–1641.
12. Jeevan DS, Anlsow P, Jayamohan J. Abnormal venous drainage in syndromic craniosynostosis and the role of CT venography. Childs Nerv Syst 2008, 24:1413–1420.

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

61+57

FACTA Universitatis. Series:, Medicine and Biology / editor-in-chief Ljiljana Šaranac. - Vol. 1, No. 1 (1993)- . - Niš : University of Niš, 1993- (Niš : Unigraf-X-Copy). - 29 cm

Dva puta godišnje. - Drugo izdanje na drugom medijumu: Facta Universitatis. Series: Medicine and Biology (Online) = 2406-0526.
ISSN 0354-2017 = Facta Universitatis. Series: Medicine and Biology

COBISS.SR-ID 32415756

FACTA UNIVERSITATIS

Series
Medicine and Biology

Vol. 20, Nº 2, 2018

Contents