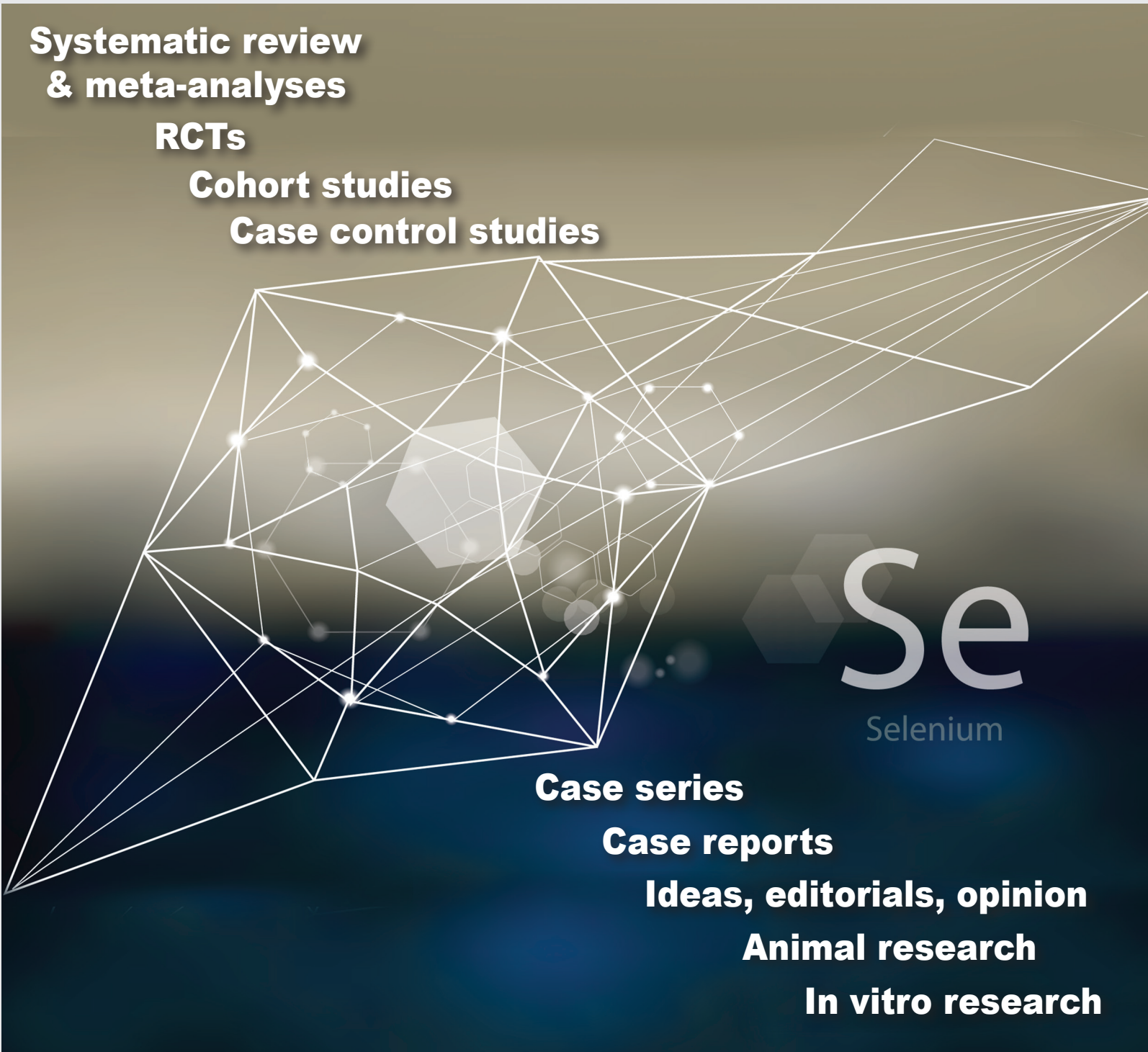
UNIVERSITY OF NIŠ

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

FACTA UNIVERSITATIS

Series
MEDICINE AND BIOLOGY
Vol. 22, N° 2, 2020



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**

The cover photo: https://depositphotos.com/stock-photos/

Publication frequency – one volume, two issues per year.

Published by the University of Niš, 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. 22, No 2, 2020

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 six 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. 22, No 2, 2020, pp. i–iv http://doi.org/10.22190/FUMB210210001S

Editorial

TWO CENTURIES AFTER DISCOVERY OF SELENIUM, STILL BETWEEN MEDICAL USE AND MISUSE IN CLINICAL PRACTICE?

Selenium (Se), atomic number 34, was discovered as a by-product of sulfuric acid in 1817 by the Swedish chemist and physician J.J. Berzelius. Its name is derived from the Greek: selene (Σελήνη), meaning Moon [1]. In Greek mythology, Selene is the goddess of the Moon. Namely when Se cools rapidly on being melted, it produces sheen similar to that of the Moon [2]. Appropriately, Se was detected in the Moon dust brought by the 1969 Apollo mission [3].

Although discovered before more than two centuries, Se and its role in health and disease remained underestimated and not fully elucidated. Endemic deficiency in large areas of central Asia clearly demonstrated its critical importance for human health and survival. There are two well-established entities of selenopenia: Kashin-Beck osteochondropathy and Keshan disease, severe cardiomyopathy characterized by fulminant heart failure [4, 5]. Concomitant with severe iodine deficiency, selenopenia leads to myxedematous endemic cretinism [6]. Se is present in food in organic form (selenocysteine and selenomethionine) and inorganic form (selenite or selenate). Recommended daily Se intake is 40-70 μg. Plasma Se levels should be kept between 60 and 120 μg/l [7]. It is an active component of selenoproteome, consisted of about 26 selenoproteins, mainly enzymes. The most important are glutathione peroxidases (GPxs; encoded by seven genes), iodothyronine deiodinases (DIs; encoded by three genes,) and three isoforms of thioredoxin reductases (TRx). GPxs protect cells from oxidative stress, the TRx system is involved in development and proliferation. Deiodinase system is essential for T4 to T3 conversion and preservation of iodine from uncontrollable wasting [2, 7]. Underactivity of selenoproteins (especially GPxs) caused by Se deficiency increases the risk for the impaired outcome of pregnancy, exaggerated inflammation, autoimmunity, infertility, cancer, and osteochondropathy [1, 2, 4, 8]. On the other hand, higher concentrations could potentiate the risk of hyperglycemia, insulin resistance, T2DM (Type 2 diabetes mellitus), glaucoma, cancer, hyperlipidemia, atherosclerosis, and increased cardiovascular mortality [2, 9].

Se antiviral effects are nowadays of special interest, as well as its immunomodulatory properties. Namely, Se's ability to improve the activity of T cells and the cytotoxicity of natural killer cells could render it effective in viral diseases [2]. Se deficiency as an environmental factor increases T-cell activation and shifts Th1/Th2 ratio to a Th1-type response, increasing typical Th1 cytokines as IL-2, TNF-α, IFN-γ, thereby reducing CD25+T regulatory cells (TREG) [1, 2, 4, 9]. It is an immune response typical for autoimmune diseases. However, a recent meta-analysis did not present sufficient data on the clinical efficacy of selenium supplementation in chronic autoimmune thyroiditis (CAT) even in adults, and the authors conclud-

Correspondence to: Ljiljana Šaranac, M.D., Ph.D.
Faculty of Medicine, Dr. Zoran Djindjić 81 Blvd., Niš, Serbia
Phone: +381 62 824 2161
E-mail: endoljilja@yahoo.com
Received February 10th, 2021

ed that further investigations are warranted [10]. Interventional studies with Se in different pathological conditions in children and adolescents are very rare. Investigation of Se levels in critical illness and its supplementation is of special clinical interest. The use of systematic reviews (SR) as a reliable source of evidence for making health decisions is increasing rapidly. The systematic review is the objective and reproducible method that uses explicit strategies to systematically search all available evidence on a specific focused topic, critically appraise methods and results of these studies, and synthesize their findings in order to provide reliable and accurate conclusions [11]. Depending on the heterogeneity between the included studies, the SR may or may not provide a quantitative synthesis of the overall effect using data presented in each included primary study through a statistical method called meta-analysis [12, 13]. Evidence-based medicine uses a hierarchy of reliable evidence, and systematic reviews and meta-analysis are placed at the top of the Evidence-Based Pyramid, representing the strongest and highest quality of evidence available. Going down the pyramid, the quality of provided scientific evidence is decreasing. When synthesizing the results of the primary studies, SR uses efficient strategies to reduce bias and random errors. Reading the SR allows taking into account a different range of findings from multiple studies on a specific topic and an efficient way to keep up-to-date with the best available evidence. It is often used as a starting point when making clinical guidelines. This approach can also point out where the evidence is lacking and can direct future research efforts to fill these gaps. In this issue of Facta Universitatis *Series* Medicine and Biology, Vuković et al. presented results of their SR on the investigation of Se levels in endocrine diseases in childhood and concluded that level of Se was mostly lower in children with goiter and those with the T1DM while several significant correlations, positive and negative, were reported across different parameters in patients with thyroid disease and with diabetes. They found that goiter and thyroid dysfunction are more prominent signs of Se deficiency in school children, based mainly on studies performed in Iran and Turkey. This is not surprising taking into account that the human thyroid represents the organ with the highest Se concentration per unit weight, among all tissues. The thyroid retains its Se content to an even higher level than the brain does [1,7].

It is noteworthy to emphasize that selenopenia in obese children is responsible for impaired thyroid structure and function that could be misdiagnosed as CAT. Improving Se status could be crucial for avoiding thyroid dysfunction. Elevation of TSH level in obese children may be due to increased leptin-mediated production of pro-TRH, impaired feedback due to a lowered number of T3 receptors in the hypothalamus of obese subjects, or with great certainty because of variations in peripheral deiodinase activity due to relative Se deficiency in obese children [14]. Nevertheless, we still recommend Se use without estimation of the patient's Se status or precise measurements of its plasma concentrations. Doctors are prone to prescribe Se to every single patient with thyroid disease, neglecting or not be fully aware of its potentially harmful effects. Despite numerous favorable functions in health protection, the narrow range of Se safety should be a reason for its limited use. Namely, both acute and chronic Se toxic effects (selenosis) are possible [2, 7]. The high amount of inorganic Se in drinking water increases the relative risk for amyotrophic lateral sclerosis [15].

In an extensive review, Duntas and Benvenga showed dose - dependent, beneficial and harmful effects of Se and the importance

of selenostasis for maintenance of overall health [2]. The Se curve of plasma concentration is U shaped. The right amount of Se is necessary for beneficial effects and the proper function of the human body machinery. As for iodine too little and too high concentration could be harmful. Taking into account its effect on potentiation the antioxidant effect, the question should arise: "Does everyone need an antioxidant activity?". Obviously, there is no universal fit. A better way is to have an individual approach, and the best way is to consider Se use in different pathological conditions on the basis of Se level determination. Endocrinologists embraced Se use in autoimmune thyroid disease (AITD), but there is a critical appraisal for such use. Se use in incipient and mild forms of thyroid disease is beneficial and routine use in all patients with AITD is questioned. There is evidence of a potential effect of Se in reducing the TPO-antibodies (Abs) titer and improving the ultrasound appearance and structure. Optimal results were found in patients with low Se levels and those with very high TPO-Abs levels [16, 17].

Is the Se supplementation a simple solution for complicated health issues? This question still needs an answer. Se as a missing link between health and prevention of different diseases certainly merits further investigation. Recent information from the USA regarding centenarian who survived deadly disease is in favor of such opinion. In 1918, Bernice Homan was just a year old when the Spanish Flu swept across the world and nearly took her life. The doctor told her mother Bernice wouldn't survive the night. Despite a bad prognosis, her mother refused to give up. She heard that pumpkin seeds were the most helpful for the flu. She made Pumpkin Seed Tea and spoon-fed Bernice the whole night until the sun came up and Bernice opened her eyes [18]. Mrs. Homan is a living breathing miracle and this touching story has a connection with the fact that Se could be critical for survival. Namely pumpkin seed contains iodine with antibacterial and Se with antiviral effects [19]. Over 100 years later, Bernice was the first resident at Willamette Oaks in the USA, to roll up her sleeves and get the COVID-19 vaccine.

References

1. Duntas LH. Selenium and the thyroid: a close-knit connection. J Clin Endocrinol Metab 2010; 95 (12): 5180-5188, doi: 10.1210/jc.2010-0191
2. Duntas LH, Benvenga S. Selenium: an element for life. Endocrine 2015; 48:756-775.
3. Flohe L, Andreesen JR, Brigelius-Flohe R, Maiorino M, Ursini F. Selenium, the element of the moon, in the life on the earth. IUBMB Life 2000; 49:411-420.
4. Guastamacchia E, Giagulli VA, Licchelli B, Triggiani V. Selenium and Iodine in autoimmune thyroiditis. Endocrine, Metabolic&Immune Disorders-Drug Targets 2015; 15:288-292.
5. Chanoine JP, Neve J, Wu S, Vanderpas J, Bourdoux P. Selenium decreases thyroglobulin concentrations but does not affect the increased thyroxine-to-triiodothyronine ratio in children with congenital hypothyroidism. J Clin Endocrinol Metab 2010; 96:1160-1163.
6. Zimmermann MB, Kohrle J. The impact of of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. Thyroid 2002; 12:867-878.
7. Ashton K, Hooper L, Harvey LJ, Hurst R, Casgrain A, Fairweather-Tait SJ. Methods of assessment of selenium status in humans: a systematic review. Am J Clin Nutr 2009; 89(Suppl):2025-2039.
8. Hofstee P, McAlpine JJ, McKeating DR, Vanderlelie JJ , Cuffe JSM, V Perkins AV. Low serum selenium in pregnancy is associated with reduced T3 and increased risk of GDM. J Endocrinol 2021; 248 (1):45–57. DOI: https://doi.org/10.1530/JOE-20-0319
9. Duntas LH. The role of iodine and selenium in autoimmune Thyroiditis. Horm Metab Res 2015; 47:721-726.
10. Winther KH, Wichman JEM, Bonnema SJ, Hegedus L. Insufficient documentation for clinical efficacy of selenium supplementation in chronic

autoimmune thyroiditis, based on a systematic review and meta-analysis. Endocrine 2017; 55:376-385.
11. Gopalakrishnan S, Ganeshkumar P, Systematic Reviews and Meta-analysis: Understanding the Best Evidence in Primary Healthcare. J Family Med Prim Care 2013; 2(1):9-14. doi: 10.4103/2249-4863.109934.
12. McNamara ER, Scales CD. Role of systematic reviews and meta-analysis in evidence-based clinical practice. Indian J Urol 2011; 27(4):520-524. doi: 10.4103/0970-1591.91445.
13. Clarke M. The Cochrane Collaboration and systematic reviews. Br J Surg 2007;94(4):391-2. doi: 10.1002/bjs.5812.
14. Radetti G, Kleon W, Buzi F, Crivellaro C, Pappalardo L, Di Lorgi N, Maghnie M. Thyroid function and structure are affected in childhood obesity J Clin Endocrinol Metab 2008; 93:4749-4754.
15. Vinceti M, Bonvicini F, Rothman KJ, Vescovi L, Wang F, The relation between amiotrophic lateral sclerosis and inorganic selenium in drinking water: a population based, case-control study. Environ Health 2010; 9:77. https://doi.org/10.1186/1476-069X-9-77
16. Gartner R, Gasnier BCH, Dietrich JW, Bjarne K, Angstwurm MWA. Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. J Clin Endocrinol Metab 2002; 87:1687-1691.
17. Duntas LH, Mantzou E, Koutras DA. Effects of a six-month treatment with selenomethionine in patients with autoimmune thyroiditis. Eur J Endocrinol 2003; 148:389-393.
18. https://www.kezi.com/content/news/103-year-old-Spanish-Flu-survivor-gets-COVID-19-vaccine-in-Eugene--573692281.html
19. Kreft I, Stibilj V, Trkov Z. Iodine and selenium contents in pumpkin (Cucurbita pepo L.) oil and oil-cake. Eur Food Res Technol 2002; 215:279–281. https://doi.org/10.1007/s00217-002-0563-5

Editor-in-Chief

Ljiljana Šaranac

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 22, No 2, 2020, pp. 25–33

UDC 616.43-008.6-53.2:546.23
http://doi.org/10.22190/FUMB210105002V

Original Article

SELENIUM LEVELS IN PEDIATRIC PATIENTS WITH ENDOCRINE DISEASES: EVIDENCE FROM A SYSTEMATIC REVIEW

Vladimir Vuković[1,2], Ljiljana Šaranac[3], Marko Jović[3], Bojko Bjelaković[3]

[1]Institute for Biomedicine, Eurac Research, Affiliated Institute of the University of Lübeck, Bolzano, Italy
[2]Centre for Disease Control and Prevention, Institute of Public Health of Vojvodina, Novi Sad, Serbia
[3]Faculty of Medicine, Niš, University of Niš, Serbia

Abstract. *Selenium (Se) is essential micronutrient involved in several physiological processes. In many regions around the world, a suboptimal intake of Se has been reported in several health conditions, also in pediatric age. Studies on association between Se level and diseases in children reported contrasting results. We took an aim to perform a systematic review of literature and provide evidence-based conclusion on the magnitude of Se deficit in endocrine diseases in children. PubMed, ISI WoS, and Scopus databases were searched to identify eligible studies, published until July 25, 2019. Methodological quality was assessed using Newcastle–Ottawa Scale. After careful selection, 13 eligible studies were included. Majority were conducted in Turkey (n=5) and Iran (n=5), and sample size varied from 61 to 628 children, with a mean (±SD) age of cases from 5.1±1.6 months up to 13.8±4.5 years. Eleven studies focused on different thyroid diseases, and two on children with type 1 diabetes mellitus (T1DM). In goitrous patients, Se level ranged from mean (±SD), 25.71±20.68 μg/L to 114.9±34.1 μg/L, while in patients with T1DM was 20.9±12.9 μg/mL and mean (95% CI)=58.4 μg/L (55.0–63.09). We may conclude that goiter and thyroid dysfunction are prominent signs of Se deficiency in children. Although deficiency of iodine and selenium are usually combined in some area, our systematic review showed that Se deficiency is important goitrogenic factor in school children. Further randomized controlled trials are needed to adequately explore the role of Se in endocrine disorders in children, across different populations and regions.*

Key words: *Selenium, endocrine, thyroid, diabetes, pediatric, systematic review.*

Introduction

Selenium (Se) is a trace mineral and one of the essential micronutrients involved in several physiological processes [1]. It is a constituent of selenoproteins many of which are engaged in protection against oxidative stress [2], and a cofactor of many enzymes involved in several major metabolic pathways [3–6]. It is involved in thyroid metabolism as a cofactor of the glutathione peroxidase (GPx), protecting the thyroid gland against oxidative stress, and iodothyronin deiodinase enzymes, converting thyroxine (T4) to triiodothyronine (T3) [4, 6–9]. Being so important in human organism, the deficiency of Se can endanger human health and lead to misbalance of many biochemical processes further resulting in disease [10, 11].

Se intake and its level in the body depend on the person's diet, where major sources of Se intake are plant foods, meat and meat products, nuts (Brazil nuts), cereals, fish and shellfish [12, 13]. In many regions around the world, people are exposed to inadequate content of Se in food because of the low Se content in the soils where plants and animals are grown [6, 14, 15]. Activity of Se-dependent thyroid enzymes, in case of Se deficiency, could cause impairment of the thyroid metabolism even in the situation of adequate iodine intake [16]. Also, deficit of Se has been associated with the poor immune response [17, 18]. In general, association of Se deficiency with many diseases has been documented, among which the increased risk of thyroid cancer, infections and immunodepression, diabetes, Keshan's disease and endemic myxedema cretinism, mostly suggested to be due to the lack of protection against the oxidative stress [15, 19, 20].

Suboptimal intake of Se has been reported in several health conditions also in the pediatric age [21]. In Hashimoto's thyroiditis in childhood, the deficiency of Se may promote initiation or progression of the disease [17, 22, 23]. Also, in other conditions of thyroid dysfunction in children, congenital hypothyroidism and goiter, different levels of serum Se have been demonstrated among cases and controls [16, 24]. Lower levels of Se and consequent oxidative damage are one of the possible factors involved in the etiology of diabetes mellitus in children [25]. Lower Se levels have also been reported in children with iron-deficiency anemia, dilated cardiomyopathy and acute lymphoblastic leukemia, when compared to the matched healthy controls [26, 27]. Low selenium levels also affected early neonatal morbidity in premature infants [28], and has been associated with hypoxia and respiratory diseases [29].

Studies on the association between Se level and diseases in children reported significantly lower levels of

Correspondence to: Vladimir Vuković M.D., Ph.D.
Centre for Disease Control and Prevention, Institute of Public Health of Vojvodina, Futoška 121, 21000 Novi Sad, Serbia
Phone: +381 21 4897 800
E-mail: vladimir.dr.vukovic@gmail.com
Received January 5th, 2021 / Accepted January 23rd, 2021

Se in cases or no difference between levels in cases and controls. Since endocrine health and hormonal balance are of great importance, particularly in the childhood, for normal growth and development, we took an aim to perform a comprehensive and systematic review of the literature in order to provide a general overview and draw evidence-based conclusion on the prevalence and magnitude of the Se deficit in endocrine diseases in children.

Material and Methods

Literature Search Strategy

In order to retrieve primary studies, a systematic bibliographic search was conducted in accord with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [30] through a multi-engine search of PubMed, ISI Web of Science (WoS), and Scopus databases. For initial search of the PubMed database, the following combination of subject headings and text words was used: (((selenium OR selenate OR "selenium derivative" OR "selenium compound*" OR "antioxidant cocktail*" OR selenite OR "sodium selenite" OR selenomethionine OR selenocysteine OR ebselen OR "selenious acid" OR "selenium-binding protein*" OR "trace element*") AND (lack OR deficit OR deficiency OR reduction OR shortage OR decreas* OR insufficien*)) AND (pediatric* OR children OR child OR preschool OR school OR infan* OR adolescen* OR young*)). The remaining two databases were searched using appropriately modified PubMed query (detailed search queries are available upon request). Our search was restricted to studies conducted on humans and published up until July 25, 2019 in English, Italian or Serbian language.

Study Selection

Records from three different electronic databases were retrieved and cross-linking was performed in order to remove duplicates. Titles and abstracts of the identified records were screened and full texts of the initially eligible articles were obtained and evaluated for final eligibility. All of the steps are documented in the PRISMA flow diagram.

Studies were considered eligible if they met the following inclusion criteria: cross-sectional, case-control or cohort study design; studies on children or adolescents ≤18 years old with endocrine disease; studies that evaluated serum/plasma levels of selenium. Other reviews, letters, commentaries, editorials, case studies, studies conducted on cell or animal model were further excluded. Justification for the exclusion of records was specified and any disagreement in opinion between reviewers was resolved through discussion.

Data Extraction, Synthesis and Quality Assessment

Extraction of the data from each eligible study was conducted and data was entered into standard Excel form in order to synthesize and present the results. Data on first author's name, year of study publication, country where the study was conducted, type of study design, type of endocrine disorder, total number of children, their age, percent of male participants, number of cases/controls, Se source (plasma or serum), measurement method of Se, measured concentration of Se (in cases/controls), reported statistical findings (if available). Due to the absence of common statistical estimates of effect in the included studies, we were unable to perform a quantitative pooling of data through meta-analysis thus we used a narrative synthesis to describe the findings.

Methodological quality of the included studies was assessed using the Newcastle–Ottawa Scale (NOS) for case-controls studies [31] and the adapted version by Herzog et al. [32] for the cross-sectional studies. Three main perspectives of the methodological quality of each included study were evaluated: selection of the study groups; comparability of the groups; and the ascertainment of outcome and exposure for cross-sectional and case-control studies, respectively. This tool uses a star system to assign a maximum of nine stars for case-control and ten for cross-sectional studies, across the evaluated domains. Studies were classified as good (≥6 stars), moderate (3-5 stars) and low (<3 stars) quality based on the maximum score. Detailed instructions for using this tool are provided elsewhere [33].

Results

Literature Search and Study Selection

A total of 4272 records were identified through the search of PubMed, ISI WoS and Scopus databases. After removing duplicates, 3312 titles and abstracts were screened, leading to a careful selection of 239 full text articles to be assessed for further eligibility. Of these, 226 articles were excluded for not meeting the inclusion criteria, leaving 13 eligible studies to be included in the qualitative synthesis [34, 35, 44–46, 36–43]. Detailed process of literature search and study selection is presented in Figure 1.

Characteristics of the Studies

Majority of the included studies were conducted in Turkey (n=5) and Iran (n=5), and by one in Poland, Ethiopia and Democratic Republic of the Congo (Table 1). Publication years ranged from 1990 [46] to the most recent one from 2018 [44]. Most of the studies used cross-sectional study design [34–41] while the remaining five were case-control studies [42–46]. Sample size varied from 61 [43] to 628 children [38] with mean age of cases spanning from 5.1±1.6 months [44] to 13.8±4.5 years [43]. Percentage of boys was different across the included studies, with a minimum of 21.95% [42] to a maximum of 71.25% [46]. Considering the investigated endocrinological disease, 11 studies focused on different thyroid disorders, namely goiter [34–41] and hypo-

thyroidisms [42,44], while the remaining two explored selenium status in children with the type 1 diabetes mellitus (T1DM) [43, 45]. Number of cases ranged from 48 [35, 39] to 280 [38] goitrous, and 35 [43] to 87 [45] children with T1DM.

Details on the selenium concentrations and main findings from the included studies are summarized in Table 2. Atomic absorption spectrometry was the most widely used method to measure the level of Se in serum [34–38, 42, 44, 46] and plasma [39–41, 43, 45]. Mean concentration of Se was different across the investigated populations and diseases. For goitrous patients, Se levels ranged from 25.71±20.68 μg/L [34] to 114.9±34.1 μg/L [36], while in patients with T1DM were 20.9±12.9 μg/mL [43] and 58.4 μg/L [45].

In general, level of Se was lower in children with goiter [35, 36, 39, 41] and in children with T1DM [43] or there was no difference reported between cases and controls in children with goiter and Hashimoto thyroiditis [38, 42]. Studies that had investigated correlation of Se level and several clinical parameters reported different findings, negative [34, 41], positive [39, 42], mixed [38, 44] some positive and some negative, and no significant correlation [35, 37, 40] in thyroid patients. In particular, a study by Gashu and colleagues reported that serum Se was negatively associated with T4, but positively with T3 concentration, and that a unit increase in concentration of Se could increase the serum T3 concentration by a factor of 0.16 [38]. On the other side, Cinaz et al. demonstrated the lack of correlation between serum Se levels and thyroid function tests in their study [35]. In two studies conducted on diabetic patients, a negative correlation was reported between Se level and glycosylated hemoglobin (HbA1c) level [43], while Salmonowicz et al. reported non-significant correlations across different parameters [45]. Overall, the body of evidence in our review was characterized by a moderate to high quality level. Methodological quality of the included studies was good, especially of those cross-sectional study design, where two studies [34, 38] scored maximum stars across all evaluated domains. Case-control studies were mostly of moderate to good methodological quality (Table 3).

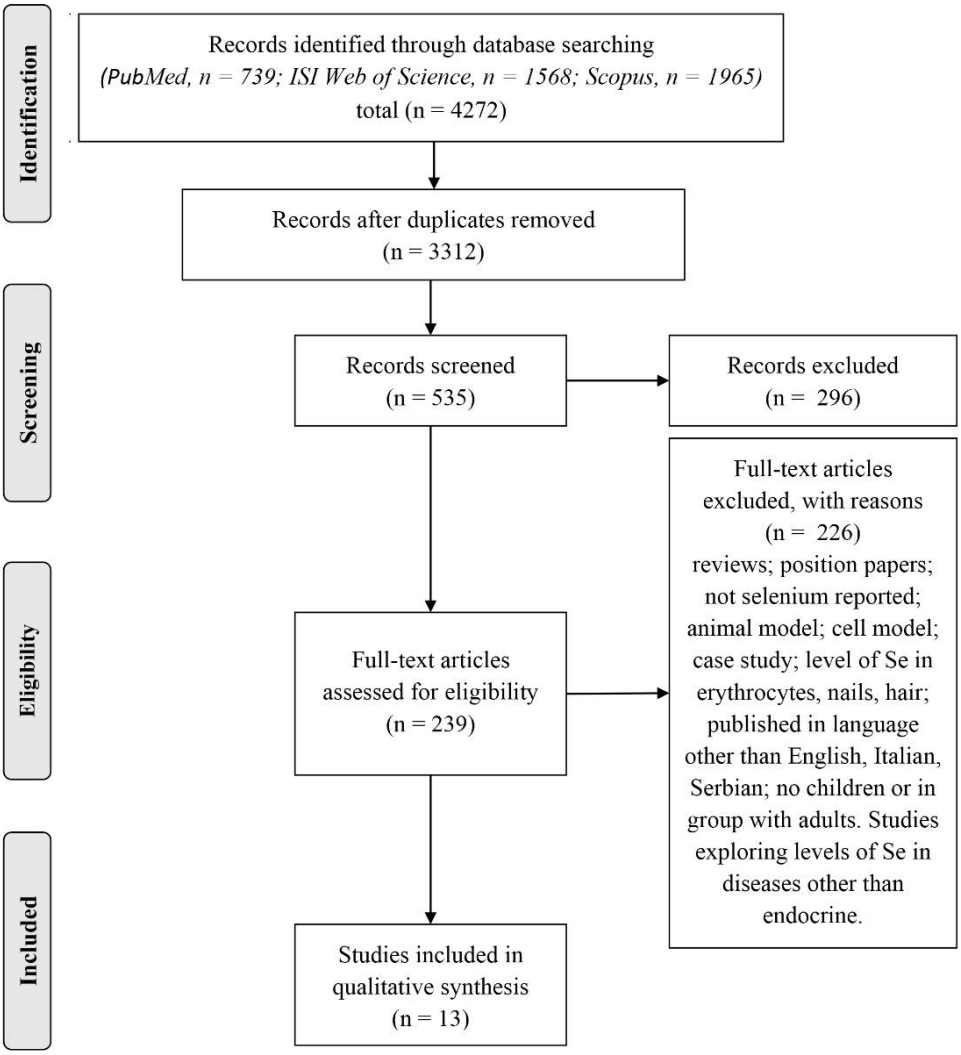


Fig. 1 Flowchart depicting literature search and study selection process

Table 1 General characteristics of the included studies

First author, year	Country	Study design	Endocrine disease	Total number of children	Age range, mean±SD (years)	Males, n (%)	Number (n) of cases and controls
Aydin, 2002 [34]	Turkey	Cross-sectional	Goiter	73	Range=7-12; mean±SD= 9.56±1.77	35 (48%)	Cases (56); controls (17)
Cinaz, 2004 [35]	Turkey	Cross-sectional	Goiter	165	Mean±SD= 9.04 ±1.91	NR	Cases (48); controls (117)
Dabbaghmanesh, 2007 [36]	Iran	Cross-sectional	Goiter	500	Range=8–13	NR	Cases (198); controls (302)
Erdogan, 2001 [37]	Turkey	Cross-sectional	Goiter (endemic)	251	Range=9–11	123 (49.01%)	Chidren from cities: Ankara (62), Kastamonu (64), Bayburt (59), and Kastamonu (66)
Gashu, 2016 [38]	Ethiopia	Cross-sectional	Goiter	628	Range=4.5–5; mean±SD= 4.74 ± 0.15	311 (49.52%)	Cases (280); controls (348)
Giray, 2001 [39]	Turkey	Cross-sectional	Goiter	121	Range= 15–18	52 (42.98%)	Cases (48); controls in-region controls (49), and out-region (24)
Hashemipour, 2008 [40]	Iran	Cross-sectional	Goiter	219	Range= 7-13; mean±SD=9.3±1.0	101 (46.12%)	Cases (108); controls (111)
Keshteli, 2009 [41]	Iran	Cross-sectional	Goiter	168	Range = 6-13; mean±SD= 9.39±1.18 (girls) and 9.47±1.12 (boys)	71 (42.26%)	Case (96); controls (72)
Nourbakhsh, 2015 [42]	Iran	Cross-control	Hashimoto's thyroiditis and hypothyroidism	82	Children with Hashimoto's thyroiditis, mean±SD= 13.0±4.2; with hypothyroidism, mean±SD= 11.4±3.3; controls, mean±SD= 12.4±3.0	18 (21.95%)	Cases: Hashimoto's thyroiditis (35), hypothyroidism (22); and controls (30)
Ozenc, 2015 [43]	Turkey	Cross-control	Type 1 diabetes mellitus (T1DM)	61	Cases, mean±SD=13.8±4.5; controls, mean±SD= 12.8±3.3	34 (55,74%)	Cases (35); controls (26)
Rostami, 2018 [44]	Iran	Cross-control	Congenital hypothyroidism	99	Cases, range= 0.25–1, mean±SD= 0.42 ± 0.13; controls, range= 0.08–1.25, mean±SD= 0.52 ± 0.12	49 (49,50%)	Cases (39); controls (60)
Salmonowicz, 2014 [45]	Poland	Cross-control	Type 1 diabetes mellitus (T1DM)	155	Children with T1DM range=2–19, mean±SD=13.0±4.0; siblings range= 4.5–16.5, mean±SD= 13.2 ± 3.7; and control groups range=10.5–18, mean±SD=14.8 ± 2.2	79 (50,97%)	Cases (87); controls: siblings of the patients with T1DM (27 children) and healthy (41) children
Vanderpas, 1990 [46]	Republic of Zaire (Democratic Republic of the Congo)	Cross-control	Endemic myxedematous cretinism	80	Cases, range=3-25; controls, range=9-18	57 (71,25%)	Cases (28); controls (52)

Table 2 Selenium concentrations and main findings from the included studies

First author, year	Biological sample for selenium measurement	Measurement method for selenium concentration	Selenium concentrations in cases and controls	Reported correlations
Aydin, 2002 [34]	Serum	Atomic absorption spectrometry	Goitrous, mean±SD= 25.71±20.68μg/L); non-goitrous, mean±SD= 47.76±22.84μg/L	Negative correlation was reported between thyroid volume and Se level (r = 0.32, ρ <0.05).
Cinaz, 2004 [35]	Serum	Spectrophotometer (Unicam 939 AA)	Goitrous, mean±SD= 52.39± 10.87 ng/mL; non-goitrous group, mean±SD= 58.94±15.42 ng/mL; p=0.002	Serum Se levels did not correlate with the thyroid function tests, and T3, T4, and TSH levels are found to be similar in goitrous and nongoitrous children.
Dabbaghmanesh, 2007 [36]	Serum	Atomic absorption spectrometry (Chemtech Anaytical CTA 2000, AAS)	Goiterous, mean±SD= 114.9 ± 34.1 μg/L; non-goitrous, mean±SD= 121.9 ± 28.7 μg/L; p<0.05	NR
Erdogan, 2001 [37]	Serum	Atomic absorption spectrometer (Hitachi Z-8200® polarized Zeeman)	Study area: Ankara, mean±SD= 54.82±15.73ng/mL; Kastamonu, mean±SD= 50.99±13.38ng/mL; Bayburt, mean±SD= 57.54±14.60ng/mL; and Trabzon, mean±SD= 52.22±14.30ng/mL	No significant correlations between serum Se concentrations and studied parameters (i.e., Thyroid volume, thiocyanate (SCN^-) overload, thyroid hormones, sensitive TSH (sTSH) levels, and urinary iodine concentrations (UICs)) was detected.
Gashu, 2016 [38]	Serum	Mass spectrometer (PerkinElmer, ELAN9000, Norwalk, CT, USA).	NR (mean Se concentrations of goitrous and non-goitrous children were not significantly different, p>0.05). Entire study population, median=61.4 μg/L, range=10.7–290.9 μg/L	Serum Se was negatively correlated to T4 concentration (r=−0.22, p<0.01).
Giray, 2001 [39]	Plasma	Spectrofluorometric method	Total goiter group, mean±SD= 67.1±10.9μg/L; total in-region control, mean±SD= 75.0±14.6μg/L; and out-region control group, mean±SD= 75.3±12.8μg/L	In "all severely deficient children" a positive correlations of urinary iodine and Se were observed (r = 0.42, p<0.05).
Hashemipour, 2008 [40]	Plasma	Atomic absorption spectrometer	Goitrous, mean±SD= 62.7 g/L; non-goitrous, mean±SD= 60.8 g/L; p = 0.42	No correlation was found between serum Se concentration and baseline data of the patients including height, weight, BMI and age.
Keshteli, 2009 [41]	Plasma	Atomic absorption spectrometer	Goitrous, mean±SD= 66.86±21.82 μg/L; non-goitrous children, mean±SD= 76.67±23.33 μg/L; p=0.006	In goitrous children, Se level was reversely correlated with age (r=−0.24, p=0.02), BMI (r=−0.20, p=0.05) and body surface area (r=−0.26, p=0.01) and was positively correlated with T4 (r=0.22, p=0.03).
Nourbakhsh, 2015 [42]	Serum	Atomic absorption spectrometer (ContrAA 700, Analytik Jena AG, Jena, Germany)	Hashimoto's thyroiditis, mean±SD= 91.6±17.7μg/L; hypothyroidism, mean±SD= 85.9±14.8 μg/L; controls, mean±SD= 97.2±29.4μg/L	Se levels did not show any significant correlation with glutathione peroxidase but it had a significant correlation with selenoprotein P (r = 0.34, p= 0.02)
Ozenc, 2015 [43]	Plasma	Atomic absorption spectrophotometry (Varian Techtron Pty. Ltd., Victoria, Australia)	T1DM, mean±SD= 20.9±12.9μg/mL; controls, mean±SD=32.6±10.2μg/mL	There was a negative correlation between Se and HbA1c levels (r=−0.44, p<0.01)
Rostami, 2018 [44]	Serum	Atomic absorption spectroscopy	Hypothyroidism, mean±SD= 10.5 ± 0.65 mg/L; controls, mean±SD=55.17 ± 8.55 mg/L; p = 0.01	No statistically significant correlations were found between Se concentration and TSH level (r = 0.13, p = 0.29), and FT4 concentration (r = 0.07, p = 0.54). There was a significant correlation between prooxidant-antioxidant balance (PAB) value and serum Se concentration, and also between Se concentration and MCV (for all parameters, p>0.05)
Salmonowicz, 2014 [45]	Plasma	Atomic absorption spectrophotometer (SOLAAR M6, Thermo Elemental, Great Britain)	Patients with T1DM, mean (95% CI)= 58.4μg/L (55.0–63.09); siblings of patients with T1DM, mean, (95% CI)=53.45μg/L (46.09–65.21); healthy controls, mean (95% CI)= 53.3μg/L (45.78–70.17)	In patients with T1DM no significant correlations were found between Se and: HbA1c (r=0.2, p=0.07), age (r = 0.01, p = 0.89), TC (r = –0.05, p = 0.72), TG (r = –0.07, p = 0.59), HDL-C (r = –0.12, p = 0.45), LDL-C (r = –0.02, p = 0.89), TC/HDL–C (r = 0.20, p = 0.22)
Vanderpas, 1990 [46]	Serum	Spectrofluorimetry	Cases, mean±SD= 443 ± 188 nmol/L; controls, mean±SD=343 ± 176 nmol/L; p>0.1	NR

NR= not reported; SD=standard deviation; T1DM=Type 1 diabetes mellitus

Table 3 Methodological quality of the included studies

First author, year	Study design	Selection	Comparability	Outcome/Exposure
Aydin, 2002 [34]	CS	*****	**	***
Cinaz, 2004 [35]	CS	****	**	***
Dabbaghmanesh, 2007 [36]	CS	*****	*	***
Erdogan, 2001 [37]	CS	***	**	***
Gashu, 2016 [38]	CS	*****	**	***
Giray, 2001 [39]	CS	***	**	***
Hashemipour, 2008 [40]	CS	****	**	***
Keshteli, 2009 [41]	CS	****	**	***
Nourbakhsh, 2015 [42]	CC	***	*	**
Ozenc, 2015 [43]	CC		*	**
Rostami, 2018 [44]	CC	****	*	**
Salmonowicz, 2014 [45]	CC	***	*	**
Vanderpas, 1990 [46]	CC	*	*	**

CS = cross-sectional; CC = case-control.

Note: A study can be awarded a maximum of four stars for case-controls and five stars for cross-sectional study within the selection category. A maximum of two stars can be given for comparability and three stars for outcome/exposure categories.

Discussion

Although discovered more than two centuries ago, Se and its role in health and disease remained underestimated and not fully elucidated. Endemic deficiency in large areas of central Asia clearly demonstrated its critical importance for survival. There are two well established entities of selenopenia: Kashin-Beck osteochondropathy and Keshan disease, a severe cardiomyopathy characterized by fulminant heart failure [47, 48]. The later mainly affects young children and women in childbearing age and it is apparent in population with particularly low Se intake (<15 μg/d), in some areas of China. It is noteworthy to mention that there is a relatively narrow range of Se intake, between deficiency (<30 μg/d) and toxicity (>900 μg/d) [49]. Numerous beneficial effects are attributed to Se and are extensively investigated in adults: antioxidant effects, correct functioning of the immune system, antiviral effects (nowadays of special interest), decrease in the risk of miscarriage, effects on bone metabolism, regulation of normal spermatozoa development and motility, beneficial effect on mood [6, 47, 50, 51]. Interventional studies with Se in different pathological conditions in pediatric age are very rare.

In the current review, we selected 13 articles with the aim to investigate interdependence of Se levels and clinical presentation of endocrine diseases in children and adolescents. Majority of them refers to thyroid disorders and thyromegaly. Only two were performed in children with T1DM. It is expected finding taking into account that thyroid represents the organ which is most abundant in Se per gram of tissue, even richer than the brain, due to a high content of selenoproteins [6]. According to these data, the most frequent and severe Se deficiency is present in some parts of Africa (Northern Zair and Ethiopia) and Asia (China, Iran and Turkey) [38, 46, 49]. In a cross-sectional study on 628 children from Gonder, town of the Amhara region of Ethiopia, a goiter was found in 44,6% of them. The majority of children (88,6%) had suboptimal iodine supply (<100 μg/l). The presence of Se deficiency (serum Se <70 μg/l) was recognized as a problem in 57.8% of children [38]. Also, serum Se was negatively associated with T4 level in young children from this region. Vanderpas et al. emphasized that combined iodine and Se deficiency could be associated with the elevated frequency of myxedematous cretinism as a consequence of thyroid involution [46]. Similarly to observations from China, the distribution of Se deficiency was highly variable such that the deficiency and toxicity occurred in populations living just about 20 km apart, depending strongly on the geochemical characteristics of the soil in these specific areas [46, 49].

As mentioned above, in some severely iodine-deficient areas a concomitant deficiency of Se aggravates hypothyroidism leading to the myxedematous cretinism [14, 46]. On the other hand, an adequate selenium supply protects the thyroid from damage due to excessive iodine exposure, thus a selenium deficit should be corrected before the introduction of iodine supplementation when both deficits coexist [14, 52, 53]. Selenium deficiency was negatively associated with serum T4, but almost all children had normal level of T3. Vanderpas et al. suggested that in iodine-deficient subjects, Se deficiency may help maintaining the T3 concentration at normal level [46]. We find that it is just opposite from what could be expected, taking into account the role of Se containing deiodinase DIO1 in conversion of T4 to T3. Improved peripheral deiodination of T4 should provide increase in T3/T4 ratio that is confirmed after Se supplementation in school children from Se deficient area, evident from the study of Contempre et al. [54]. From 11 studies concerning the interdependence of Se level and thyroid status, two demonstrated a positive correlation, one to T4 and another to T3. In the study of Gashu et al., Se concentration was negatively associated to T4 concentration, but positively to serum T3 concentration [38]. It is difficult to explain contradictory data about T4 and T3 levels, so further studies conducted on larger cohorts are necessary.

Endemic goiter is present in almost all regions of Turkey, while the highest prevalence is reported in the Black Sea region, reaching over 50%, particularly in

some high-altitude mountain villages. Iodization of salt was introduced in Turkey in 1968, but IDD (Iodine Deficiency Disorders) are far from eradication in this country [39]. For comparison, iodization of salt in former Yugoslavia started in 1956 and then reevaluated and reinforced in 1993, when the amount of iodine was doubled [55]. Giray et al. evaluated Se concentrations in goitrous children with severely and moderately iodine deficiency in comparison with normal and mildly iodine deficient non goitrous children serving as control, and found significantly lower Se values in the goitrous group. The same study showed lower level of antioxidant blood enzymes glutathione peroxidase, catalase and superoxide dismutase, suggesting alterations in antioxidant defense system and multiple micronutrient deficiency [39]. All Turkish studies reported high goiter persistence, despite normalization of the iodine status. Goitrous children from Turkey had moderate to severe Se deficiency in iodine depleted as well as in the iodine repleted areas [34, 39]. Similar to Turkish experience, the Iranian studies in former endemic areas showed high percentage of goiter in school children. The deficiency of both trace elements, iodine and selenium is even more severe than what was reported in Turkey. The most affected endocrine gland by Se deficiency was thyroid. Studies from Turkey, as well as from Iran referred goiter in children as the most prominent clinical sign of selenopenia (9 out of 11 studies). Unfortunately, even in the cross-sectional studies, Se level was not measured in the whole group, but only in goitrous children, selected by palpation of the gland when some severely Se deficient children might be omitted. In selected children with thyromegaly the widest difference in Se level between goitrous and non-goitrous children was found in the study of Aydin et al., 25.71 μg/L and 47.76 μg/L, respectively [34]. The authors found significant negative correlation between thyroid volume and Se levels. On the other side, Gashu [38] and Hashemipour [40] did not find significant difference in Se levels between goitrous and non-goitrous children. Obviously, the role of other goitrogens in food or another trace-element deficiency should be additionally evaluated.

Extremely low Se level was found by Rostami et al. in the group of hypothyroid children [44]. Only one pediatric study from Iran, among selected in our review, referred to autoimmune thyroid disease. It failed to find significant difference in Se levels between patients with Hashimoto's thyroiditis and normal controls [42]. It is expected that having a normal Se level provides effective thyroid function and diminishes oxidative stress in thyroid cells, thus protecting thyroid from autoimmunity. In adult patients with chronic autoimmune thyroiditis from Germany and Greece, a Se supplementation decreased thyroid peroxidase antibodies [56, 57]. A striking majority of these patients reported an improvement in mood and well-being after 6 months of combined treatment (LT4+Se) [57]. In children, this effect was not confirmed [42]. However, a recent meta-analysis did not present sufficient data on clinical efficacy of selenium supplementation in chronic autoimmune thyroiditis in adults, and the authors concluded that further investigations are warranted [51].

Only two studies on children with T1DM were included as eligible in our systematic review [43, 45]. Hyperglycemia in diabetes increases or potentiates the oxidative stress. In the Turkish study a significantly lower levels of Se were found in diabetic children in comparison with controls and it correlated negatively with the HbA1c. Expectantly, the level of Se-containing enzyme, GPx was significantly higher in diabetic children [43]. We may explain this finding by enhanced the need for GPx antioxidant activity that consumes more Se in diabetic subjects. In the study of Polish authors, on a larger group of patients, difference in the GPx activity between diabetic children and non-diabetic controls was not confirmed [45]. It would be of interest to determine and to compare Se levels in different stages of diabetes in children (prediabetes, at admission, in acute metabolic decompensation, remission period, permanent stable or brittle diabetes). These results could be of immense practical importance for the prevention strategies.

Strengths and Limitations

To the best of our knowledge, this is the first review about the selenium deficiency in endocrine diseases to focus specifically on pediatric population, and thus has several strengths. First, we used a comprehensive methodological approach and careful selection of the studies, and also did an extensive data extraction from the included studies and provided a synthesis of evidence on this issue. Second, our search covered wide time interval and several different populations of children across different regions and countries. Further, the methodological quality of included studies was of moderate to high level, which was confirmed by a critical evaluation of their study design using appropriate evaluation scale.

However, there are some limitations that should be considered when interpreting our results. We only searched for published studies in English, Italian and Serbian language, thus we cannot exclude the possibility of publication bias and also, this might have affected the geographical representation of the studies. We did not limit our search to any particular endocrine disease since we wanted to present the general overview of the Se levels in endocrine disorders in children, available so far. Observational nature of the included studies is another limitation that should be acknowledged. Further randomized controlled trials are needed to adequately explore the role of Se in disorders of the endocrine system.

We were unable to perform a quantitative pooling of the data through a meta-analysis due to the lack of effect estimates in the included studies. Additionally, most of the included studies investigated populations from low and middle income countries thus our findings might not be widely applicable to other populations, particularly those from high-income countries, due to the pos-

sible difference in life-style, habits and particularly in diet. In fact, lack of information on dietary habits and Se intake from food as well as the genetic background of the participants might have influenced our findings, but these were not available to us since they were not explored in the included studies. This further underlines the need for more research in that direction. In particular, since the incidence of endocrine diseases, especially diabetes is rising across the world, a trans-regional multicenter studies on children to explore Se levels across different populations and regions are highly desirable.

Conclusions

We may conclude that thyroid disorders, goiter and thyroid dysfunction are prominent signs of Se deficiency in school children. Critical importance of thyroid function on early brain development, somatic growth and maturation, as well as for appropriate energy level for an active life, is impetus for further thorough investigation of selenostasis in wide population, especially in children and adolescents. For thyroid autoimmunity, there are insufficient data in the pediatric age. Although deficiency of iodine and selenium are usually combined in some area, our systematic review showed that Se deficiency is important goitrogenic factor in school children. Deficiency of other trace elements, like Zn (cofactor of TSH receptor) and iron (cofactor of thyroid peroxidase enzyme), necessary for appropriate thyroid function, should be further investigated.

The role of Se in autoimmunity is attractive and promising, but it is underinvestigated and its clinical use is not recommended routinely. Some selenopenic subjects with autoimmune diseases could have beneficial effects. Wider use of Se is not advised because of its potential toxic effects. High Se intake may lead to selenosis or increase risk of type 2 diabetes mellitus, glaucoma, cancer, cardiovascular mortality and peripheral arterial disease [6]. Thus, it is particularly important to ensure optimal and balanced diet, in both macro and micronutrients, for better metabolic health from an early age.

Acknowledgments. This work was 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 the Faculty of Medicine Nis, University of Niš, Serbia and was scientifically supported by the Association of Preventive Pediatrics of Serbia (Udruženje za preventivnu pedijatriju Srbije) 18000 Niš - Mediana, Blvd Zorana Djindjica 48, Clinical Centre Nis, Serbia.

References

1. Margalioth EJ, Schenker JG, Chevion M. Copper and Zinc levels in normal and malignant tissues. Cancer 1983; 52:868–872. doi: 10.1002/1097-0142(19830901)52:5<868::AID-CNCR2820520521>3.0.CO;2-K
2. Köhrle J, Jakob F, Contempré B, Dumont JE. Selenium, the thyroid, and the endocrine system. Endocr Rev 2005; 26:944-84. doi: 10.1210/er.2001-0034
3. Hincal F. Trace elements in growth: Iodine and selenium status of Turkish children. J Trace Elem Med Biol 2007; 21:40–43. doi: 10.1016/j.jtemb.2007.09.012
4. Mickiewicz B, Villemaire ML, Sandercock LE, Jirik FR, Vogel HJ. Metabolic changes associated with selenium deficiency in mice. BioMetals 2014; 27:1137–1147. doi:10.1007/s10534-014-9774-z
5. Jonklaas J, Danielsen M, Wang H. A pilot study of serum selenium, Vitamin D, and thyrotropin concentrations in patients with thyroid cancer. Thyroid 2013; 23:1079–1086. doi: 10.1089/thy.2012.0548
6. Duntas LH, Benvenga S. Selenium: an element for life. Endocrine 2015; 48:756–775. doi: 10.1007/s12020-014-0477-6
7. Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D. Selenium supplementation in the treatment of Hashimoto's thyroiditis: A systematic review and a meta-analysis. Thyroid 2010; 20:1163–1173. doi: 10.1089/thy.2009.0351
8. Beckett GJ, Arthur JR. 3 The iodothyronine deiodinases and 5′-deiodination. Baillieres Clin Endocrinol Metab 1994; 8:285–304. doi: 10.1016/S0950-351X(05)80253-8
9. Arthur JR, Beckett GJ, Mitchell JH. The interactions between selenium and iodine deficiencies in man and animals. Nutr Res Rev 1999; 12:55–73. doi: 10.1079/095442299108728910
10. Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system. J Nutr 2003; 133:1457–1459. doi: 10.1093/jn/133.5.1457s
11. Rayman MP. The importance of selenium to human health. Lancet 2000; 356(9225):233–241 doi: 10.1016/S0140-6736(00)02490-9
12. Kobayashi Y, Ogra Y, Ishiwata K, Takayama H, Aimi N, Suzuki KT. Selenosugars are key and urinary metabolites for selenium excretion within the required to low-toxic range. Proc Natl Acad Sci U S A 2002; 99:15932–15936. doi: 10.1073/pnas.252610699
13. Athanasopoulou H, Doulgeraki A. The importance of selenium in pediatrics. Trace Elem Electrolytes 2013; 30:100–104. doi: 10.5414/TEX01286
14. Zimmermann MB, Köhrle J. The impact of iron and selenium deficiencies on iodine and thyroid metabolism: Biochemistry and relevance to public health. Thyroid 2002; 12:867–878. doi: 10.1089/105072502761016494
15. Rayman MP. Selenium and human health. Lancet.2012; 379 (9822):1256–1268. doi: 10.1016/S0140-6736(11)61452-9
16. Wu Q, Rayman MP, Lv H, Schomburg L, Cui B, Gao C, et al. Low population selenium status is associated with increased prevalence of thyroid disease. J Clin Endocrinol Metab 2015; 100:4037–4047. doi: 10.1210/jc.2015-2222
17. Effraimidis G, Wiersinga WM. Mechanisms in endocrinology: Autoimmune thyroid disease: Old and new players. Eur J Endocrinol 2014; 170:R241-52. doi:10.1530/EJE-14-0047
18. Köhrle J, Gärtner R. Selenium and thyroid. Best Pract Res Clin Endocrinol Metab 2009; 23(6):815–827. doi: 10.1016/j.beem.2009.08.002
19. Cardoso BR, Ong TP, Jacob-Filho W, Jaluul O, Freitas MIDÁ, Cozzolino SMF. Nutritional status of selenium in Alzheimer's disease patients. Br J Nutr 2010; 103:803–806. doi: 10.1017/S0007114509992832
20. Thomson CD, Campbell JM, Miller J, Skeaff SA, Livingstone V. Selenium and iodine supplementation: Effect on thyroid function of older New Zealanders. Am J Clin Nutr 2009; 90 1038–1046. doi: 10.3945/ajcn.2009.28190
21. Chanoine JP. Selenium and thyroid function in infants, children and adolescents. BioFactors 2003; 18:137–143. doi: 10.1002/biof.5520190306
22. De Luca F, Santucci S, Corica D, Pitrolo E, Romeo M, Aversa T. Hashimoto's thyroiditis in childhood: presentation modes and evolution over time. Ital J Pediatr 2013; 39:8. doi: 10.1186/1824-7288-39-8
23. Vanderpump MPJ. The epidemiology of thyroid disease. Br Med Bull 2011; 99:39–51. doi:10.1093/bmb/ldr030

24. Kishosha PA, Galukande M, Gakwaya AM. Selenium Deficiency a Factor in Endemic Goiter Persistence in Sub-Saharan Africa. World J Surg 2011; 35:1540–1545. doi: 10.1007/s00268-011-1096-5
25. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003; 17: 24–38. doi: 10.1002/jbt.10058
26. Hincal F, Basaran N, Celiker A, Bilgic A, Ozbarlas N, Baysal M. Selenium levels of children with dilated cardiomyopathy in Turkey. In: Abdulla M, Vohora S, Athar M, editors. Trace and toxic elements in nutrition and health. New Delhi: Wiley Eastern; 1995. pp. 175–8.
27. Yetgin S, Hincal F, Baçaran N, Ciliv G. Serum Selenium Status in Children with Iron Deficiency Anemia. Acta Haematol 1992; 88:185–188. doi: 10.1159/000204683
28. Burjonrappa SC, Miller M. Role of trace elements in parenteral nutrition support of the surgical neonate. J Pediat Surg 2012; 47: 760–771. doi: 10.1016/j.jpedsurg.2012.01.015
29. Senkevich OA, Koval'skiy YG. The Importance of Selenium in Children's Health and Reproduction. Importance of Selenium in the Environment and Human Health. IntechOpen 2020. doi:10.5772/ intechopen.88864
30. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009; 339:b2535–b2535. doi:10.1136/bmj.b2535
31. The Newcastle-Ottawa Scale Coding Manual. Available: www.ohri.ca/%0Aprograms/clinical_epidemiology/nos_manual.pdf
32. Herzog R, Álvarez-Pasquin MJ, Díaz C, Del Barrio JL, Estrada JM, Gil Á. Are healthcare workers intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. BMC Public Health. 2013; 13:154. doi:10.1186/1471-2458-13-154
33. Wells G, Shea B, O'Connell D. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000 [cited 2 Jan 2021]. Available: http://www.ohri.ca/programs/ clinical_epidemiology/oxford.asp
34. Aydin K, Kendirci M, Kurtoğlu S, Karaküçük E, Kiriş A. Iodine and selenium deficiency in school-children in an endemic goiter area in Turkey. J Pediatr Endocrinol Metab 2002; 15:1027–1031.
35. Cinaz P, Karakasü DS, Çamurdan MO, Bideci A, Ayvalı ED, Yücel C. Goiter Prevalence, Serum Selenium, and Urine Iodine Status in a Previously Iodine-Deficient Area in Turkey. Biol Trace Elem Res 2004; 100:185–194. doi: 10.1385/BTER:100:3:185
36. Dabbaghmanesh MH, Sadegholvaad A, Ejtehadi F, Omrani G. Low serum selenium concentration as a possible factor for persistent goiter in Iranian school children. BioFactors 2007; 29:77–82. doi :10.1002/biof.552029207
37. Erdogan G, Erdogan MF, Sav H, Güllü S, Kamel N. Endemic Goiter, Thiocyanate Overload, and Selenium Status in School-Age Children. Biol Trace Elem Res 2001; 79:121–130. doi: 10.1385/BTER:79:2:121
38. Gashu D, Stoecker BJ, Adish A, Haki GD, Bougma K, Aboud FE, et al. Association of serum selenium with thyroxin in severely iodine-deficient young children from the Amhara region of Ethiopia. Eur J Clin Nutr 2016; 70:929–934. doi: 10.1038/ejcn.2016.27
39. Giray B, Hincal F, Teziç T, Ökten A, Gedik Y. Status of Selenium and Antioxidant Enzymes of Goitrous Children Is Lower Than Healthy Controls and Nongoitrous Children with High Iodine Deficiency. Biol Trace Elem Res 2001; 82:35–52. doi: 10.1385/ BTER:82:1-3:035
40. Hashemipour M, Siavash M, Amini M, Aminorroaya A, Rezvanian H, Kachuei A, et al. Goiter Persistence After Iodine Replenishment, the Potential Role of Selenium Deficiency in Goitrous Schoolchildren of Semirom, Iran. Exp Clin Endocrinol Diabetes 2007; 116:75–79. doi: 10.1055/s-2007-990298
41. Keshteli AH, Hashemipour M, Siavash M, Amini M. Selenium Deficiency as a Possible Contributor of Goiter in Schoolchildren of Isfahan, Iran. Biol Trace Elem Res 2009; 129:70–77. doi: 10.1007/ s12011-008-8296-3
42. Nourbakhsh M, Ahmadpour F, Chahardoli B, Malekpour-Dehkordi Z, Nourbakhsh M, Hosseini-Fard SR, et al. Selenium and its relationship with selenoprotein P and glutathione peroxidase in children and adolescents with Hashimoto's thyroiditis and hypothyroidism. J Trace Elem Med Biol 2016; 34:10–14. doi: 10.1016/j.jtemb.2015.10.003
43. Özenç S, Saldir M, Sarı E, Çetinkaya S, Yeşilkaya Ş, Babacan O, et al. Selenium, zinc, and copper levels and their relation with HbA1c status in children with type 1 diabetes mellitus. Int J Diabetes Dev Ctries 2015; 35:514–518. doi: 10.1007/s13410-015-0327-y
44. Rostami S, Fathollahpour A, Abdi M, Naderi K. Alteration in Prooxidant-Antioxidant Balance Associated with Selenium Concentration in Patients with Congenital Hypothyroidism. J Med Biochem 2018; 37:355–363. doi: 10.1515/jomb-2017-0052
45. Salmonowicz B, Krzystek-Korpacka M, Noczyńska A. Trace Elements, Magnesium, and the Efficacy of Antioxidant Systems in Children with Type 1 Diabetes Mellitus and in Their Siblings. Adv Clin Exp Med 2014; 23:259–268. doi: 10.17219/acem/37074
46. Vanderpas JB, Contempré B, Duale NL, Goossens W, Bebe N, Thorpe R, et al. Iodine and selenium deficiency associated with cretinism in northern Zaire. Am J Clin Nutr 1990; 52:1087–1093. doi: 10.1093/ajcn/52.6.1087
47. Guastamacchia E, Giagulli V, Licchelli B, Triggiani V. Selenium and Iodine in Autoimmune Thyroiditis. Endocrine, Metab Immune Disord Targets 2015; 15:288–292. doi: 10.2174/ 1871530315666150619094242
48. Chanoine J-P, Nève J, Wu S, Vanderpas J, Bourdoux P. Selenium Decreases Thyroglobulin Concentrations But Does Not Affect the Increased Thyroxine-to-Triiodothyronine Ratio in Children with Congenital Hypothyroidism 1. J Clin Endocrinol Metab 2001; 86: 1160–1163. doi: 10.1210/jcem.86.3.7312
49. Ashton K, Hooper L, Harvey LJ, Hurst R, Casgrain A, Fairweather-Tait SJ. Methods of assessment of selenium status in humans: A systematic review. Am J Clin Nutr 2009: 89: 2025S-2039S. doi:10. 3945/ajcn.2009.27230F
50. Duntas LH. Selenium and the Thyroid: A Close-Knit Connection. J Clin Endocrinol Metab 2010; 95:5180–5188. doi: 10.1210/jc.2010-0191
51. Winther KH, Wichman JEM, Bonnema SJ, Hegedüs L. Insufficient documentation for clinical efficacy of selenium supplementation in chronic autoimmune thyroiditis, based on a systematic review and meta-analysis. Endocrine 2017; 55:376–385. doi: 10.1007/s12020-016-1098-z
52. Bartalena I, Tanda M, Piantanida E, Lai A, Compri E, Lombardi V. Environment and thyroid autoimmunity. In: Wiersinga W, Drexhage H, Weetman A, editors. The Thyroid and Autoimmunity. Stuttgart: Thieme; 2007. pp. 60–73.
53. Saranac L, Zivanovic S, Bjelakovic B, Stamenkovic H, Novak M, Kamenov B. Why Is the Thyroid So Prone to Autoimmune Disease. Horm Res Paediatr 2011; 75:157–165. doi: 10.1159/000324442
54. Contempré B, Duale NL, Dumont JE, Ngo B, Diplock AT, Vanderpas J. Effect of selenium supplementation on thyroid hormone metabolism in an iodine and selenium deficient population. Clin Endocrinol (Oxf) 1992; 36:579–583. doi: 10.1111/j.1365-2265.1992. tb02268.x
55. Sinadinovic J, Han R. Iodine deficiency, endemic goiter and prophylaxis. Monography (In Serbian). Belgrade: Politop Belgrade Ed; 1995.
56. Gärtner R, Gasnier BCH, Dietrich JW, Krebs B, Angstwurm MWA. Selenium Supplementation in Patients with Autoimmune Thyroiditis Decreases Thyroid Peroxidase Antibodies Concentrations. J Clin Endocrinol Metab 2002; 87:1687–1691. doi: 10.1210/jcem.87.4.8421
57. Duntas L, Mantzou E, Koutras D. Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. Eur J Endocrinol 2003; 148:389–393. doi: 10.1530/ eje.0.1480389

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 22, No 2, 2020, pp. 34–38

UDC 577.17:616-097
http://doi.org/10.22190/FUMB191004017R

Review Article

LEPTIN: FROM APPETITE SUPPRESSION TO AUTOIMMUNITY

Milica Ranđelović*, Tatjana M. Stoimenov-Jevtović

Faculty of Medicine, University of Niš, Niš, Serbia

Abstract. *The hormone leptin is released by adipocytes accordingly to current energy stores to suppress appetite. Apart from this, leptin acts as a proinflammatory cytokine and strongly stimulates inflammation. Immune-modulating properties are partly achieved by affecting T-cell maturation, polarization, and viability. Leptin rises inflammatory cells count, increases proinflammatory cytokine secretion, and impairs regulatory T-lymphocytes differentiation. Leptin secretion and signalization disturbances have recently started to be observed in the context of autoimmunity. In this review, we discuss signaling pathways affected by the satiety hormone, its effect on T-lymphocyte maturation, differentiation and polarization, and relation to other immune-modulating agents. In the end, we highlight the rising evidence connecting hyperleptinemia state which is almost always related to obesity, with autoimmune disorders and take a brief overview of possible mechanisms behind leptin's potency to induce self-reactivity.*

Key words: *leptin, leptin resistance, leptin receptor, autoimmunity.*

Introduction

Leptin (also: obese, satiety, starvation hormone) is a hormone made of 167 amino acids and released by adipocytes accordingly to current energy stores. The main role of the satiety hormone is to suppress appetite by delivering information about peripheral energy supplies towards CNS, more precisely to hypothalamic nuclei. In this manner leptin indirectly modulates the metabolic rate of the body [1–3].

The leptin gene (*LEP; OB*) is located at chromosome 7, while chromosome 1 carries the leptin receptor gene (*LEPR; DB*). Mutations in any of these genes result in obesity and multiple metabolic disorders related to leptin deficiency (*ob/ob*) or leptin resistance (*db/db*) [4]. The inability of leptin receptors to recognize and adequately respond to leptin stimulation is called leptin resistance. It is always coupled with hyperleptinemia as an attempt of the body to influence the hunger center in the hypothalamus and prevent further energy intake [5]. There are several mechanisms of leptin resistance development, but the particularly interesting one is where the mutation occurs only in receptors transporting leptin through the blood-brain barrier (BBB). The disorder results in peripheral hyperleptinemia with disabled leptin delivery to hypothalamic nuclei and dysregulated appetite suppression leading to obesity [6]. Although the highest density of LepRb is found in hypothalamic nuclei managing the appetite and energy expenditure, distribution of this receptor shows that other areas of CNS, liver, pancreas, perivascular intestinal tissue, heart, and immune cells are also liable to leptin [7]. The *LEPR* expression in immunologically active cells give leptin the immunomodulatory role and a whole new meaning to peripheral hyperleptinemia [8]. Considering the abundance of microinflammation in obese, the possible proinflammatory effect of leptin and hyperleptinemia has recently started to be examined.

Correspondence to: Milica Ranđelović M.D., *Ph.D. Student
Faculty of Medicine, Niš, Dr. Zorana Djindjića Blvd. 81,
18000 Niš, Serbia
Phone: +381 63 643886
E-mail: mimi20691@gmail.com
Received October 4th, 2019 / Accepted January 8th 2021

Leptin Signaling Pathways

So far, we have been familiar with six isoforms of the leptin receptor gene, of which one is "long" (LepRb), while the rest are "short" (LepRa, LepRc, LepRd, LepRe, LepRf) [9]. LepRb is a transmembrane form able to convey leptin signal towards the nucleus. Short receptor forms take part in leptin transport through BBB, leptin metabolism, and elimination [10].

Leptin binding to LepRb is followed by the activation of three signaling pathways: JAK-STAT, ERK, and PI3K. The first step in leptin-dependent signal transduction is autophosphorylation of janus kinase 2 (JAK2) attached to the intracellular part of LepRb. JAK2 directly phosphorylates three tyrosine residues: Tyr985, Tyr1077 and Tyr1138, also located at the intracellular portion of the receptor. Phosphorylated tyrosine residues further initiate activation of next-generation messengers (e.g. Tyr1138→STAT3 - signal transducer and activator of transcription 3) which travel to the nucleus and trigger transcription of various genes [11]. So far, studies have confirmed that leptin affects transcription of *socs3*, *pomc*, *cart*, *agrp*, and *npy*. SOCS3 (suppressor of cytokine signaling 3) plays an important role in the autoregulation of leptin signaling ince its binding to phosphorylated Tyr985 leads toJAK2 inhibition [12]. Phosphorylated Tyr985 also positively regulates the ERK pathway which is highly important in the differentiation, metabolism, and viability of T-helper lymphocytes (Th) [6]. The third, and the

fastest leptin signaling pathway starts with phosphorylation of insulin-substrate receptor (IRS) by JAK2, that via PI3K triggers two axes: Akt-FoxO1 and mTORC1. Their dominant effect on cell enzymatic systems over transcription regulation results in shortly notable changes [13].

Considering intracellular pathways activated by the obese hormone, as well as the LepRb distribution, leptin turns into much more than the plain messenger in the chain of appetite control. Apart from the primary role in energy expenditure adjustment, its involvement in immunological processes has gained importance lately, especially in regulatory T-lymphocytes (Tregs) maturation and favored proinflammatory over anti-inflammatory response [11, 14].

Leptin as a Proinflammatory Agent

The satiety hormone brings the signal about sufficient energy supplies to CNS so it can coordinate immune functions, as directing energy towards immune cells maturation and specialization or uplifting and maintaining the immune reaction. Further, LepRb is found in certain types of immune cells (neutrophils, monocytes, macrophages, T-lymphocytes, B-lymphocytes, mastocytes, dendritic cells, natural killer cells) which implies leptin's direct effect on counted cells metabolism and functions [8, 15]. Although various immune cells are affected by leptin, it dominantly supports proinflammatory T-cells subtypes (Th1, Th17, Th22, Th9) and simultaneously suppress anti-inflammatory Th2 cells and Tregs [16]. Also, proinflammatory cytokines: LPS, TNF-α, and Il-1 were shown to increase leptin secretion, so its involvement in inflammation became even more certain [17, 18].

Leptin stimulates the expression of IL-7, also familiar as a thymocyte growth factor, in medullary thymic epithelial cells. This role in T-lymphocyte maturation is confirmed in leptin-deficient conditions which are always coupled with thymus atrophy [19, 20]. Leptin also affects matured, peripheral T-cells by managing their proliferation, differentiation, and viability [16]. Hence, leptin increases the proliferation of naïve cells and their differentiation towards proinflammatory phenotypes, while memory T-cells production remains suppressed under leptin impact [21, 22]. Also, T-cells viability is significantly improved by leptin-dependent mTOR activation [23].

Th1/Th2 polarization is dependent on leptin signalization and it significantly decreases in leptin-deficient conditions. However, Th1 response is prominently supported and Th2 response is suppressed by the satiety hormone [24]. Additionally, leptin promotes the production of several proinflammatory cytokines: Il-1, TNF-α, INF-γ, IL-2, IL-6, IL-12, IL-17, IL-21, and simultaneously decreases secretion of IL-10 and IL-4, known to suppress inflammation and restore pre-inflammatory, physiological condition [25].

In addition to proinflammatory, leptin shows autoimmune features as well. Tregs are Th subset supervising lymphocyte reactivity, peripheral tolerance to own antigens, and maintenance of an adequate inflammatory reaction with consequent resolution [26]. Considering the origin, Tregs are divided into two subgroups: naturally occurring Tregs (nTregs) originating from precursor cells in the thymus, and inducible Tregs (iTregs) formed from naïve T-helper cell under certain conditions [27]. Leptin can affect nTregs differentiation in the following mechanism: leptin induces hypoxia inducible factor (HIF) -1α expression, leading to FoxP3 (master regulator of differentiation and function in Tregs) degradation and Tregs inhibition [28]. Oppositely, leptin-stimulated HIF-1α activity in Th17 subset ameliorates glycolysis and increases energy for proliferation, maturation, and activity [24, 29, 30]. More importantly, HIF-1α provokes the secretion of pro-autoimmune cytokines (Il-17, IL-21,and IL-22) in Th17 precursor and its differentiation. Also, the ERK pathway activated by leptin-dependent Tyr985 phosphorylation maintains Th17 inflammatory response [31, 32]. The main producing cytokine in this cell subtype, IL-17, was shown to promote autoimmune response and inflammation [33, 34]. Figure 1 shows leptin impact on nTreg and Th17 precursors.

The urgency of understanding leptin's proinflammatory action and a strong inhibiting effect on inflammatory process resolution rises considering the number of obese patients with central leptin resistance and multiple metabolic disorders. Since leptin cannot pass BBB, the patient feels hunger, keeps ingesting food, and stores the energy, making adipocytes secrete more leptin which expresses its actions peripherally, on immune cells. Joining hyperleptinemia to existing metabolic disorders (dyslipidemia, hyperinsulinemia, hypertension, increased production of proinflammatory cytokines with TNF-α, IL-1 and IL-6 on the lead, etc.), makes a perfect base for multiple focal microinflammation with the tendency to autoimmune reaction development, and decreased potency of immune system monitoring.

Leptin in Autoimmune Diseases

The leptin role in autoimmunity may be discussed from three aspects: (a) the Tregs suppression, (b) the Th17 stimulation, and (c) an increase in proinflammatory cytokines secretion [35, 36]. Such an environment makes a perfect lining for autoimmune reaction, as soon as plenty of metabolic disturbances are also seen contemporary with leptin sensitivity disorders, as mentioned above. Yet, there are only a few clinical studies connecting leptin with autoimmune diseases since the role of the starvation hormone as an immunomodulator has recently started to be examined.

Hyperleptinemia related to obesity was shown to be part of the pathogenesis in several autoimmune diseases, such as rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, autoimmune thyroiditis, and type 1 diabetes mellitus (T1DM). Moreover, inflammatory bowel diseases (IBD) and systemic lupus erythematosus (SLE) activity seemed to be dependent on leptin concentration as well [37].

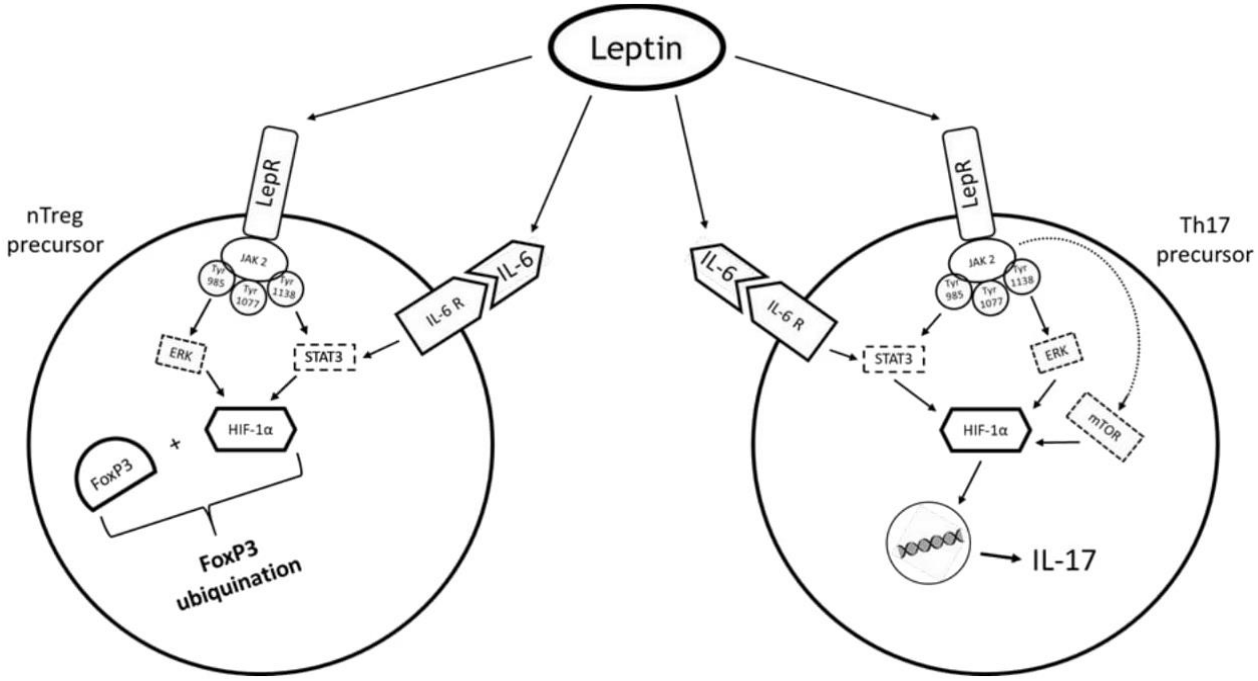


Fig. 1 Leptin impairs nTreg maturation and promotes Th17 differentiation.
Figure legend: Leptin increases HIF-1α in T-helper precursor cells directly by binding to its own receptor (LepRb), and indirectly by stimulating IL-6 production. In nTreg precursors HIF-1α binds to FoxP3 and promotes its degradation, delaying the cell maturation. Oppositely, the differentiation of Th17 is encouraged by HIF-1α *relaying to its stimulating effect on IL-17 production*. FoxP3 and IL-17 are regulators of differentiation in these two cell subtypes.
Abbreviations: LepR – leptin receptor, IL-6 R – interleukin-6 receptor; JAK2 – janus kinase 2; HIF-1α - intracellular hypoxia inducible factor 1α; STAT3 - signal transducer and activator of transcription 3; ERK – extracellular signal regulated kinase; FoxP3 – forkhead box P3 protein; mTOR – mammalian target of rapamycin.

A positive association between RA and obesity has been observed earlier [38]. A milieu of proinflammatory cytokines in the environment of dyslipidemia has a certain impact on synovial destruction and chondrocyte phenotype loss in RA. As a potent adipokine, leptin stimulates the secretion of NOS, IFN-γ,IL-1, and metalloproteinases in chondrocytes, as well as the activity of autoreactive T lymphocytes, causing the deterioration of the disease [39]. Clinical studies observed that RA activity was positively related to leptin serum level since the secretion of pathogenic enzymes and cytokines was strongly ameliorated by leptin [40, 41]. Although the meta-analysis in 2016 confirmed a positive correlation between serum leptin level and disease activity in humans [42], the understanding of leptin's impact on the disease activity is not yet fully understood.

In MS both serum and liquor leptin levels were elevated and correlated with Th17 count and IFN-γ activity in neural structures [43]. An increased serum leptin level in children is considered to be one of several obesity-related factors related to a twofold higher risk of MS onset in adulthood, as reported in several longitudinal studies [44, 45].

Further, in animals fed with high-fat diet leptin caused a significant increase in IFN-γ production and T1DM rapid onset [14]. As animal studies have shown, leptin administration is capable of inducing spontaneous T1DM in non-obese animals [46], while human studies reveal hyperleptinemia state in children suffering from T1DM [47]. However, the complexity standing behind the leptin's effect on glucose metabolism on the one hand, and leptin's proinflammatory effects on the other, should be briefly considered while summarizing a protentional leptin's role in T1DM onset and course.

Leptin-dependent Th17 inflammatory reaction and IL-17 hyperproduction were among the leading causes of autoimmune thyroiditis [48, 49], MS [50], SLE [51, 52], and many other systemic and organic autoimmune diseases [53]. Although the crucial role of this adipokine in the development remains unclear, its impact on differentiation and survival of autoreactive T lymphocytes cannot be neglected. A meta-analysis including 1333 patients suffering from SLE and 1048 healthy controls concluded that serum leptin level was significantly increased in SLE patients [54]. In particular, deterioration in renal function seems to be in strong positive relation with leptin secretion [55].

Weight loss, followed by basal leptin decrease, showed amelioration in the course of the autoimmune diseases and increased Tregs count [56]. Finally, studies also confirmed that experimental models carrying *ob/ob* genotype were protected from certain autoimmune dis-

eases, like experimental autoimmune encephalomyelitis (equal to MS in humans) [57], SLE [58], and chronic inflammation in distinct tissues [59–61].

Conclusion

Low grade, sterile inflammation coupled with extracellular matrix remodeling and fibrosis in adipose tissue occurring in obesity leads to dysregulation of adipokines secretion and permanently active immune response. Disturbance in adipokines relation is followed by systemic inflammation and obesity comorbidities. For example, leptin, resistin, TNF-α, and IL-6 serum levels are elevated in obesity, leading to oxidative stress, angiogenesis, and thrombosis [62–64]. On the other hand, adiponectin, known by its anti-inflammatory properties is decreased in conditions of adipose inflammation [65]. With increased proinflammatory immune modulators supporting not only inflammation but autoreactivity as well, obesity becomes one of the risk factors for autoimmune disorders.

Theoretically, cell types and pathways affected by the satiety hormone make leptin a remarkable player in autoimmunity [11, 14, 29]. Its dominant effect on Th1 versus Th2 differentiation, Tregs suppression, and macrophage stimulation inflames immune response. On the other hand, conditions with the lack of leptin, like lipid dystrophia or deletion of the leptin gene, are linked to a higher risk of fatal outcome due to infection. This could be attributed to irregular thymic function, T lymphocyte differentiation, disturbances in the function of other immune cells like macrophages, neutrophils, and NK cells. Current findings highlight leptin's role in the proper development of immune response. Also, conditions with elevated leptin secretion, like leptin resistance and obesity, are followed by multiple immune defects often ending with cardiovascular diseases and autoimmune disorders. Clinical studies on humans are needed in order to reveal the exact leptin's role in the interplay of many other mediators secreted in the obesity state. One is sure for now: leptin is much more than just a plain appetite suppressor.

References

1. Denver RJ, Bonett RM, Boorse GC. Evolution of Leptin Structure and Function. Neuroendocrinology 2011; 94(1):21–38.
2. Münzberg H, Morrison CD. Structure, production and signaling of leptin. Metabolism 2015; 64(1):13–23.
3. Allison MB, Myers MG. 20 Years of Leptin: Connecting leptin signaling to biological function. J Endocrinol 2014; 223(1):T25–35.
4. Flier JS, Maratos-Flier E. Leptin's Physiologic Role: Does the Emperor of Energy Balance Have No Clothes? Cell Metab 2017; 26(1):24–26.
5. Liu J, Yang X, Yu S, Zheng R. The Leptin Resistance. In: Wu Q, Zheng R, editors. Neural Regulation of Metabolism. Singapore: Springer Singapore; 2018; p. 145–163.
6. Zhou Y, Rui L. Leptin signaling and leptin resistance. Front Med 2013; 7(2):207–222.
7. Caron E, Sachot C, Prevot V, Bouret SG. Distribution of leptin-sensitive cells in the postnatal and adult mouse brain. J Comp Neurol 2010; 518(4):459–476.
8. Tartaglia LA. The Leptin Receptor. J Biol Chem 1997; 272(10): 6093–6096.
9. Wada N, Hirako S, Takenoya F, Kageyama H, Okabe M, Shioda S. Leptin and its receptors. J Chem Neuroanat 2014;61–62: 191–199.
10. Schaab M, Kratzsch J. The soluble leptin receptor. Best Pract Res Clin Endocrinol Metab 2015; 29(5):661–670.
11. Wauman J, Zabeau L, Tavernier J. The Leptin Receptor Complex: Heavier Than Expected? Front Endocrinol 2017; 8.
12. Engin A. Diet-Induced Obesity and the Mechanism of Leptin Resistance. In: Engin AB, Engin A, editors. Obesity and Lipotoxicity. Cham: Springer International Publishing; 2017: p. 381–397.
13. Kwon O, Kim KW, Kim M-S. Leptin signalling pathways in hypothalamic neurons. Cell Mol Life Sci 2016; 73:1457–1477.
14. La Cava A. Leptin in inflammation and autoimmunity. Cytokine 2017; 98:51–58.
15. Procaccini C, La Rocca C, Carbone F, De Rosa V, Galgani M, Matarese G. Leptin as immune mediator: Interaction between neuroendocrine and immune system. Dev Comp Immunol 2017; 66:120–129.
16. Saucillo DC, Gerriets VA, Sheng J, Rathmell JC, MacIver NJ. Leptin Metabolically Licenses T Cells for Activation To Link Nutrition and Immunity. J Immunol 2014; 192:136–144.
17. de Heredia FP, Gómez-Martínez S, Marcos A. Obesity, inflammation and the immune system. Proc Nutr Soc 2012; 71:332–338.
18. Abella V, Scotece M, Conde J, Pino J, Gonzalez-Gay MA, Gómez-Reino JJ, et al. Leptin in the interplay of inflammation, metabolism and immune system disorders. Nat Rev Rheumatol 2017; 13:100–109.
19. Sreenivasan J, Schlenner S, Franckaert D, Dooley J, Liston A. The thymoprotective function of leptin is indirectly mediated via suppression of obesity. Immunology 2015; 146:122–129.
20. Gruver AL, Ventevogel MS, Sempowski GD. Leptin receptor is expressed in thymus medulla and leptin protects against thymic remodeling during endotoxemia-induced thymus involution. J Endocrinol 2009; 203:75–85.
21. Hsu H-C, Mountz JD. Metabolic syndrome, hormones, and maintenance of T cells during aging. CurrOpin Immunol 2010; 22:541–548.
22. Maciolek JA, Alex Pasternak J, Wilson HL. Metabolism of activated T lymphocytes. CurrOpin Immunol 2014; 27:60–74.
23. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 2011; 12:21–35.
24. Gerriets VA, Danzaki K, Kishton RJ, Eisner W, Nichols AG, Saucillo DC, et al. Leptin directly promotes T-cell glycolytic metabolism to drive effector T-cell differentiation in a mouse model of autoimmunity. Eur J Immunol 2016; 46:1970–1983.
25. Vadacca M, Margiotta DP, Navarini L, Afeltra A. Leptin in immuno-rheumatological diseases. Cell Mol Immunol 2011; 8: 203–212.
26. Ali N, Rosenblum MD. Regulatory T cells in skin. Immunology 2017; 152:372–381.
27. Pacella I, Piconese S. Immunometabolic Checkpoints of Treg Dynamics: Adaptation to Microenvironmental Opportunities and Challenges. Front Immunol 2019; 10.
28. Yang J, Yang X, Zou H, Li M. Oxidative Stress and Treg and Th17 Dysfunction in Systemic Lupus Erythematosus. Oxid Med Cell Longev 2016; 2016:1–9.
29. Park H-K, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism 2015; 64: 24–34.
30. Huynh A, DuPage M, Priyadharshini B, Sage PT, Quiros J, Borges CM, et al. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. Nat Immunol 2015; 16:188–196.
31. Cassano S, Pucino V, La Rocca C, Procaccini C, De Rosa V, Marone G, et al. Leptin modulates autophagy in human CD4+CD25− conventional T cells. Metabolism 2014; 63:1272–1279.
32. Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23–IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol 2014; 14:585–600.

33. Isailovic N, Daigo K, Mantovani A, Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. J Autoimmun 2015; 60:1–11.
34. Beringer A, Noack M, Miossec P. IL-17 in Chronic Inflammation: From Discovery to Targeting. Trends Mol Med 2016; 22:230–241.
35. Naylor C, Petri WA. Leptin Regulation of Immune Responses. Trends Mol Med 2016; 22:88–98.
36. Pucino V, De Rosa V, Procaccini C, Matarese G. Regulatory T Cells, Leptin and Angiogenesis. In: Marone G, Granata F, editors. Chemical Immunology and Allergy. Basel: S. KARGER AG; 2013: p. 155–169.
37. Francisco V, Pino J, Campos-Cabaleiro V, Ruiz-Fernández C, Mera A, Gonzalez-Gay MA, et al. Obesity, Fat Mass and Immune System: Role for Leptin. Front Physiol 2018; 9.
38. Dar L, Tiosano S, Watad A, Bragazzi NL, Zisman D, Comaneshter D, et al. Are obesity and rheumatoid arthritis interrelated? Int J Clin Pract 2018; 72:e13045.
39. Lago F, Gomez R, Conde J, Scotece M, Dieguez C, Gualillo O. Functions of Adipose Tissue and Adipokines in Health and Disease. In: Aimaretti G, editor. Update on Mechanisms of Hormone Action - Focus on Metabolism, Growth and Reproduction. InTech; 2011.
40. Tian G, Liang J-N, Wang Z-Y, Zhou D. Emerging role of leptin in rheumatoid arthritis: Emerging role of leptin in RA. Clin Exp Immunol. 2014; 177:557–570.
41. Carrión M, Frommer KW, Pérez-García S, Müller-Ladner U, Gomariz RP, Neumann E. The Adipokine Network in Rheumatic Joint Diseases. Int J Mol Sci 2019; 20:4091.
42. Lee YH, Bae S -C. Circulating leptin level in rheumatoid arthritis and its correlation with disease activity: a meta-analysis. Z FürRheumatol 2016; 75:1021–1027.
43. Guerrero-García J de J, Carrera-Quintanar L, López-Roa RI, Márquez-Aguirre AL, Rojas-Mayorquín AE, Ortuño-Sahagún D. Multiple Sclerosis and Obesity: Possible Roles of Adipokines. Mediators Inflamm 2016; 2016:1–24.
44. Umano GR, Pistone C, Tondina E, Moiraghi A, Lauretta D, Miraglia del Giudice E, et al. Pediatric Obesity and the Immune System. Front Pediatr 2019; 7.
45. Munger KL, Bentzen J, Laursen B, Stenager E, Koch-Henriksen N, Sørensen TI, et al. Childhood body mass index and multiple sclerosis risk: a long-term cohort study. MultScler J 2013; 19(10):1323–1329.
46. Matarese G, Sanna V, Lechler RI, Sarvetnick N, Fontana S, Zappacosta S, et al. Leptin Accelerates Autoimmune Diabetes in Female NOD Mice. Diabetes 2002; 51:1356–1361.
47. Luna R, Garcia-Mayor RV, Lage M, Andrade MA, Barreiro J, Pombo M, et al. High serum leptin levels in children with type 1 diabetes mellitus: contribution of age, BMI, pubertal development and metabolic status. Clin Endocrinol (Oxf) 1999; 51:603–610.
48. Drobniak A, Kanecki K, Grymowicz M, Radowicki S. Serum leptin concentration in women of reproductive age with euthyroid autoimmune thyroiditis. Gynecol Endocrinol 2016; 32:128–131.
49. Wang S, Baidoo SE, Liu Y, Zhu C, Tian J, Ma J, et al. T cell-derived leptin contributes to increased frequency of T helper type 17 cells in female patients with Hashimoto's thyroiditis: Leptin and Th17 cells in HT patients. Clin Exp Immunol 2013; 171:63–68.
50. Huitema MJD, Schenk GJ. Insights into the Mechanisms That May Clarify Obesity as a Risk Factor for Multiple Sclerosis. Curr Neurol Neurosci Rep 2018; 18.
51. Yu Y, Liu Y, Shi F-D, Zou H, Matarese G, La Cava A. Cutting Edge: Leptin-Induced RORγt Expression in CD4 $^{+}$ T Cells Promotes Th17 Responses in Systemic Lupus Erythematosus. J Immunol 2013; 190:3054–3058.
52. Lourenço EV, Liu A, Matarese G, La Cava A. Leptin promotes systemic lupus erythematosus by increasing autoantibody production and inhibiting immune regulation. Proc Natl Acad Sci 2016; 113: 10637–10642.
53. Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulos K. The Role of IL-17 and Th17 Lymphocytes in Autoimmune Diseases. Arch Immunol Ther Exp 2015; 63:435–449.
54. Lee YH, Song GG. Association between circulating leptin levels and systemic lupus erythematosus: an updated meta-analysis. Lupus 2018; 27:428–435.
55. Chougule D, Nadkar M, Venkataraman K, Rajadhyaksha A, Hase N, Jamale T, et al. Adipokine interactions promote the pathogenesis of systemic lupus erythematosus. Cytokine 2018; 111:20–27.
56. Liu Y, Yu Y, Matarese G, La Cava A. Cutting edge: fasting-induced hypoleptinemia expands functional regulatory T cells in systemic lupus erythematosus. J Immunol Baltim Md 1950: 2012;188:2070–2073. doi: 10.4049/jimmunol.1102835
57. Ouyang S, Hsuchou H, Kastin AJ, Mishra PK, Wang Y, Pan W. Leukocyte infiltration into spinal cord of EAE mice is attenuated by removal of endothelial leptin signaling. Brain Behav Immun 2014; 40:61–73.
58. Fujita Y, Fujii T, Mimori T, Sato T, Nakamura T, Iwao H, et al. Deficient Leptin Signaling Ameliorates Systemic Lupus Erythematosus Lesions in MRL/Mp- *Fas^{lpr}* Mice. J Immunol 2014; 192:979–984.
59. Tarzi RM, Cook HT, Jackson I, Pusey CD, Lord GM. Leptin-Deficient Mice Are Protected from Accelerated Nephrotoxic Nephritis. Am J Pathol 2004; 164:385–390.
60. Zhou Y, Yu X, Chen H, Sjöberg S, Roux J, Zhang L, et al. Leptin Deficiency Shifts Mast Cells toward Anti-Inflammatory Actions and Protects Mice from Obesity and Diabetes by Polarizing M2 Macrophages. Cell Metab 2015; 22:1045–1058.
61. Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Portincasa P, et al. Normalization of adiponectin concentrations by leptin replacement in ob/ob mice is accompanied by reductions in systemic oxidative stress and inflammation. Sci Rep 2017; 7:2752.
62. Katsiki N, Mikhailidis DP, Banach M. Leptin, cardiovascular diseases and type 2 diabetes mellitus. Acta Pharmacol Sin 2018; 39:1176–1188. doi: 10.1038/aps.2018.40
63. Park HK, Kwak MK, Kim HJ, Ahima RS. Linking resistin, inflammation, and cardiometabolic diseases. Korean J Intern Med 2017; 32:239–247.
64. Forsbladd'Elia H, Pullerits R, Carlsten H, Bokarewa M. Resistin in serum is associated with higher levels of IL-1Ra in post-menopausal women with rheumatoid arthritis. Rheumatology 2008; 47:1082–1087.
65. ArunkumarAchari, Sushil Jain. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci 2017; 18:1321.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 22, No 2, 2020, pp. 39–48

UDC 616.432-008. 61:618.177
http://doi.org/10.22190/FUMB200902007K

Review Article

PROLACTIN AND HYPERPROLACTINAEMIA IN FEMALE REPRODUCTIVE ENDOCRINOLOGY – AN UPDATE

Ranko M. Kutlešić[1,2], Jasmina Popović[1,2], Milan Stefanović[1,2], Predrag Vukomanović[1,2], Jelena Milošević Stevanović[1,2], Danka Mostić Stanišić[3]

[1]Clinic of Gynaecologyand Obstetrics, Clinical Centre Niš, Niš, Serbia
[2]Faculty of Medicine University of Niš, Niš, Serbia
[3]Institute of Gynaecology and Obstetrics Belgrade, Clinical Centre of Serbia, Belgrade, Serbia

Abstract. *Hyperprolactinaemia is one of the most frequent causes of anovulation, resulting in infertility and hypoestrogenic state with consequences on overall women's health. Recent investigations on biological actions of prolactin, especially prolactin of extrapituitary origin, expand our knowledge on prolactin role in the human organism and open new questions connected with female reproductive function and treatment of female infertility. This article represents the review of current knowledge on prolactin physiology, etiopathogenesis, clinical features, assessment , differential diagnosis ,and teatment of hyperprolactinaemia in the female patient.*

Key words: *prolactin, hyperprolactinaemia, physiology, female infertility, treatment.*

Introduction

Prolactin (PRL) was first discovered in the late 1920s by different authors who demonstrated the ability of the injected bovine pituitary extract to cause lactation in rabbits [1].

The hormone was purified in 1932 by Riddle et al. and was named "pro-lactin" due to its it's lactogenic action [2].

PRL was not able to be separated from growth hormone (GH) till 1970's when adequate radioimmunoassay was developed by Friesen et al [3, 4].

Today, PRL is known to act at many different tissues with numerous biological activities. Hyperprolactinaemia is one of the most common problems in human endocrinology, especially connected with reproduction. In fact, PRL is viewed as a hormone on a global level, but its paracrine/autocrine action as cytokine is also the subject of numerous investigations.

Physiology of PRL Secretion

PRL belongs to the PRL/GH/placental lactogen family. Those hormones share a similar structure, function, and binding properties, as well as the origin from a common ancestral gene from which they have diverged 400 million years ago [5].

The prolactin gen is located on chromosome 6 in the human genome [6], with different promoter regions directing pituitary and extrapituitary PRL synthesis, which is the unique characteristic of humans [7].

Correspondence to: Ranko Kutlešić M.D., Ph.D.
Faculty of Medicine, Niš, Dr. Zorana Djindjića Blvd. 81,
18000 Niš, Serbia
E-mail: mkutlesic5@gmail.com
Received September 2nd, 2020 / Accepted November 29th, 2020

This gene is encoding prohormone for prolactin, which is consisted of 227 amino acids, with a signaling peptide of 28 amino acids. PRL is a polypeptide hormone, aminoacid chain consisting of 199 amino acids, 50% of them are in the form of α-helix and the rest forms the loop. The tertiary structure was predicted according to the current three-dimensional model: prolactin contains four long α-helices, arranged in antiparallel fashion [5, 8].

The molecular weight of PRL is around 23 kDa, which represents the main and biologically the most potent form of PRL in circulation - monomeric or *small* PRL. There are also the variants of PRL molecule: 60 kDa form – *big PRL* (dimmer) and *big-big PRL* of 150 – 170 kDa. Posttranslational processing is taking place in the anterior pituitary, including polymerization, phosphorization, glycolysis as well as formatting complexes with immunoglobulins (the most often with IgG in humans) or forming covalent and noncovalent bonds [5]. Such macromolecular forms have clinical implications, which will be discussed later.

PRL may have pituitary and extra-pituitary origin. The main sources of prolactin in humans are anterior pituitary lactotrophs. Synthesis and secretion of pituitary PRL are regulated by both inhibiting, and releasing factors (Table 1). Inhibiting factors include dopamine, gamma-aminobutyric acid (GABA) and somatostatin [5]. Hypothalamic dopamine is considered to be the main prolactin inhibiting factor in humans exerting its action through D2 and D4 receptors located on cell membranes of lactotrophs. Such dopamine action results in down-regulation of PRL gene expression reduced PRL secretion and decreased lactotroph proliferation [9].

Different molecules release pituitary prolactin (table 1), thyrotropin-releasing hormone (TRH) being clinically the most important, but "PRL-releasing factor" has not been identified as it is the case for other pituitary hormones [10].

Table 1 PRL inhibiting and releasing factors

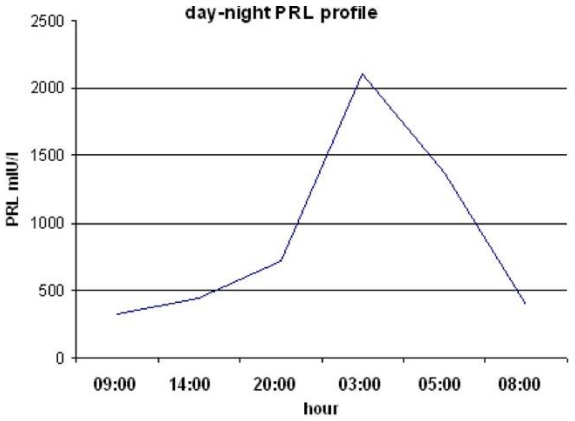
PRL inhibiting factors	
dopamine from hypothalamus	
gamma-aminobutyric acid (GABA)	
somatostatin	
PRL releasing factors	
thyrotropin-releasing hormone (TRH)	vasoactive intestinal polypeptide (VIP)
endogenous opioides	neurotensin
oxytocin	galanin
vasopressin	salsolino
serotonin	
Physiological "PRL-releasing factor"?	

Pituitary PRL is secreted in a pulsatile fashion, with peaks 20–30 minutes apart. This fact has clinical implications: taking three samples in 20–30 min. apart could help to make the correct diagnosis of hyperprolactinaemia.

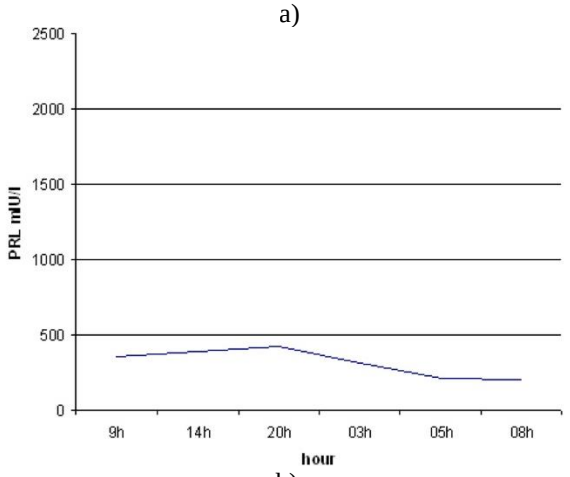
Pituitary prolactin secretion is characterized by a unique circadian rhythm. Higher levels occur during the sleep, with two peaks: around 17-20h and higher one between 02h and 04 h (about 4–5 hours after the beginning of the sleep). These facts also have clinical implications: higher PRL levels during the sleep cause "nocturnal hyperprolactinaemia" and possible galactorrhoea, especially in chronic stress (Figure 1).

PRL serum levels in humans are also fluctuating during the menstrual cycle, increasing during the follicular phase with periovulatory peak [12], so it was recommended to measure PRL serum levels in the early follicular phase, as we do in our everyday clinical practice [13].

Extra-pituitary prolactin is structurally identical to pituitary PRL. It is produced in the ovaries, uterus and endometrium, breast, prostate, lymphocytes, haematopoetic cells, adipose tissue, skin, thymus, lymphatic system, endothelium, and the brain. The action is still under investigation, the most probably autocrine/paracrine nature, but it was well established that its regulation is site- specific and quite different from the pituitary PRL [5, 14].



a)



b)

Fig. 1 Day and night profile of PRL serum levels in our patient with so-called "normoprolactinaemic galactorrhoea" caused by nocturnal hyperprolactinaemia due to chronic stress (being refuge during the civil war). Morning PRL levels are within physiological limits, while nocturnal PRL serum levels are higher than normal (a). Galactorrhoea and nocturnal hyperprolactinaemia ceased after the normalization of the living conditions (b) [11].

Actions of PRL

PRL acts through its receptor (PRL-R): a single membrane- bound protein of the cytokine receptor family, expressed in the pituitary and in the numerous other tissues as the mammary gland, endometrium, ovaries, heart, lung, thymus, liver, pancreas, spleen adrenal gland, skeletal muscle, bone (osteoblasts), skin, and brain [5].

PRL has more than 80 functions and over 300 separate biological activities, the most investigated are reproductive and homeostatic activities [5].

Reproductive action of PRL includes roles in mammogenesis, lactogenesis, and galactopoiesis (development and maturation of the breast during pregnancy, induction, and maintaining of lactation) as well as its role in follicular development and maintenance of the *corpus luteum* (luteotropic action of *physiological* levels of PRL) [15]. PRL is also involved in oogenesis and adequate implantation [15].

The possible PRL role in spermatogenesis is supported by findings of male infertility in knock-out PRL-R mice [9, 16].

The reproductive role of PRL is connected to its action as brain neuropeptide: animal experiments showed that reproductive behaviour was under the influence of PRL, which could stimulate sexual receptivity during the estrus [17], while inhibited sexual behaviour during the lactation [18].

As brain neuropeptide, PRL also has a role in mediating the positive influence of social interactions between mother and child or between mating partners on the mental and physical state and beneficial effects of such interactions on adaptive processes related to emotional and physiological stress coping in both sexes [19].

PRL is also known as "stress hormone": increases secretion of ACTH, induces adrenal hypertrophy and storage of cholesterol esters, and stimulates the secretion of androgens (inclouding DHEA), cortisol, and aldosterone in adrenals [12].

Extrapituitary PRL acts as a cytokine on numerous sites. PRL produced by lymphocytes and haematpoietic cells is supposed to be involved in the immune response to stress [5, 12, 14, 20].

PRL promotes bone growth and mineralization in foetus, whereas it is involved in bone resorption in pregnancy, providing micronutrients to foetus [21].

PRL has vasoconstrictive action and a possible role in the development of pre-eclampsia or peripartal cardiomyopathy [15].

During the pregnancy, PRL is supposed to be included in fetal osmoregulation and inhibiting water transport across the human amnion [22].

Numerous actions of the extrapituitary PRL are still under investigation. The PRL roles in human physiology are illustrated in the best way by the following words [5]: *"It has been well recognized that prolactin ensures survival of the species through its reproductive role and survival of the individuals of the species in its homeostatic roles. While we know a great deal about the chemistry, biological actions, and controls in its reproductive role, there is a paucity of similar information in its homeostatic roles".*

Etiopathogenesis of Hyperprolactinaemia

Hyperprolactinaemia could be physiological, pathological, and iatrogenic. The main causes of hyperprolactinaemia are listed in the Table 2.

Physiological causes could elevate PRL synthesis and release in different manners.

Endogenous oestrogens enhance PRL secretion by regulation of PRL gene expression, downregulation of dopamine receptor expression, and stimulation of lactotroph cell hyperplasia [5].

During the pregnancy PRL serum levels progressively rises to 4 000 – 10 000 mIU/l (200 – 500 ng/ml) [23].

Suckling – induced PRL release during breastfeeding is caused by lowered dopamine tonus, but PRL releasing factors as TRH, oxytocin, etc (Table 1) are also proposed to be involved [5, 24].

Neural mechanisms interfering with dopamine transmission are involved in PRL release during the suckling in lactation and nipple stimulation [5], which is not observed during the breast examination, breast ultrasound, or mammography. [25-27].

Table 2 Etiology of hyperprolactinaemia

Physiological hyperpoalctinaemia:
- pregnancy, puerperium and lactation, stress (psychic, physic, surgery and anaesthesia, hypoglicaemia etc), during the sleep, nipple stimulation and coitus, exercises and in 2.5% of healthy people,

Pathological hyperpolactinaemia:
- prolactinomas, other pituitary adenomas, and pituitary conditions (acromegaly, hypophysitis, macroprolactinaemia etc)
- hypothalamic conditions and pituitary stalk compression (tumours, granulomas, infiltrative lesions, tuberculosis, sarcoidosis, cranial irradiation, trauma with stalk section, Rathke cleft cyst, craniopharyngiomas etc)
- idiopathic hyperprolactinaemia
- chronic renal failure
- hypothyroidism
- hepatic failure
- chest: neurogenic chest wall trauma, surgery scars, herpes zoster
- epileptic seizures
- autoimmune diseases
- 6-pyruvoyl tetrahydropterin synthase deficiency
- ectopic PRL production by different tumours

Pharmacological hyperprolactinaemia:
- dopamine receptor antagonists and dopamine synthesis inhibitors
- dopamine depleting agents (antihypertensives: methyl dopa, reserpine, verapamil, etc)
- hormones and neuropeptides – stimulators of prolactin release (TRH, estrogens – oral contraceptives, etc)
- opiates and opiate antagonists, morphine derivatives, aesthetics, opium smoking, opioid addiction, cocaine and marijuana
- antihistamines H2 (cimetidine, meclizine, etc)
- antipsychotics / neuroleptics, antidepressants, anticonvulsants,
- antiemmetics (domperidone and metoclopramide)
- cholinergic agonist
- catecholamine depletor

Oral contraceptives containing high doses of oestrogen (>= 35 mcg) could rise PRL serum levels by alteration of dopamine regulation [28], which is not a case with modern contraceptives with lower amounts of oestrogen [29].

Stress-induced changes in dopamine and serotonin may affect PRL release causing hyperprolactinaemia [20]

It was already mentioned that hypothalamic dopamine acts through D2 receptors on lactotrophs as the main PRL inhibiting factor. Therefore, the main reasons for elevated serum PRL levels in pathological and pharmacological could be as follows:

- high PRL production (by prolactinoma),
- disruption of the dopamine transport to the pituitary (due to stalk compression by tumours or inflammation),
- decreased dopamine PRL inhibition and elevated estrogen levels in hepatic failure,
- decreased PRL clearance and increased PRL secretion in renal failure,
- blockade of endogenous dopamine receptors by a variety of drugs,
- PRL synthesis stimulated by medications.

Clinical Features of Hyperprolactinaemia

Hyperprolactinaemia causes hypogonadism in women and men. Syndrome "galactorrhoea – amenorrhoea" was first described by Hypocrite. Women with hyperprolactinaemia could experience cycle irregularity (amenorrhoea and oligomenorrhoea) or even to have regular cycles, but the consequence is infertility in the most. In fact, hyperprolactinaemia disturbed ovulation in women: on one end of the spectrum of the ovulation disorders caused by hyperprolactinaemia is amenorrhoea due to complete anovulation; on another end of the spectrum is luteal phase deficiency in women with almost regular cycles, but still with the problem to conceive.

Hyperprolactinaemia in women could cause the development of the hypoestrogenic state resulting in osteoporosis. In fact, hyperprolactinaemia could increase bone resorption and inhibits bone formation, both in men and women [30].

Such hypoestrogenemic state is also associated with multiple impairement of female sexual functions (sexual desire and arousal, lubrication, orgasm, sexual satisfaction, and dyspareunia) [31].

Galactorrhoea is present in 10% to 90% of hyperprolactinaemic women and in 14 – 33% of men [32]. Nipple discharge could be colorless, yellowish, white like breast milk in lactation or even dark. "Normoprolactinaemic galactorrhoea" is a condition characterized by galactorrhoea associated with normal PRL serum levels measured in usual fashion. Nocturnal hyperprolactinaemia is a possible reason for that condition, for diagnostic purposes day-night prolactin profile should be obtained (Figure 1).

Hyperandogenemia could be present in 25% of hyperprolactinaemic women, due to the increase in adrenal DHEA, the consequence is mild hirsutism. Hyperandrogenemia could be also present in patients with prolactinomas [33].

The connection between hyperprolactinaemia and female pattern hair loss is still unclear [34], but we have seen such cases in our own clinical practice.

In men, hyperprolactinaemia causes hypogonadism: impotency, loss of libido ,and gynecomastia. Anaemia, decreased energy and muscle mass may be also present as secondary manifestations of hypogonadism [35, 36].

Abnormal pituitary findings were found in 50-80% of patients with monomeric hyperprolactinaemia [37]. Prolactinomas are the main causes of hyperpolactinaemia and the most common type of pituitary adenomas (about 40%) [38].

In patients with prolactinomas, neurological manifestations are present in cases with macroprolactinomas (pituitary adenoma > 10 mm in diameter): visual disturbances if the tumor compresses optical chiasma, which is not the case with microprolactinoma (pituitary adenoma < 10 mm in diameter).

The existence of hyperprolactinaemia was reported in numerous autoimmune disorders including: systemic lupus erythematosus, rheumatoid arthritis, sclerodermia etc, which is explained by immunomodulatory effects of PRL acting as cytokine on the level of T- and B-lymphocytes, enhancing inflammatory response and immunoglobulin production [39].

Hyperprolactinaemia has a negative influence on glycoregulation, it was demonstrated that hyperpolactinaemia in patients with prolactinoma was associated with a higher risk of hyperglycaemia accompanied by obesity. Bromocriptine and cabergoline have favorable effects on glucose metabolism, but the exact mechanism of its action on glycemic control and favorable cardio-metabolic profile is still unclear and it seems to be that this action is more complex than "the historical explanation of "resetting" the circadian clock" [40].

Metabolic consequences of dopamine agonists treatment for hyperprolactinaemia were investigated in 14 consecutive patients with prolactinoma: insulin sensitivity tended to improve after 6 months of the treatment with dopamine agonists (bromocriptine and cabergoline) [41].

It was recommended that hyperprolactinaemic premenopausal women with abnormal lipide profile and positive familial history of coronary disease, should be subjects of investigation for hyperinsulinaemia, and if it exists, PRL serum levels and insulin resistance should be normalized by adequate therapy [42].

Hyperprolactinaemia is also associated with endothelial dysfunction, development of perimenopausal atherosclerosis, and risk for cardiovascular disease, probably connected with vasoconstrictive characteristics of prolactin, but further studies are needed for definitive conclusions [43].

Osteoporosis could be developed in a hyperprolactnaemic patient due to hypoestrogenemic state and decreased osteocalcin levels [44].

It was also hypothesised that multiple pregnancies and iatrogenic hyperpolactinaemia could increase the risk for otosclerosis [45].

Hyperprolactinaemia and Female Infertility

Hyperprolactinaemia causes anovulation and infertility. High PRL levels suppress GnRH (via reduction of kisspeptins) and decrease LH pulse, ovarian oestrogens, and progesterone production [46, 47]. The consequences are menstrual irregularities (amenorrhoea or oligomenorrhoea), infertility, decreased libido, and galactorrhoea [38, 46].

A novel hypothesis states that kisspeptin could be of crucial importance for the explanation of anovulation and infertility in hyperpolactinaemic state [48-50]. Kisspeptins, neuropeptides encoded by KISS1 gene, are potent stimulators of GnRH neurons and important for pubertal maturation and regulation of reproduction [50]. Kisspeptin is expressed in hypothalamic arcuate and anteroventral periventricular nuclei. In the hyperprolactinaemic mouse model with continuous infusion of prolactin subcutaneously, the kisspeptin content was reduced and administration of kisspeptin intraperitoneal injection once daily for 20 days restored estrous cyclicity, induced ovulation, and increased LH and FSH levels in circulation [51]. Nevertheless, kisspeptins are not recommended for the therapy of amenorrhoea [52].

Kisspeptin administered in brain chambers
↓
Activity of tuberoinfudibular DA neurons are modulated
↓
DA tonus decreases
↓
PRL increases

Scheme 1 Influence of kisspeptin on PRL release [53]

Hyperprolactinaemia inhibits expression of kisspeptins
↓
Kisspeptin decreases
↓
Stimulation of GnRH neurons is absent
↓
Anovulation

Scheme 2 Influence of PRL on GnRH production [48, 50, 54].

In the human ovary hyperprolactinaemia inhibits development of corpus luteum, granulose cell luteinization, and steroidogenesis. PRL serum levels higher than 2000 mIU/l (about 100 ng/ml) inhibit progesterone secretion [55, 56].

Women with PCOS have elevated PRL serum levels in 30% of cases, thought to be a consequence of elevated estrogen levels and reduction of dopamine tonus [57].

However, the relationship between PCOS and hyperprolactinaemia remains unclear. Some authors stated that PCOS and hyperprolactinaemia are distinct entities [58, 59].

Hyperprolactinaemia is reported to be present in patients with endometriosis, moreover, there was a positive correlation of serum prolactin levels and stadium of endometriosis [60, 61].

Patients with endometriosis have exgaggerated nocturnal PRL secretion, it was stated *"this is the part of pathophysiology of that disease"* [62]. Human decidua produces prolactin and prolactin receptors are found in endometriotic tissue, therefore, it was postulated that the patients with endometriosis have at least occult hyperprolactinaemia according to TRH (thyrotropin -releasing hormone) stimulation, with higher serum prolactin levels in patients who had not achieved pregnancy during the treatment for endometriosis [63-65].

On the other side, there are reports denying the connection of prolactin with endometriosis [66, 67].

The influence of prolactin on fertility depends on serum concentrations: as the level of prolactin increases, cycle abnormalities can progress sequentially from an inadequate luteal phase to intermittent anovulation with oligomenorrhoea to total anovulation and amenorrhoea. The consequence is infertility; therefore, it is crucial to control prolactin levels in hyperpolactinaemic infertile patients.

Hyperprolactinaemia and Pregnancy

It was reported that up to one- third of hyperolactinaemic patients achieved pregnancy in unstimulated cycles, though it must be noted that most of the studies on that issue were conducted before the introduction of macroprolactin screening in routine clinical practice. Nevertheless, during the pregnancy of previous hyperprolactinaemic patients, there is no increased percentage of spontaneous abortions, nor increased perinatal mortality and morbidity. Breastfeeding is also allowed. There are no proven harmful effects of dopamine agonists as bromocriptine and quinagolide, including teratogen effects and effects on fetal osmoregulation. It was recommended that dopamine agonist therapy should be stopped when pregnancy is diagnosed [38].

Another concern during the pregnancy is a possible increase of the size of prolactinomas. The rise of microprolactinomas is extremely rare during the pregnancy: in 5% of all cases is asymptomatic, only in 2% of pregnant women the rising microprolactinoma could cause headache or visual disturbances [68, 69].

Macroprolactinomas rise more often: 15% of pregnant women with macroprolactinomas have symptoms due to increased tumour: headache and visual disturbances [69, 70, 71]. In such cases, it is possible to use NMR for diagnosing and commence the therapy with dopamine agonists which could control the rise of the tumour in most of the patients [72, 73]. It is recommended that during the pregnancy follow-up should be based on the occurrence of the symptoms: NMR of the pituitary sella should be done in case of the appearance of the symptoms. Routine measurements of the PRL serum levels during the pregnancy are not necessary [38].

A rare, but serious complication of the prolactinoma during the pregnancy is tumour necrosis and bleeding inside of the tumour, resulting in insipid diabetes and pituitary insufficiency after the delivery [70].

Clinical experience showed that some previously hyperprolactinaemic patients after the delivery have normalized PRL serum levels and commenced spontaneous menstrual cycles without therapy, moreover get pregnant spontaneously, even they had many problems achieving the first pregnancy. This is explained by spontaneous tumor necrosis during the pregnancy or sponatneous recovering of the primary dysfunction related to hyperprolactinaemia [74-76].

Diagnostic Evaluation of Hyperprolactinaemia

The diagnosis of hyperprolactinaemia is established by single measurements of serum PRL levels in two separated occasions (with serum sampling at least two hours apart from sleeping or eating). The serum should be obtained without excessive venipuncture stress and a level higher than the upper limit confirmed the diagnosis (> 530 mIU/l - according to World Health Organization Standard 84/500), as it was recommended previously [38, 77, 78].

When the obtained level of prolactin raised any doubt, the sampling should be repeated on another day at 15–20 min intervals to avoid prolactin pulsatile secretion.

PRL serum levels higher than 250 ng/ml are suggestive of the presence of a macroprolactinoma. PRL levels <100 ng/ml are associated with pseudoprolactinomas, drug-induced hyperprolactinaemia or systemic disease [79], but this is not always the rule [80, 81].

In cases of highly elevated PRL serum levels "hook effect" should be considered, therefore PRL serum levels should be measured in diluted blood serum. Clinically, such cases are present with symptoms of hyperprolactinaemia and falsely low PRL serum levels in undiluted spacemen [82, 83].

Diagnostic evaluation of hyperprolactinaemia includes: medical history, physical examination, assessing the clinical features and laboratory findings (especially PRL serum levels), as well as imaging studies of the pituitary and sella turcica (preferably pituitary NMR). Screening for macroprolactinaemia is also desirable, in asymptomatic patients is highly recommended [38].

Taking a proper medical history is essential in cases of pharmacological hyperprolactinaemia, but the concomitance of a pathologic cause should be always kept in mind. PRL measurements should be repeated after the discontinuation of the medication after 3 to 4 days (corroborated by a psychiatrist). If the discontinuing of the medication is not possible, pituitary NMR should be performed. In cases of confirmed drug-induced hyperpolactinaemia, the alternative medication should be tried, if possible [38, 84].

A similar situation is in the case of the rare concomitance of primary hypothyroidism and prolactinomas, which should be suspected when high PRL serum levels persist despite normalization of thyroid function [80].

Macroprolactinaemia

In the case of obvious discrepancy between high PRL serum levels and the absence of clinical symptoms and signs of hyperprolactinaemia, macroprolactinaemia should be suspected.

Macroprolactinaemia is a condition where 60% of circulating PRL is made of macroprolactin, form of circulating PRL with lesser biological activity.

In patients with hyperprolactinaemia about 25% have macroprolactinaemia (ranges from 10% to 35%) [85-89] but the precise prevalence of macroprolactinaemia in hyperprolactinaemic and the general population is still unknown [90].

The gold standard for diagnosing macroprolactinaemia is gel-filtration chromatography, which is expensive and time-consuming, so polyethylene glycol (PEG) serum precipitation is used as a screening method. The diagnosis of macroprolactinaemia is made when a PEG precipitation ratio is greater than 60% or recovery of less than 40% after PEG [91].

Macroprolactin is not considered to have significant biological activity, but it retains partial or total immunoreactivity with anti-PRL antibodies used in commercial immunoassays. Detection of macroprolactin is clinically important to avoid incorrect diagnosis and unnecessary investigations.

Nevertheless, later investigations revealed that some patients with macroprolactinaemia could have symptoms of hyperprolactinaemia or abnormal findings of the anterior pituitary, but in far lesser degree compared with patients with true hyperprolactinaemia [93]. Therefore, the presence of menstrual disorders, galactorrhoea ,and/or infertility in hyperprolactinaemic patients does not exclude macroprolactinaemia. Galactorrhoea was found in 46%, menstrual disorders in 39%, infertility associated with galactorrhoea or with menstrual irregularities in 28% of macroprolactiaemic patients [85].

Some authors recommend that the screening for macroprolactiaemia should be done in all hyperprolactinaemic patients [90, 93], the others consider that it is mostly indicated for asymptomatic patients, in apparent idiopathic hyperprolactinaemia and any patients without an obvious cause for the hyperprolactinaemia [46].

Idiopathic Hyperprolactinaemia

Idiopathic hyperprolactinaemia is defined as hyperprolactinaemia of unknown etiology, ie when its secondary causes have been ruled out and pituitary nuclear magnetic resonance (NMR) is normal [77, 94, 95]. The prevalence of idiopathic hyperpolactinaemia varies from 3.6% among patients with hyperpolactinaemia, to as much as 87.97% in hyperprolactinaemic infertile women [88].

Possible explanations for idiopathic hyperprolactinaemia include: immunological causes (formation of antipituitary antibodies) [96], the existence of very small microprolactinomas that could not be detected with current imaging techniques [78], or differences in prolactin receptor function (novel hypothesis) [97].

Treatment of Hyperprolactinaemia in Women

Indications for lowering high serum PRL levels are: anovulation and subsequent infertility, treatment of galactorrhoea that represent a problem to the patient, and treatment of the manifestation of the hypoestrogenic state to prevent osteoporosis and improve the quality of life.

Treatment options for hyperprolactinaemia are listed in Table 3.

Table 3 Treatment options for hyperprolactinaemia in clinical practice

Dopamine agonist
▪ ergot derivates: bromocriptine, cabergoline, pergolide, lisuride etc.
▪ nonergot derivate: quinagolide
Surgical treatment for prolactinomas

Dopamine agonists are the first line for the medical treatment of hyperprolactinaemia in women with anovulation as sole reason for infertility, being successful in restoring ovulation in up to 80% of infertile patients with hyperprolactinaemia.

Bromocriptine is safe for the treatment of the patients with hyperprolactinaemic anovulation and infertility, due to its safety in early pregnancy. Bromocriptine has been used for almost 50 years with no reported fetal harmful effects. The long term follow-up of the children born by mothers taking bromocriptine in early pregnancy showed the absence of any adverse effects [98].

In our practice, we start bromocriptine with a dose of 1.25 mg/day, or even less, before sleeping due to side effects. It can be titrated to a maximum of 7.5 mg/day for the treatment of hyperprolacatinaemic anovulation and infertility. The half-life of bromocriptine is about 6 hours, so we consider that daily dose should be divided into two or three smaller doses, which offer better control of circadian variations of prolactin serum levels, as well as better control of nocturnal prolactin levels. Side effects of bromocriptine included nausea, dizziness, headache, and postural hypotension, as common side effects of dopamine agonists .

The unusual side effects of the bromocriptine therapy are hallucinations due to similarity of the bromocriptine molecule to LSD (in 1-2%). We have seen such effects in our patients: one had scintillations and another had seen a fire on the electric stow. The more often problem is possibility of low concentration during the bromocriptine therapy and the patient should be warned on such effect.

Cabergoline is also an effective dopamine agonist. It was reported that cabergoline is better tolerated and more successful in achieving pregnancy than bromocriptine [99, 100].

The half-life of the cabergoline is 65 hours, so it is administered once (0.8 mg) or twice (0.4 mg) a week, with a maximal dose of 1–2 mg/week. The incipient dose of cabergoline could be as small as 0.25 mg twice a week, with a gradually increase to 0.5 mg twice a week according to the effects of therapy. It is more comfortable for the patient than bromocriptine. Cabergoline is safe during early pregnancy according to fetal anomalies [101-103], but the studies of other possible effects on fetal development are still missing. The problem with cabergoline is also a development of cardiac valvular disease due to possible thickened of cardiac valves caused by cabergoline effects on mitogenesis and fibroblast proliferation, which has been seen in patients with Parkinson's disease taking higher doses of cabergoline (4 mg/day – much higher than the dose for hyperprolactinaemia) [104, 105].

Nevertheless, the development of constrictive pericarditis during cabergoline therapy for hyperprolactinaemia (and smaller doses) has been also reported, so echocardiography in 6 to 12 months intervals is mandatory for patients receiving cabergoline [106].

Quinagolide, a nonergot dopamine agonist, is also successful in the treatment of infertile patients with hyperprolactinaemia [107].

Quinagolide has a higher affinity to D2 receptors and a longer half-life (22 hours) than bromocriptine, so it is administered once a day [108]. The initial dose is 25 – 50 micrograms once a day; with a gradual increase to 75 micrograms once a day.

Quinagolide is well tolerated and safe in early pregnancy [109].

In patients with prolactinomas, dopamin agonists are successful in achieving normal PRL serum levels in 71% and tumour shrinkage in 80% of all cases [110]. In the case of the rising prolactinoma, it was recommended to use cabergoline in preference to other dopamine agonists because it has a higher efficacy in normalizing PRL levels, as well as better results in pituitary tumour shtrinkage. Cabergoline greater efficacy may be explained by its higher affinity for dopamine receptor binding sites [38].

Surgical treatment (nasosphenoidal approach) is reserved for growing macroprolactinomas or those associated with neural manifestations. Microprolactinomas do not need such interventions. Moreover, it was recommended that asymptomatic microprolactinomas should not be treated with dopamine agonists. It was suggested that patients with amenorrhoea and microadenoma should be treated with dopamine agonists or oral contrarceptives [38]. The growth of the tumour during the therapy with oral contraceptives is extremely rare, but the patient should be warned on the symptoms and possibility of the rise of the intracranial pressure [68, 69, 74].

Conclusion

Hyperoprlactinaemia is one of the most frequent causes of anovulation, resulting in infertility and hypoestrogenic state with consequences on overall women's health. There are numerous etiological factors of hyperprolactinaemia, therefore, taking proper medical history and clinical assessment are crucial for correct differential diagnosis and proper therapy. Macroprolactinaemia and "hook

effect" must be considered in the evaluation of hyperprolactinaemia. Indications for the treatment of hyperprolactinaemic female patient are infertility, galactorrhoea that represents the problem for the patient, and hypoestrogenic state. Dopamine agonists are successful in lowering PRL serum levels and restoring ovulation and represent the main option for the treatment of the female patient with hyperprolactinaemia.

References

1. Tucker HA. Hormones, mammary growth, and lactation: A 41-year perspective. J Dairy Sci 2000; 83:874–884. doi: 10.3168/jds.S0022-0302(00)74951-4.e
2. Riddle O, Bates R, Dyksom S. The preparation, identification and assay of prolactin – a hormone of the anterior pituitary. Am J Physiol 1932; 98:511–546. doi: 10.1152/ajplegacy.1933.105.1.191
3. Friesen H, Guyda H, Hardy J. Biosynthesis of human growth hormone and prolactin. J Clin Endocrinol Metab 1970; 31:611–624. doi: 10.1210/jcem-31-6-611
4. Lewis UJ, Singh RN, Seavey BK. Human prolactin: Isolation and some properties. Biochem Biophys ResCommun. 1971; 44:1169–1176. doi: 10.1016/S0006-291X(71)80209-7
5. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: Structure, function, and regulation of secretion. Physiol Rev. 2000; 80:1523–1631. doi: 10.1152/physrev.2000.80.4.1523
6. Owerbach D, Rutter WJ, Cooke NE, Martial JA, Shows TB. The prolactin gene is located on chromosome 6 in humans. Science 1981; 212:815–816.
7. Berwaer M, Martial JA, Davis JR. Characterization of an up-stream promoter directing extrapituitary expression of the human prolactin gene. Mol Endocrinol 1994; 8:635–642.
8. Keeler C, Dannies PS, Hodsdon E. The tertiary structure and Backbone dynamics of human prolactin. J Molecul Biol 2003; 328: 1105–1121.
9. Rastrelli G, Corona G, Maggi M. The role of prolactin in andrology: What is new? Rev Endocr Metab Disord 2015; 16:233–248. doi: 10.1007/s11154-015-9322-3.
10. Grattan DR. 60 years of neuroendocrinology: The hypothalamo-prolactin axis. J Endocrinol 2015; 226:T101–T122. doi: 10.1530/JOE-15-0213.
11. Kutlešić R, Milosavljević M, Branković D, Lukić B, Janjić Z. Galactorrhoea associated with normal basal PRL levels and nocturnal hyperprolactinaemia. Proceedings XLIII Gynaecological Obstetric week; Gynaecological Obstetric section SLD: Belgrade, 1999. (Serbian)
12. Capozzi A, Scambia G, Pontecorvi A, Lello S. Hyperprolactinemia: Pathophysiology and therapeutic approach. Gynecol Endocrinol 2015; 31:506–510. doi: 10.3109/09513590.2015.1017810
13. Tanner MJ, Hadlow NC, Wardrop R. Variation of female prolactin levels with menopausal status and phase of menstrual cycle. Aust N Z J Obs Gynaecol 2011; 51:321–324. doi: 10.1111/j.1479-828X.2011.01321.x
14. Marano R.J., Ben-Jonathan N. Minireview: Extrapituitary prolactin: an update on the distribution, regulation, and functions. Mol Endocrinol 2014; 28:622–633. doi: 10.1210/me.2013-1349.
15. Ignacak A, Kasztelnik M, Sliwa T, Korbut RA, Rajda K, Guzik TJ. Prolactin-not only lactotrophin. A "new" view of the "old" hormone. J Physiol Pharm 2012; 63:435–443.
16. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 1998; 19:225–268.
17. Harlan RE, Shivers BD, Pfaff DW. Midbrain microinfusions of prolactin increase the estrogen-dependent behavior, lordosis. Science 1983; 219: 1451–1453.
18. Sodersten P, Hansen S, Eneroth P. Inhibition of sexual behavior in lactating rats. J Endocrinol 1983; 99: 189–197.
19. Bridges RS, Ronsheim PM. Prolactin (PRL) regulation of maternal behavior in rats: bromocriptine treatment delays and PRL promotes the rapid onset of behavior. Endocrinology 1990; 126:837–848.
20. Levine S, Muneyyirci-Delale O. Stress-induced hyperprolactinemia: pathophysiology and clinical approach. Obs Gynecol Int 2018; 2018:9253083. doi: 10.1155/2018/9253083
21. Zaidi M, Sun L, Liu P, et al. Pituitary-bone connection in skeletal regulation. Horm Mol Biol Clin Investig 2016; 28:85–94. doi: 10.1515/hmbci-2016-0015
22. Leontic EA, Tyson JE. Prolactin and fetal osmoregulation: water transport across isolated human amnion. Am J Physiol Regulatory Integrative Comp Physiol 1977; 232: R124-R127.
23. Mah PM, Webster J. Hyperprolactinemia: etiology, diagnosis, and management. Semin Reprod Med 2002; 20:365–374.
24. Crowley WR. Neuroendocrine regulation of lactation and milk production. Compr Physiol 2015; 5:255–291. doi: 10.1002/cphy.c140029
25. Saraç F, Tütüncüoğlu P, Ozgen AG, et al. Prolactin levels and examination with breast ultrasound or mammography. Adv Ther 2008; 25:59–66. doi: 10.1007/s12325-008-0002-5
26. Jarrell J, Franks S, McInnes R, Gemayel K, Guyda H, Arronet GH, Naftolin F. Breast examination does not elevate serum prolactin. Fertil Steril 1980; 33:49–51. doi: 10.1016/S0015-0282(16)44476-6
27. Hammond KR, Steinkampf MP, Boots LR, Blackwell RE. The effect of routine breast examination on serum prolactin levels. Fertil Steril 1996; 65:869–870. doi: 10.1016/S0015-0282(16)58229-6
28. Alvarez-Tutor E, Forga-LLenas L, Rodriguez-Erdozain R, Goñi-Iriarte MJ, Mendendez-Torre E, Alvarez-Tutor J. Persistent increase of PRL after oral contraceptive treatment. Alterations in dopaminergic regulation as possible etiology. Arch Gynecol Obs 1999; 263:45–50. doi: 10.1007/s004040050261.
29. Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba A.J. Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: Prospective, randomized, crossover trial. J Clin Endocrinol Metab 2007; 92:456–461. doi: 10.1210/jc.2006-1988
30. Mazziotti G, Frara S, Giustina A. Pituitary Diseases and Bone. Endocr Rev 2018; 39:440–488. doi: 10.1210/er.2018-00005
31. Krysiak R, Drosdzol-Cop A, Skrzypulec-Plinta V, Okopien B. Sexual function and depressive symptoms in young women with elevated macroprolactin content: a pilot study. Endocrine 2016; 53:291e8.
32. Vance ML, Thorner M. Prolactinoma. Endocrinol Metab Clin North Am 1987; 16:731–754.
33. Moria Y, Kortbawi R, El-Asmar N, Arafah BM. Increased androgen secretion in patients with prolactinomas: The impact of altered HPA function. Pituitary 2019; 22:170–178. doi: 10.1007/s11102-019-00948-w
34. Carmina E, Azziz R, Bergfeld W, et al. Female pattern hair loss and androgen excess: A report from the multidisciplinary androgen excess and pcos committee. J Clin Endocrinol Metab 2019; 104: 2875–2891. doi: 10.1210/jc.2018-02548
35. Majumdar A, Mangal NS. Hyperprolactinemia. J Hum Reprod Sci 2013 ;6:168–175. doi: 10.1007/s11102-014-0563-z.
36. Tirosh A, Benbassat C, Lifshitz A, Shimon I. Hypopituitarism patterns and prevalence among men with macroprolactinomas. Pituitary 2015; 18:108–115.
37. Isik S, Berker D, Tutuncu YA, et al. Clinical and radiological findings in macroprolactinemia. Endocrine 2012; 41:327e33.
38. Melmed S, Casanueva FF, Hoffman AR, et al. Diagnosis and treatment of hyperprolactinemia: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96:273–288. doi: 10.1210/jc.2010-1692
39. Cejkova P, Cerna M. Immunomodulatory role of prolactin in diabetes development. Autoimmune Rev 2009; 9: 23–27.
40. Lamos EM, Levitt DL, Munir KM. A review of dopamine agonist therapy in type 2 diabetes and effects on cardio-metabolic parameters. Prim Care Diabetes 2016; 10:60–65. doi: 10.1016/j.pcd.2015.10.008
41. Berinder K, Nyström T, Höybye C, Hall K, Hulting AL. Insulin sensitivity and lipid profile in prolactinoma patients before and after

normalization. Pituitary 2011; 14:199–207. doi: 10.1007/s11102-010-0277-9
42. Yavaz D, Deyneti O, Aksal O, et al. Endothelial function, insulin sensitivity and inflamatory markers in hyperprolactinemic pre-menopausal women. Eur J Endocrinol 2003; 149:187-190.
43. Georgiopoulos GA, Stamatelopoulos KS, Lambrinoudaki I, et al. Prolactin and preclinical atherosclerosis in menopausal women with cardiovascular risk factors. Hypertension 2009; 54:98–105.
44. Sartorio A, Conti R, Ambrosi B. Osteocalcin levels in patients with microprolactinoma before and during medical treatment. J Endocrinol Invest 1990; 13:419–422.
45. Horan KC. The effect of sex hormons on bone metabolism of the otic capsule – an overwiev. Horm Res 2008; 252:56–60.
46. Vilar L, Abucham J, Albuquerque JL, et al. Controversial issues in the management of hyperprolactinemia and prolactinomas—An overview by the Neuroendocrinology Department of the Brazilian Society of Endocrinology and Metabolism. Arch Endocrinol Metab 2018; 62:236–263. doi: 10.20945/2359-3997000000032
47. Kokay IC, Petersen SL, Grattan DR. Identification of prolactin-sensitive GABA and kisspeptin neurons in regions of the rat hypothalamus involved in the control of fertility. Endocrinology 2011; 152:526–535.
48. Sonigo C, Young J, Binart N. Hyperprolactinémie et infertilité. Approche physiopathologique Med Sci (Paris) 2013; 29:242–244.
49. Kaiser UB. Hyperprolactinemia and infertility: new insights. J Clin Invest 2012; 122:3467–3468. doi:10.1172/JCI64455
50. Roa J, Navarro VM, Tena-Sempere M. Kisspeptins in reproductive biology: consensus knowledge and recent developments. Biol Reprod 2011; 85:650–660.
51. Sonigo C, Bouilly J, Carré N, et al. Hyperprolactinemia-induced ovarian acyclicity is reversed by kisspeptin administration. J Clin Invest 2012; 122:3791–3795. doi: 10.1172/JCI63937.
52. Gordon CM, Ackerman KE, Berga SL, et al. Functional hypothalamic amenorrhea: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2017; jc:2017-00131. doi: 10.1210/jc.2017-00131
53. Szawka RE, Ribeiro AB, Leite CM, et al. Kisspeptin regulates prolactin release through hypothalamic dopaminergic neurons. Endocrinolog 2010; 151:3247–3457.
54. Araujo-Lopez R, Crampton JR, Aquino NS et al. Prolactin regulates kisspeptin neurons in the arcuate nucleus to suppress LH secretion in female rats. Endocrinology 2014; 155:1010–1020. doi: 10.1210/en.2013-1889
55. Adashi EY, Resnick CE. Prolactin as an inhibitor of granulosa cell luteinization: implications for hyperprolactinemia-associated luteal phase dysfunction. Fertil Steril 1987; 48: 131–139
56. McNeilly AS, Glasier A, Jonassen J, Howie PW. Evidence for direct inhibition of ovarian function by prolactin. J Reprod Fertil 1982; 65:559–569.
57. Falaschi P, del Pozo E, Rocco A, et al. Prolactin release in polycystic ovary. Obstet Gynecol.1980; 55:579–582.
58. Filho RB, Domingues L, Naves L, Ferraz E, Alves A, Casulari LA. Polycystic ovary syndrome and hyperprolactinemia are distinct entities. Gynecol Endocrinol 2007; 23:267–272.
59. Robin G, Catteau-Jonard S, Young J, Dewailly D. Physiopathological link between polycystic ovary syndrome and hyperprolactinemia: myth or reality? Gynecol Obstet Fertil 2011; 39:141–145.
60. Lima AP, Moura MD, Rosa e Silva AA. Prolactin and cortisol levels in women with endometriosis. Braz J Med Biol Res 2006; 39: 1121–1127.
61. Cunha-Filho JS, Gross JL, Lemos NA, et al. Hyperprolactinemia and luteal insufficiency in infertile patients with mild and minimal endometriosis. Horm Metab Res 2001; 33:216–220.
62. Radwanska E, Henig I, Dmowski WP. Nocturnal prolactin levels in infertile women with endometriosis. J Reprod Med 1987; 32: 605–608.
63. Gregorion G, Bakes P, Vitoratos N. Evaluation serum prolactin levels in patients with endometriosis and infertility. Gynecol Obstet Invest 1999; 48:48–51.
64. Muse K, Wilson EA, Jawal MJ. Prolactin hyperstimulation in response to thyrotropin-releasing hormone in patients with endometriosis. Fertil Steril 1982; 38:419–422.
65. Matalliotakis I, Panidis D, Vlassis G, et al. PRL, TSH, and their response to the TRH test in patients with endometriosis before, during and after treatment with Danazol. Gynecol Obstet Invest 1996; 42:183–186.
66. Arumugam K. Serum prolactin levels in infertile patients with endometriosis. Malays J Pathol 1991; 13:43–45.
67. Panidis D, Vavilis D, Rousso D, Panidou E, Kalogeropoulos A. Provocative tests of prolactin before, during and after long-term danazol treatment in patients with endometriosis. Gynecol Endocrinol 1992; 6:19–24.
68. Robert E, Massati L, Piscitelli G, Ferrari CI. Pregnancy outcome after treatment with the ergot derivate, cabergoline. Reprod Toxicol 1996; 10: 533.
69. Holmgren U. Women with prolactinoma: effect of pregnancy and lactation on serum prolactin and on tumor growth. Acta Endocrinol 1986; 111: 452–453.
70. Molitch ME, Russell EJ. The pituitary "incidentaloma". An Intern Med 1990; 112: 925–931.
71. Kupersmith MJ, Rosenberg C, Kleinberg D. Visual loss in pregnant women with pituitary adenomas. Ann Inter Med 1994; 121:473.
72. Well C. The saffiety of bromocriptine in hyperprolactinemic female infertility; a literature review. Curr Med Res Opin 1986; 10: 172–195.
73. Pittini L, Minisci M. Effecive mannagement of supraselar exeding prolactinoma during pregnancy. Clin Exp Obstet Gyn 1987; 14: 152–154.
74. Yamoda M, Myake A, Koike K. Sponataneous pregnancy after a pregnancy induced by treatment in hyperprolactinemic women. Eur J Obstet Gynecol Repr Biol 1990; 35:125–129.
75. Crosignani PG, Mattei AM, Severini V, Cavioni V, Maggioni P, Testa G. Long-term effects of time, medical treatment and pregnancy in 176 hyperprolactinemic women. Eur J Obstet Gynecol Reprod Biol 1992; 44:175–180.
76. Jeffcoate WJ, Pound N, Sturrock ND, Lambourne J. Longterm follow-up of patients with hyperprolactinaemia. Clin Endocrinol (Oxf) 1996; 45:299–303.
77. Casanueva FF, Molitch ME, Schlechte JA, et al. Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas. Clin Endocrinol (Oxf) 2006; 65:265–273.
78. Mancini T, Casanueva FF, Giustina A. Hyperprolactinemia and prolactinomas. Endocrinol Metab Clin North Am 2008; 37:67–99. doi: 10.1016/j.ecl.2007.10.013
79. Vilar L, Freitas MC, Naves LA, et al. Diagnosis and management of hyperprolactinemia: results of a Brazilian multicenter study with 1234 patients. J Endocrinol Invest 2008; 31:436–444.
80. Vilar L, Naves LA, Gadelha MR. Pitfalls in the diagnosis of hyperprolactinemia. Arq Bras Endocrinol Metab 2003; 47:347-357.
81. Molitch ME. Disorders of prolactin secretion. Endocrinol Metab Clin North Am 2001; 30:585–610.
82. Schofl C, Schofl-Siegert B, Karstens JH, Bremer M, Lenarz T,Cuarezma JS. Falsely low serum prolactin in two cases of invasive macroprolactinomas. Pituitary 2002; 5:261–265.
83. Hoffman KL, Parsons GH, Allerdt LJ, Brooks JM, Miles LE. Elimination of 'hook-effect' in two-site immunoradiometricassays by kinetic rate analysis. Clin Chem 1984; 30:1499–1501.
84. Vilar L, Fleseriu M, Bronstein MD. Challenges and pitfalls in the diagnosis of hyperprolactinemia. Arq Bras Endocrinol Metab 2014; 58:9-22.
85. Vallette-Kasic S, Morange-Ramos I, Selim A, et al. Macroprolactinemia Revisited: A Study on 106 Patients. J Clin Endocrinol Metab 2002; 87:581–588.
86. Elenkova A, Genov N, Abadzhieva Z, Vasilev V, Kirilov G, Zacharieva S. Macroprolactinemia in patients with prolactinomas: prevalence and clinical significance. Exp Clin Endocrinol Diabetes 2013; 121:201e5.
87. Bjoro T, Makrid L, Wergerland R, Turter A, Kvistborg A, Sand T. Frequency of hyperprolactinemia due to large molecular weight prolactin (150-170 kD PRL). Scand J Clin Lab Investig 1995; 55:139e47.
88. Thirunavakkarasu K, Dutta P, Sridhar S, et al. Macroprolactinemia in hyperprolactinemic infertile women. Endocrine 2013; 44:750–755. doi: 10.1007/s12020-013-9925-y

89. Kasum M, Pavičić-Baldani D, et al. Importance of macroprolactinemia in hyperprolactinemia. Eur J Obstet Gynecol Reprod Biol 2014; 183:28–32.
90. Kasum M, Oreskovic S, Čehić E, Šunj M, Albert Lila, Ejubović E. Laboratory and clinical significance of macroprolactinemia in women with hyperprolactinemia. Taiwan J Obstet Gynecol 2017; 56:719e-724. doi: 10.1016/j.ejogrb.2014.10.013.
91. Shimatsu A and Naoki Hattori N. Macroprolactinemia: Diagnostic, Clinical, and Pathogenic Significance. Clin Dev Immunol 2012; 167132. doi: 10.1155/2012/167132
92. Saleem M., Martin H., Coates P. Prolactin biology and laboratory measurement: an update on physiology and current analytical issues. Clin Biochem Rev 2018; 39:3–16.
93. Fahie-Wilson M, Smith TP. Determination of prolactin: the macroprolactin problem. Best Pract Res Clin Endocrinol Metab 2013; 27:725–742. doi: 10.1016/j.beem.2013.07.002
94. Martin TL, Kim M, Malarkey WB. The natural history of idiopathic hyperprolactinemia. J Clin Endocrinol Metab. 1985;60(5):855–858.
95. Rabinovich IH, Cámara Gómez R, García Mouriz M, et al. on behalf of the Neuroendocrinology Group of the SEEN. Clinical guidelines for diagnosis and treatment of prolactinoma and hyperprolactinemia. Endocrinol Nutr. 2013; 60:308–319. doi: 10.1016/j.endonu.2012. 11.005.
96. De Bellis A, Colao A, Pivonello R, et al. Antipituitary antibodies in idiopathic hyperprolactinemic patients. Ann NY Acad Sci 2007; 1107:129–135.
97. Chang S, Copperman AB. New insights into human prolactin pathophysiology: genomics and beyond. Curr Opin Obstet Gynecol 2019; 31:207–211. doi: 10.1097/GCO.0000000000000545.
98. Raymond JP, Goldstein E, Konopka P, Leleu MF, Merceron RE, Loria Y. Follow-up of children born of bromocriptine-treated mothers. Horm Res 1985; 22:239–246.
99. Wang AT, Mullan RJ, Lane MA, et al. Treatment of hyperprolactinemia: a systematic review and meta-analysis. Syst Rev 2012; 1:33. doi: 10.1186/2046-4053-1-33
100. dos Santos Nunes V, El Dib R, Boguszewski CL, Nogueira CR. Cabergoline versus bromocriptine in the treatment of hyperprolactinemia: a systematic review of randomized controlled trials and meta-analysis. Pituitary 2011; 14:259–265.doi: 10.1007/ s11102-010-0290-z.
101. Ricci E, Parazzini F, Motta T, et al. Pregnancy outcome after cabergoline treatment in early weeks of gestation. Reprod Toxicol 2002; 16:791–793.
102. Stalldecker G, Mallea-Gil MS, Guitelman M, et al. Effects of cabergoline on pregnancy and embryo-fetal development: retrospective study on 103 pregnancies and a review of the literature. Pituitary 2010; 13:345–350. doi: 10.1007/s11102-010-0243-6
103. Colao A, Abs R, Bárcena DG et al., Chanson P, Paulus W, Kleinberg DL. Pregnancy outcomes following cabergoline treatment: extended results from a 12-year observational study. Clin Endocrinol (Oxf) 2008; 68:66–71.
104. Herring N, Szmigielski C, Becher H, Karavitaki N, Wass JAH. Valvular heart disease and the use of cabergoline for the treatment of prolactinoma. Clin Endocrinol 2009; 70:104–108.
105. Webster J, Piscitelli G, Polli A, Ferrari CI, Ismail I, Scanlon MF. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinaemic amenorrhoea. Cabergoline Comparative Study Group. N Engl J Med 1994; 331:904–909.
106. Löndahl M, Nilsson A, Lindgren H, Katzman P. A case of constrictive pericarditis during cabergoline treatment for hyperprolactinaemia. Eur J Endocrinol 2008; 158:583–585. doi: 10.1530/EJE-07-0584.
107. Colao A. Pituitary tumours: the prolactinoma. Best Pract Res Clin Endocrinol Metab 2009; 23:575–596.
108. Rasmussen C, Brownell J, Bergh T. Clinical response and prolactin concentration in hyperprolactinemic women during and after treatment for 24 months with the new dopamine agonist, CV 205-502. Acta Endocrinol (Copenh) 1991; 125(2):170–176
109. Di Sarno A, Landi ML, Marzullo P, et al. The effect of quinqgolide and cabergoline, two selective dopamine receptor type 2 agonists in the treatment of prolactinomas. Clin Endocrinol 2000; 53(1):53–60.
110. Berinder K, Stackenäs I, Akre O, Hirschberg AL, Hulting AL. Hyperprolactinaemia in 271 women: up to three decades of clinical follow-up. Clin Endocrinol (Oxf) 2005; 63:450–455.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 22, No 2, 2020, pp. 49–55

UDC 616.831-005.1-036
http://doi.org/10.22190/FUMB200421008M

Original Article

EPIDEMIOLOGY AND PROGNOSTIC FACTORS IN PATIENTS WITH SUBDURAL HEMATOMA

Aleksandar Miljković[1], Filip Milisavljević[1], Ivan Bogdanović[1], Srbislav Pajić[2]

[1]Clinical Center of Serbia, Neurosurgery Division, Belgrade, Serbia
[2]The Centre for Medical Emergencies and Neurotrauma Emergency Center, KSC, Belgrade, Serbia

Abstract. *SDH was first described in 1658 and in 1914 trauma was recognized as one of the causes. Acute SDH is more common in younger population while chronic is common in the elderly with the peak of incidence of 7.35/100000 per year in the age group 70-79 years. Trauma is one of the main causes of SDH although in 30-50% of patients direct trauma to the head can be omitted. Other predisposing factors include: anticoagulant therapy, epilepsy, and hypertension. The aim of the study was to determine risk factors and prognostic factors for the occurrence of SDH, also to show what age group is most at risk for developing chronic and acute SDH. Identify the diagnostic steps in proving SDH and the best method of treatment. The study includes 267 patients treated in the period from 1.1.2019 to 31.12.2019 at the Clinic for Neurosurgery CCS. SDH was diagnosed by neurological examination and brain CT in all patients and all were treated conservatively or surgically. The analytical statistics were used parametric and non-parametric tests of difference. The study included 185 men and 82 women of middle age 68 ± 17.19 years. Most patients were between 6-8 decades. Multiple changes in CT were observed in 63.3% of patients. Chronic SDH had 50.5% of patients and 45.6% had acute SDH. The most common symptoms were headache, psycho-organic syndrome, and hemiparesis. Patients with a GCS score of more than 8 had a better prognosis and outcome. Surgical treatment was the main course of treatment in our study. CT with / without contrast is the gold standard in detecting SDH. Men are at higher risk for the occurrence of SDH. Symptoms can occur later in the clinical presentation so we need to take caution when performing neurological examination. Factors that can lead us to suspect possible SDH are: age, gender, type of injury, clinical presentation, and time of occurrence.*

Key words: *subdural hematoma, trauma, headache, CT, anticoagulation, hemiparesis.*

Introduction

Chronic subdural hematoma (cSDH) is an encapsulated collection of old blood, mainly or totally liquefied and localized between the dura mater and the arachnoid. It was first described by J.J. Wepfer in 1658 and then found himself in the popular novel Pierette written by Honore de Balzac in 1840. Balzac described the traumatic origin and surgical treatment of these hematomas even though they had not yet been recognized as a separate clinical entity by physicians. This view was challenged by Virchow in 1857. giving his term "pachymeningitis hemorrhagica intern" to describe this disease, considering it to be of inflammatory origin. It wasn't until 1914. traumatic origin of subdural hematomas firmly established by Trotter, who gave them the name "subdural hemorrhagic cysts". Hulke described the first successful operation of a chronic subdural hematoma in 1883. In the era before computed tomography (CT) diagnosis was made by angiography or diagnostic trepanation. The introduction of CT diagnostics has set the gold standard in detecting SDH. SDH is a dynamic lesion and its presentation depends on age, shortly after bleeding (acute phase) it is hyperdense due to the presence of fresh blood, after a few weeks (subacute phase) isodensate due to fibrinolysis and after 4 weeks (chronic phase) hypodense due to fluid resorption. Chronic and acute SDH have different presentations on brain CT scan (Figure 1).

Acute SDH generally occurs in the younger population, after severe trauma, followed by structural brain damage, and is presented within 72 hours. In contrast, chronic SDHs often occur in the elderly population after a trivial injury, without any brain injury, and usually take weeks or months before becoming clinically evident. The incidence peak of cSDH is in the sixth and seventh decades of life. The incidence is estimated to be 1.72 / 100,000 per year, rising rapidly with aging to 7.35 / 100,000 per year in the 70-79 age group [1-22]. Other studies have shown there is a rising incidence of chronic subdural hematomas because there are more people in older populations. It has long been known that subdural hematomas are more likely to develop in the elderly even after minor traumas. Generalized cerebral atrophy and increased vein fragility associated with aging are major risk factors. With aging, there is a decrease in brain mass and an increase in space between the brain and skull bones from 6% to 11% of total intracranial space, which leads to vein stretching and increased brain movement within the cranium [1-11, 15-29].

Correspondence to: Srbislav Pajić M.D., Ph.D
Center for Medical Emergencies and Neurotrauma Emergency Center KSC
Pasterova 2, 11000 Belgrade, Serbia
Phone: +381 64 9030226
E-mail: nevus-ng@hotmail.com
Received April 21st, 2020 / Accepted January 8th, 2021

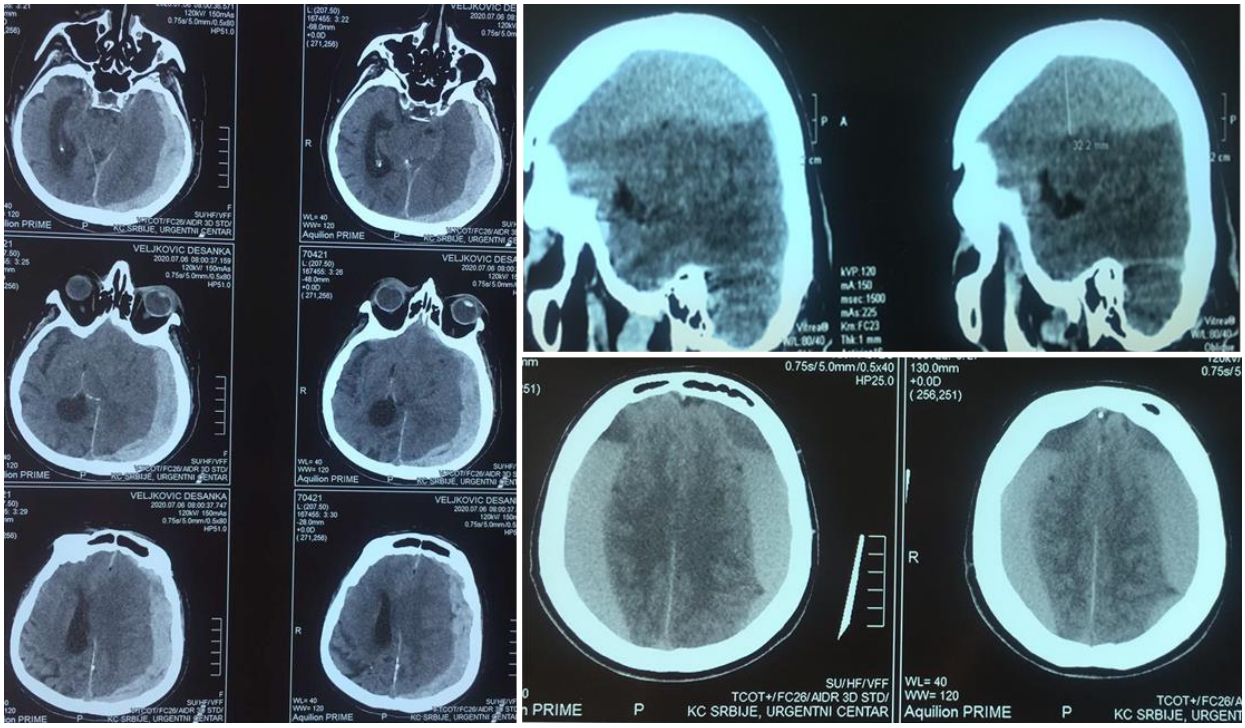


Fig. 1 Acute and chronic subdural hematoma

Trauma is one of the most important risk factors. However, direct head trauma may be absent in 30-50% of cSDH cases. Attention should be paid to indirect head injuries as well as trivial injuries that patients often forget to mention. Other predisposing factors are anticoagulant therapy, epilepsy, alcoholism, hypertension, etc. In the elderly, the most common clinical presentation is in the form of mental disorders (50-70%). It can manifest as varying degrees of state of consciousness. The diagnosis can be overlooked in psychiatric or neurological patients in whom any change in behavior or functional status is usually attributed to their pre-existing illness [1-5, 9-17, 23-33]. Hemiparesis can be found in about 58% of cases in some studies [1-7, 17-26]. Limb weakness is usually mild and contralateral, although cases of ipsilateral symptoms have been seen. The incidence of headache varies from 14% to 80% in different studies [1-3, 7-19, 29-35]. The first reason why headache is less common in the elderly compared to younger patients is partly due to increased intracranial space for hematoma accommodation [1-14].

The second reason is that confusion first occurs in the elderly so they report to the doctor before a headache occurs. Another common symptom is syncope, which occurs in about 74% of cases. Epilepsy has traditionally been considered a rare presentation, although it was detected in as many as 6% of cases as an initial symptom. We often do not suspect the diagnosis of SDH at the time of the initial presentation in most cases. Other possible diagnoses at the time of presentation are tumor, subarachnoid hemorrhage, and stroke. The most important step in the diagnosis is SDH is a high index of suspicion. It should be considered in a patient with/without a history of head injury presenting:

- Changes in mental status or worsening of a pre-existing neurological or psychiatric deficit,
- Focal neurological deficit,
- Headache with or without focal neurological deficit.

SDH treatment is surgical evacuation, although small hematomas can be withdrawn spontaneously or with conservative therapy, but patients should be closely monitored. The most common surgical complications are symptomatic recurrences of hematoma (8-37%) and epi-seizures (about 11%), while mortality and morbidity (15.6%) vary depending on the literature [1-9, 13-19, 24-26, 36-38].

Materials and Methods

This study includes a retrospective review of a database of patients (267 patients) with verified acute and chronic subdural hematoma received in the period 1/1/2019 to 31/12/2019 at the Emergency Center in Belgrade and treated at the neurosurgical department. The analysis included patients who met the following conditions:

1. Clinically, by examination and CT scan verified the presence of SDH,
2. Treated conservatively or surgically

The analysis used:

1. Complete medical histories,
2. Findings obtained by computed tomography (CT),
3. Glasgow coma and outcome scales (GCS, GOS)

Parametric (t-test) and non-parametric difference tests (χ^2 and Median test) were used in analytical statistics. Parametric tests were applied to examine the differences and interactions of observation features in which the condition for performing a parametric test was met. Non-parametric tests were applied to observation fea-

tures in which the conditions for performing analog parametric tests were not met, as well as to observation features that are naturally analyzed by these tests.

Results

Our database contained 267 patients of both gender, 185 males (69.3%) and 82 females (30.7%). A total is 267 patients, with a mean age of 68.35 ± 17.19 years. The youngest patient was 6 months old and the oldest 97 years old. The median age in the observed group was 73. There was a statistically significant difference in favor of the male sex (p <0.001). Most patients were between 60 and 80 years of age, with an incidence peak between 7 and 8 decades. A statistically significant difference in age concerning sex can be observed (p<0.001). The distribution by sex and age is shown in Figures 2 and 3.

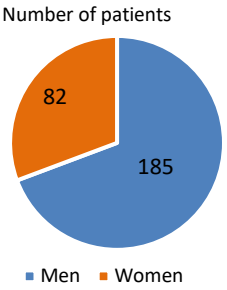


Fig. 2 Patient distribution by gender

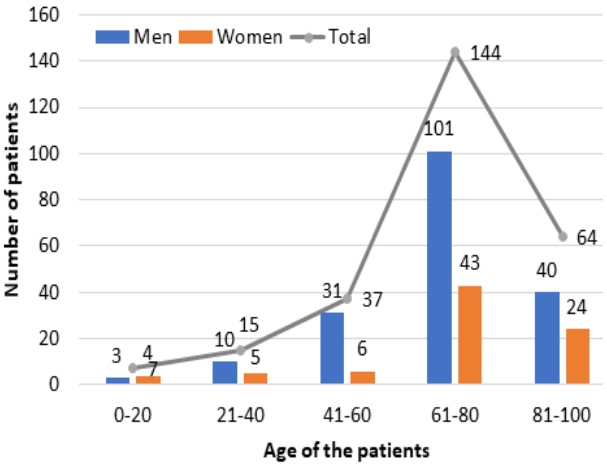


Fig. 3 Patients age distribution

With regard to the manner in which SDH occurred, patients were divided into three groups:

1) SDH resulting from a head impact by blunt force trauma, fall, or other direct head impact,
2) SDH resulting from direct head impact in a traffic accident, and
3) spontaneous SDH.

The distribution of patients concerning the onset of SDH can be seen in Figure4. A statistically significant difference in the benefit of patients in the first group was observed (p <0.001). At admission, there were 7 patients (2.7%) with polytrauma while the rest were only with/without head injury. Multiple changes in CT were observed in 169 patients (63.3%). These changes were in the form of head bone fractures (20.2%), brain contusion (25.84%), epidural hematoma (2.6%), subarachnoid hemorrhage (13.1%), and intracerebral hemorrhage (1.4%) (Figure 5). A highly statistically significant difference in the occurrence of contusions together with SDH during head injury was observed in this group of patients. Based on the findings on CT, we divided in terms of localization, time of onset of SDH, and displacement of central structures, which can be seen in Figures 6 and 7. Chronic SDH had 50.5% of our patients, while acute had 45.6% of patients. Above the left hemisphere, SDH had 45%, over the right hemisphere 39% and over both hemispheres had 16% of patients. In our study, we observed more patients with left-sided hematoma occurrence without compressive effect and displacement of central structures, as well as more patients with chronic SDH at admission (p <0.001).

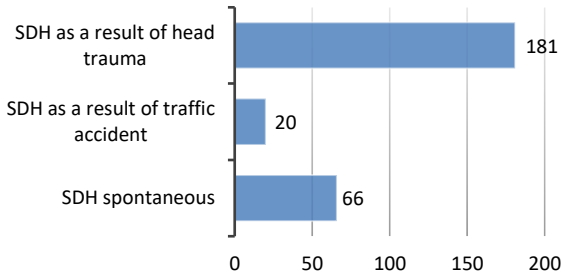


Fig. 4 Patients distribution regarding the mechanism of SDH formation

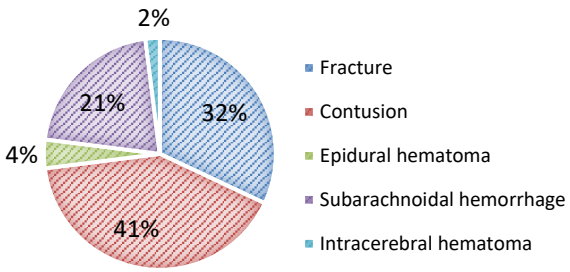


Fig. 5 Most common brain injuries associated with SDH

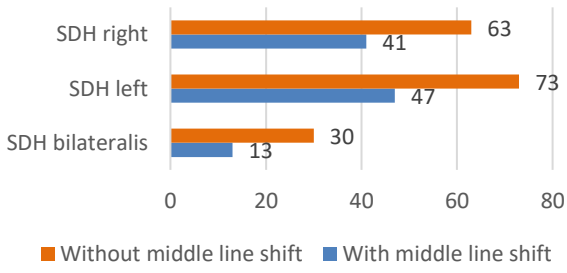


Fig. 6 SDH localization with/without compression and midline displacement

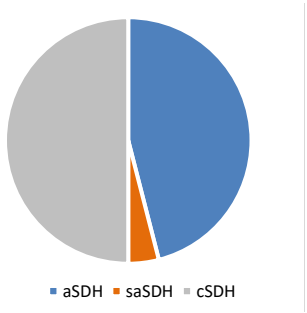


Fig. 7 Type of SDH versus time of onset in our study

Table 1 Symptom presentation of admitted patients in our study

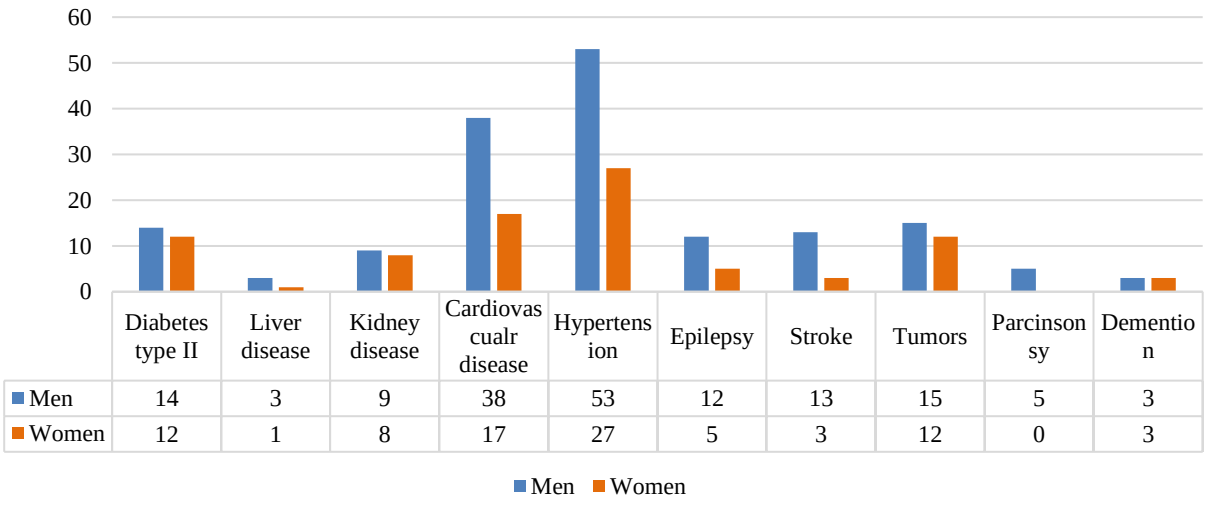
Distribution of symptoms admitted in patients in our study	
Cranial nerves	
Normal	166
Deficit	58
Palsy of n. VII on the right side	15
Palsy of the n. VII on the left side	10
Palsy of the n. III	1
Extremities	
No deficit	124
Decerebration	41
Patient with right sided hemiparesis	56
Patient with left sided hemiparesis	44
Bihemiparesis	2
Different types of hemiparesis at admission:	
the left-sided weakness of mild grade	33
the left-sided weakness of moderate severity	4
the left-sided weakness of severe grade	7
the right-sided weakness of mild grade	38
the right-sided weakness of moderate severity	11
the right-sided weakness of severe grade	7
Headache	86
Nausea	61
Vomiting	24
Psychoorganic syndrome	66
Syncope	35

The clinical presentation is manifested with limb weakness, psychoorganic syndrome, cranial nerve disorder, or syncope. The most common symptoms were headache, psychoorganic syndrome, and hemiparesis. Most patients on admission showed no signs of failure on cranial nerves or limbs ($p<0.001$). Table 1 shows the most common symptoms in our study.

The predisposing factors mentioned in the introduction may play an important role in developing SDH after trauma. As more than 50% of patients over 50 were expected to have associated comorbidities: diabetes, hypertension, epilepsy, dementia, stroke, etc. We observed that about 30% of patients had different types of cardiovascular disease and hypertension. Figure 8 shows the distribution of patients with their comorbidities by gender.

According to the protocol defined for these patient conditions, the Glasgow Coma Score was determined at the time of admission, and the conditions during discharge were determined by the Glasgow Outcome Scale. Based on the number of GCS points on admission, patients were divided into two groups: those with less and those with a score greater than 8, and in relation to the outcome on discharge in five groups. As expected, patients with a GCS score greater than 8 had a better prognosis and the best possible outcome ($p <0.001$). High statistical significance was shown in relation to the outcome in favor of men ($p <0.001$). Tables 2 and 3 show the ratio of GCS and GOS score in relation to gender and age group. Besides the GCS scores on admission, a previous use of anticoagulant therapy also influenced the outcome of the patients. Among our patients, 62 had received anticoagulant therapy prior to admission, and the study did not show statistical significance for the benefit of patients without anticoagulant therapy in terms of outcome (Figure 9).

After assessing the patient's condition, 173 patients (65%) underwent surgical treatment while 94 (35%) patients were treated with conservative therapy (Figure10). Twice as many more men had a heamatoma for surgery than females. We decided on surgical treatment in most of our patients ($p <0.001$). In our study operated patients



	Diabetes type II	Liver disease	Kidney disease	Cardiovascular disease	Hypertension	Epilepsy	Stroke	Tumors	Parcinsonsy	Dementio n
Men	14	3	9	38	53	12	13	15	5	3
Women	12	1	8	17	27	5	3	12	0	3

Fig. 8 Distribution of comorbidities according to gender

were shown to have a better treatment outcome than patients treated conservatively (p <0.001) (Figure 11).

Table 2 GCS and GOS scores by age group in our study

Distribution of the GCS and GOS score considering the age						
	GCS<9	GCS>9				
0-20	0	7				
21-40	3	12				
41-60	13	24				
61-80	30	114				
81-100	16	48				
	GOS 1	GOS 2	GOS 3	GOS 4	GOS 5	
0-20	0	1	0	0	6	
21-40	3	0	1	0	11	
41-60	10	2	2	1	22	
61-80	34	7	16	6	81	
81-100	18	9	7	3	27	

Table 3 GCS and GOS scores relative to gender in our study

GCS and GOS score considering gender					
	GCS<9	GCS >8			
M	36	149			
W	26	56			
	GOS 1	GOS 2	GOS 3	GOS 4	GOS 5
M	38	9	19	8	111
W	27	10	7	2	36

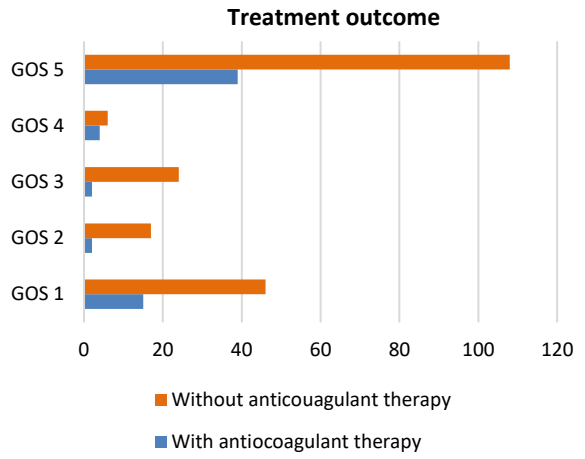


Fig. 9 Treatment outcomes in patients with and without anticoagulant therapy

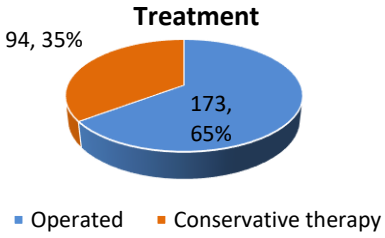


Fig. 10 Distribution of operated patients and patients treated with conservative therapy

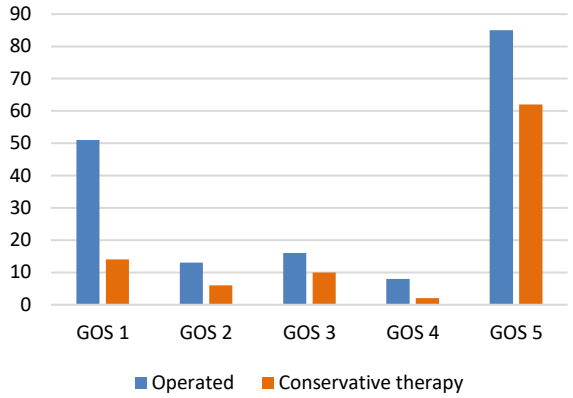


Fig. 11 Treatment outcome of patients treated with surgical and conservative therapy

Discussion

Our group consisted of 185 (69%) men and 82 (30.7%) women, which tells us that SDH occurs 2.25 times more frequently in men. Similar studies conducted recently show that the occurrence of SDH is 3 times more common in men. In the study it was shown that in 56% of cases patients were from age groups from 5 to 6 decades; another study states that more than 50% of cases were from a group of patients over 60 years of age. Our study showed that the majority of patients are consisted of people between the ages of 6 and 8 decades, which are consistet with different studies. Acute SDH has been shown to occur in 5-25% after severe head trauma depending on the study. Men were also shown to have a higher incidence of chronic SDH. In our study, it was shown that 1.44 times more acute SDH was frequent in men than women, and 4 times more chronic SDH was frequent in men than women. When we talk about literature, it was discrabed 1.3 to 2 for acute SDH in men, and 3 to 5 times more in men for chronic SDH.

Mortality is less than 20% for those under 40, about 65% for the 40-80 age group, and patients over 80 have the highest mortality [1, 4, 7, 9, 15, 21, 26, 29, 33, 36, 38]. Our study found mortality of less than 30% both of acute and chronic SDH. Causes of SDH are head trauma, coagulopathy or anticoagulant therapy, spontaneous, etc [1-5, 7-13, 16-28]. Trauma plays a major role in the onset of acute SDH. They are most injured in crashes, violence, or motor accidents [1-9, 23-33]. When it comes to chronic SDH, about 50% of patients do not have a head injury, and if they do, it is usually a minor injury. In 25% of patients who have had a head injury, symptoms of chronic SDH occur within 1-4 weeks, in the other 25% between 5 weeks and 3 months and only 1/3 of patients have no asymptomatic period [1-14].

Proven risk factors for developing SDH are: alcoholism, epilepsy, anticoagulant therapy, cardiovascular disease, diabetes mellitus, etc [1-7, 9-14].The literature and different studies found that 10-25% of patients are on Aspirin therapy, while in our study, Aspirin and/or anticoagulant therapy was used by 23% of patients [3-5, 11-16,

22-27]. In our study, the most important factors for cardiovascular problems and hypertension were singled out, which can be explained by the fact that the majority of patients were elderly. It is a well-known fact that clinical presentation is dominated by headache, confusion, hemiparesis, disorders of consciousness, while to a lesser extent weakness, seizures and incontinence occur.

Headache occur in 90% and confusion in 56% of cases, while in 75% of cases, headache is accompanied by nausea and vomiting [1, 4, 6, 9, 12, 16, 19, 20, 22, 24, 28, 30]. Hemiparesis and syncope occur in approximately 58% and 40% of cases, respectively. Hemiparesis is ipsilateral in 40% of cases [10-18, 22, 25, 31, 32]. In our study, the dominant clinical signs of SDH occurred to a lesser extent, which may be explained by the rapid arrival of patients after trauma before the symptoms could be developed. On admission, 205 patients (76.7%) had a GCS score greater than 8, while 62 (23.2%) patients had a score of less than 9. It has been shown in various studies that one of the most important prognostic factors for treatment outcome is certainly a GCS score [1-9, 25-33]. This is supported by the fact that as many as 82% of patients with a score less than 8 from our study died, while in the group of patients with a score greater than 8 that percentage was less than 1. Seventy one percent of our patients had complete recovery (GOS 5). The better treatment outcome in our study was experienced by men, which can be explained by their majority share in our study. Surgical treatment is taken as the main choice of treatment (65% patient in our study), and 35% of patients was treated with conservative therapy in our study but other studies have shown that around 23% of patients did not undergo surgery because the size of the hematoma was small [2-7, 15-23, 34-38]. In almost all studies, the operative approach is considered to be the main form of therapy for patients with SDH associated with midline shift, while conservative therapy is reserved for patients who are asymptomatic and with a small hematoma on brain CT [9-12, 24, 27, 33, 37].

Conclusion

Since the introduction of CT diagnostics, detection of both acute and chronic SDHs has progressed significantly and is now considered the gold standard in the detection of SDH. Men are at higher risk for developing SDH due to both lifestyle and associated comorbidities. Trauma has been shown to be one of the most common causes of SDH especially acute in the younger population, so care should be taken when screening as symptoms can often occur later. Efforts should be made to educate medical professionals because the diagnosis of SDH is first made with suspicion and only then with diagnostic procedures. Significant factors for suspecting a possible diagnosis of SDH are age, gender, type of injury, clinical presentation, and time of developing symptoms. In the population group between 6 and 8 decades, the most common is chronic, while in the younger age group 40, acute SDH is more common. The most common symptoms are headache, confusion, hemiparesis, state of consciousness. Surgical treatment is certainly an option of choice except in patients with very small hematoma and asymptomatic clinical imaging.

References

1. Adhiyaman V, Asghar M, Ganeshram KN, Bhowmick BK. Chronic subdural hematoma in the elderly. Postgrad Med J 2002; 78:71–75
2. Fogelholm R, Waltimo O. Epidemiology of chronic subdural haematoma. Acta Neurochir 1975; 32:247–250.
3. Ellis GL. Subdural haematoma in the elderly. Emerg Med Clin North Am 1990; 8:281–294.
4. Jones S, Kafetz K. A prospective study of chronic subdural haematomas in elderly patients. Age Ageing 1999; 28:519–521
5. Rozzelle CJ, Wofford JL, Branch CL. Predictors of hospital mortality in older patients with subdural haematoma. J Am Geriatr Soc 1995; 43:240–244.
6. Traynelis VC. Chronic subdural haematoma in the elderly. Clin Geriatr Med 1991; 7:583-598.
7. Luxon LM, Harrison MJG. Chronic subdural haematoma. Q J Med 1979; 189:43–53.
8. Cameron MM. Chronic subdural haematoma: a review of 114 cases. J Neurol Neurosurg Psychiatry 1978; 41:834–839.
9. Mulligan P, Raore B, Liu S, Olson JJ. Neurological and functional outcomes of subdural hematoma evacuation in patients over 70 years of age. J Neurosci Rural Pract 2013; 4:250–256.
10. O'Phelan K. Traumatic Brain Injury, Definitions and Nomenclature. In: Zollman FS, ed. Manual of Traumatic Brain Injury Management. Chicago, Illinois: Demos Medical Publishing; 2011. p. 3–9.
11. Lombard LA. Characterization of Traumatic Brain Injury Severity. In: Zollman FS, ed. Manual of Traumatic Brain Injury Management. Chicago, Illinois: Demos Medical Publishing; 2011. p. 16–22.
12. Alexander DN. Imaging in Moderate to Severe Traumatic Brain In: Zollman FS, ed. Manual of Traumatic Brain Injury Management. Chicago, Illinois: Demos Medical Publishing; 2011. p. 157–165.
13. Rosenow JM. Neurosurgical Management of Skull Fractures and Intracranial Hemorrhage In: Zollman FS, ed. Manual of Traumatic Brain Injury Management. Chicago, Illinois: Demos Medical Publishing; 2011. p. 166–174.
14. Narayan RK. Head Injury In: Grossman RG, ed. Principles of Neurosurgery. New York: Raven Press, Ltd; 1991. p. 235–286.
15. van Havenbergh T, van Calenbergh F, Goffin J, Plets C. Outcome of chronic subdural haematoma: analysis of prognostic factors. Br J Neurosurg 1996; 10:35–39.
16. Kotwica Z, Brzezinski J. Acute subdural haematoma in adults: an analysis of outcome in comatose patients. Acta Neurochir (Wien) 1993; 121:95–99.
17. Morinaga K, Matsumoto Y, Hayashi S, et al. [Subacute subdural hematoma: findings in CT, MRI and operations and review of onset mechanism]. No Shinkei Gek 1995; 23:213–216. Japanese.
18. Matsuyama T, Shimomura T, Okumura Y, Sakaki T. Rapid resolution of symptomatic acute subdural hematoma: a case report. Surg Neurol 1997; 48:193–196.
19. Gennarelli TA, Thibault LE. Biomechanics of acute subdural hematoma. J Trauma 1982; 22:680–686.
20. Foelholm R, Waltimo O. Epidemiology of chronic subdural haematoma. Acta Neurochir (Wien) 1975; 32:247–250.
21. Servadei F. Prognostic factors in severely head-injured adult patients with acute subdural haematoma's. Acta Neurochir (Wien). 1997; 139:279–285.

22. Massaro F, Lanotte M, Faccani G, Triolo C. One hundred and twenty-seven cases of acute subdural haematoma operated on. Correlation between CT scan findings and outcome. Acta Neurochir (Wien) 1996; 138:185–191.
23. Phuenpathom N, Choomuang M, Ratanalert S. Outcome and outcome prediction in acute subdural hematoma. Surg Neurol 1993; 40:22–25.
24. Kapsalaki EZ, Machinis TG, Robinson JS 3rd, Newman B, Grigorian AA, Fountas KN. Spontaneous resolution of acute cranial subdural hematomas. Clin Neurol Neurosurg 2007; 109: 287–291.
25. Mori K, Maeda M. Surgical treatment of chronic subdural hematoma in 500 consecutive cases: clinical characteristics, surgical outcome, complications, and recurrence rate. Neurol Med Chir (Tokyo) 2001; 41:371–381.
26. Stanisic M, Lund-Johansen M, Mahesparan R. Treatment of chronic subdural hematoma by burr-hole craniotomy in adults: influenza of some factors on postoperative recurrence. Acta Neurochir (Wien) 2005; 147:1249–1256; discussion 1256–1257.
27. Suman S, Meenakshisundaram S, Woodhouse P. Bilateral chronic subdural haematoma: a reversible cause of parkinsonism. J R Soc Med 2006; 99: 91–92.
28. Giray S, Sarica FB, Sen O, Kizilkilic O. Parkinsonian syndrome associated with subacute subdural haematoma and its effective surgical treatment: a case report. Neurol Neurochir Pol 2009; 43: 289–292.
29. Luxon LM, Harrison MJ. Chronic subdural haematoma. Q J Med 1979; 48:43–53.
30. Stein SC, Young GS, Talucci RC, et al. Delayed brain injury after head trauma: significance of coagulopathy. Neurosurgery 1992; 30:160–165.
31. Wilms G, Marchal G, Geusens E, et al. Isodense subdural hematomas on CT: MRI findings. Neuroradiology 1992; 34:497–499.
32. Brain Trauma Foundation, AANS, Joint Section of Neurotrauma and Critical Care. Guidelines for the management of severe head injury. J Neurotrauma 1996; 13:641–734.
33. Wong CW. Criteria for conservative treatment of supratentorial acute subdural hematomas. Acta Neurochir (Wien) 1995; 135: 38–43.
34. Ducruet AF, Grobelny BT, Zacharia BE, et al. The surgical management of chronic subdural hematoma. Neurosurg Rev 2012; 35: 155–169; discussion 169.
35. Lega BC, Danish SF, Malhotra NR, Sonnad SS, Stein SC. Choosing the best surgery for chronic subdural hematoma: a decision analysis. J Neurosurg 2010; 113:615–621.
36. Chesnut RM, Marshall LF, Klauber MR, et al. The role of secondary brain injury in determining the outcome of severe head injury. J Trauma 1993; 34:216–222.
37. Itshayek E, Rosenthal G, Fraifeld S, Perez-Sanchez X, Cohen JE, Spector S. Delayed posttraumatic acute subdural hematoma in elderly patients on anticoagulation. Neurosurgery 2006; 58: E851–856.
38. Stroobandt G, Fransen P, Thauvoy C, Menard E. Pathogenetic factors in chronic subdural hematoma and causes of recurrence after drainage. Acta Neurochir (Wien) 1995; 137:6.

FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol. 22, No 2, 2020, pp. 56–60

UDC 618.19-006.6:616.36-006-033.2
http://doi.org/10.22190/FUMB201110009I

Case Report

LATE LIVER METASTASIS 20 YEARS AFTER THE INITIAL DIAGNOSIS: A CASE REPORT OF METACHRONOUS HORMONE DEPENDENT TUMORS OF BREAST AND ENDOMETRIUM

Ivan Ilić, Maja Jovičić Milentijević, Aleksandar Milićević, Milica Stanković, Aleksandra Radičević

Favulty of Medicine, University of Niš, Niš, Serbia

Abstract. *Breast cancer is the most common cancer and a significant cause of morbidity and mortality in the female population worldwide. The liver is the third most common metastatic site for invasive breast malignancy besides bones, lungs, and brain. Breast cancer has been linked with metachronous bone, endometrial, colon/rectal, connective tissue (sarcoma), leukemia, lung, ovary, or thyroid cancer. Studies have shown an increased risk of secondary malignancies in women treated for breast malignancy in connection to adjuvant treatment in certain cases.*
We present a case of a 71 -year -old woman who was diagnosed with breast cancer 20 years ago. The primary diagnosis was invasive lobular breast cancer localized in the left lower lateral quadrant. Micromorphological, histochemical and immunohistochemical analyses rendered diagnosis inconclusive due to lack of tissue so after 4 months rebiopsy was performed. Clinico-pathological correlation of the second biopsy was in favor of liver metastasis of partially hormone-dependent breast cancer. Immunohistochemistry was vital for the diagnosis of the liver biopsy, in particular GATA3 positivity and vimentin negative staining which helped us exclude endometrial cancer metastasis which was diagnosed before the initial liver biopsy. GATA 3(+)/vimentin(-) panel proved to be superior to GCDFP-15 and mammaglobin in proving the breast origin of the secondary tumor deposit.
Liver metastasis from primary breast cancer can in certain cases occur many years after the initial diagnosis which shows the importance and necessity for long- term follow-up of these patients, while considering the possibility of metachronous tumors as well.

Key words: *breast cancer, liver metastases, immunohistochemistry, metachronous tumors.*

Introduction

Breast cancer is the most common cancer and a significant cause of morbidity and mortality in the female population worldwide. In around 20% - 30% of cases distant metastases occur [1].

Breast cancer usually disseminates to bones, lungs, liver, and brain by hematogenous spread; the liver is the third most common metastatic site for invasive breast malignancy [2].

The status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) in primary and metastatic breast cancers was evaluated and links between ER, PR, and HER-2 with various localisations of breast cancer (including liver) were investigated. ER+ or PR+/HER-2- (luminal A) subtypes were present in 75.0% of the cases [3].

A panel of immunohistochemical markers necessary for confirming the breast origin of the metastatic deposit consists of: GATA3, mammaglobin and gross cystic disease fluid protein 15 (GCDFP-15). In clinical practice, GATA3 immunohistochemistry stains a greater number of primary and secondary breast carcinomas than both mammaglobin and GCDFP-15 [4].

If the tumor reoccurs five or more years after the initial diagnosis, it would indicate a long -dormant period of undetectable metastases. Recent studies have focused on predicting the risk of late breast cancer recurrence by investigating clinical factors, subtypes, genes, and immune status. In a recent study early to mid recurrence was associated with more aggressive clinical progression and less favourable parameters (greater tumor diameter, more frequent lymph node metastases, higher tumor grades, later stages, and negative ER and PR hormone status) compared to survivors and the patients with late breast cancer recurrence [5].

Metastasising breast carcinomas need to be differentiated from synchronous/metachronous gynecological malignant tumors with an estrogen receptor/cytokeratin 7-positive (ER+/CK7+) and mammaglobin-positive immunohistochemical profile, which can be achieved by using GATA3 immunohistohemical stain which proved to be highly useful in diagnosing breast carcinomas, both primary and metastatic [6].

Here we report a case of a patient with breast cancer liver metastasis 20 years after primary diagnosis, which was confirmed on a liver biopsy by pathohistological and immunohistochemical analysis.

Correspondence to: Ivan Ilić M.D., Ph.D.
Faculty of Medicine, Dr. Zoran Djindjić Blvd., 81, 18000 Niš, Serbia
Phone: +381 63 7268033
E-mail: ilicko81@gmail.com
Received November 10th, 2020 / Accepted January 23rd, 2021

Materials and Methods

Liver biopsy was formalin- fixed (10% neutral buffered formalin), processed, paraffin- embedded, and then stained using standard hematoxylin and eosin(H&E) stain. The specimen was also stained histochemically using alcian blue- periodic acid-Schiff (AB-PAS) staining method at pH 2.5.

The tissue from the paraffin moulds was cut into 4 µm thick sections and placed on aptaca slides. In brief, 4 µm thick tissue sections were deparaffinized in xylene and rehydrated in a graded series of ethanol, and deionized water. Following adequate heat-induced antigen retrieval procedure, the endogenous peroxidase activity was quenched, and the slides were rinsed thoroughly with phosphate- buffered saline. The primary antibodies (Cytokeratin mouse clone AE1/AE3, mouse monoclonal CA125-clone M11, rabbit HER2-polyclonal, mouse monoclonal Estrogen receptor-clone 1D5, mouse monoclonal Progesteron receptor-clone PgR 636, and mouse monoclonal vimentin-clone V9 were applied and the slides were incubated in a water bath for one hour at room temperature. Appropriate positive and negative controls were included in every immunostaining procedure. For the visualization reaction, a standard immunoperoxidase detection system was applied according to the manufacturer's instructions (DAKO LSAB2R system-HRP, Dako, Denmark), and diaminobenzidine was used as a chromogen. Slides were afterward counterstained with Mayer's hematoxylin, dehydrated, and mounted.

Using autostainer Ventana Benchmark GX tissue sections were stained with monoclonal ready to use antibodies GATA3-mouse clone L50-823, GCDFP-15 (EP1582Y) Rabbit Monoclonal Antibody, and p16-CINtec.

Case Report

We present a case of a 71- year- old woman who was diagnosed with breast cancer 20 years ago. The primary diagnosis was invasive lobular breast cancer localized in the left lower lateral quadrant. Soon after primary diagnosis left mastectomy was performed, and the patient received radiation therapy afterwards. During a detailed follow-up 3 years ago she was diagnosed with endometrial cancer and treated accordingly. During the time of the liver biopsy on scintigraphy, multiple tumor deposits were noted.

Initially, the pathohistological examination of a liver biopsy sample showed the focal presence of dense infiltrates of inflammatory and reactive cells predominantly with ovoid and irregular morphology, abundant and occasionally eosinophile granular cytoplasm resembling histiocytes. Histochemical and immunohistochemical staining of these cells showed positivity for toluidine blue while it was negative on AB PAS, CK7, S100, CD 68, and mammaglobin. Additional immunohistochemical analyses weren't possible due to lack of tissue, so a rebiopsy was suggested.

After 4 months, the rebiopsy was performed which showed a marginal area of proliferative connective tissue with individual and small groups of discohesive cells (Figure 1.A, 1.B). The nuclei of these cells were localized marginally and mucins were not observed in the cytoplasm of individual cells (Figure 1.C). Immunohistochemical staining showed CKAE1/E3 (Figure 1.E), GATA3 (Figure 2.A), ER positivity and Ca125 (Figure 2.B, 2.E), GCDFP-15 (Figure 1.D), p16 (Figure 2.F), PR (Figure 2.C), HER2 (Figure 2.D), vimentin negative staining (Figure 1.F).

Immunohistochemistry, micromorphology and clinicopathological correlation were in favor of the liver metastasis of partially hormone dependent breast cancer (strong nuclear expression of estrogen receptors on most tumor cells and negative expression of progesteron receptors as well as HER 2 receptor negative staining).

Discussion

Breast cancer most frequently metastases to the lymph nodes, liver, bones, and lungs. Liver metastases from breast cancer may present asymptomatically or with digestive tract system symptoms like bloating, ascites, palpable abdominal mass, jaundice, or weight loss [1].

Breast cancer has been linked with metachronous bone, endometrial, colon/rectal, connective tissue (sarcoma), leukemia, lung, ovary, or thyroid cancer. The most common second primary cancer in breast cancer patients was that of the opposite breast (23.9%), it was detected on an average of 7 years after the first cancer was detected. The risk of endometrial, thyroid, and ovarian cancer was higher than that of the general population [7].

Studies have shown an increased risk of secondary malignancies in women treated for breast malignancy in connection to adjuvant treatment in certain cases. Higher endometrial cancer incidence has been observed in adjuvant trials of tamoxifen [8]. Endometrial cancer was not entirely caused by tamoxifen but also influenced by parameters like reproductive and genetic factors, obesity, and in some cases unknown factors that could lead to estrogen excess [9]. Women in the postmenopausal period with symptoms that arouse suspicion for endometrial hyperplasia or cancer and who are using tamoxifen should be evaluated in terms of therapy and diagnostics [10].

A majority of breast cancers (more than 90%) are diagnosed as no special type (NST) invasive carcinoma, which is characterized by tubule formation that resembles adenocarcinoma, the main differential diagnosis of metastatic breast carcinoma in the liver is primary intrahepatic cholangiocarcinoma. Histologically, breast carcinoma consists of atypical epithelial cells with lightly monomorphic or highly pleomorphic nuclei arranged in tubular, glandular, cribriform, or solid patterns [11]. In our case tumor cells had a discohesive pattern resembling lobular carcinoma unlike endometrial adenocarcinoma which has a glandular pattern.

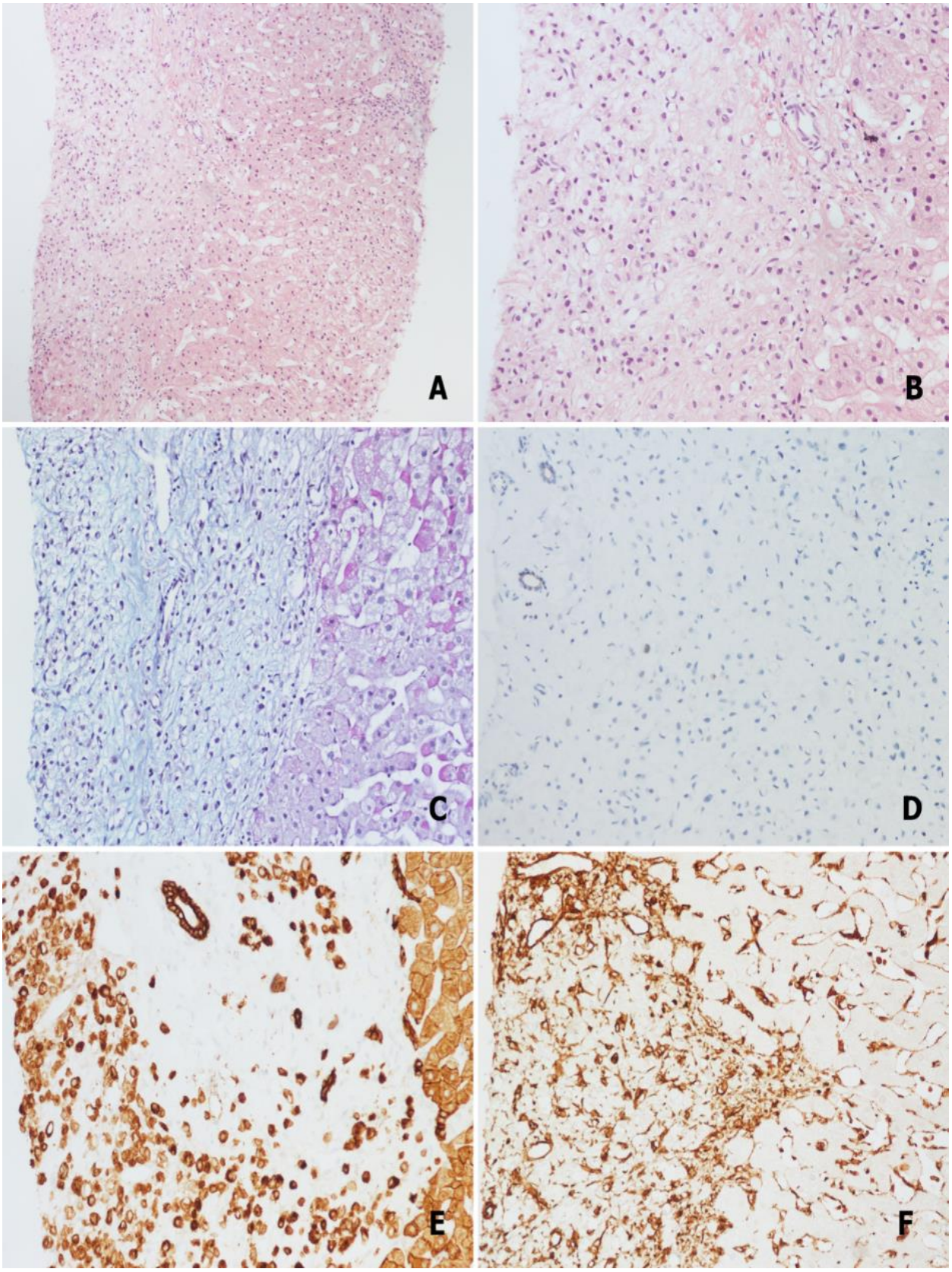


Fig. 1 **A**. Well demarcated metastatic focus on liver needle biopsy (HE x100); **B**. Higher magnification shows a relatively uniform population of cells some with "signet ring" features (HE x200); **C.** AB PAS histochemical stain showed cells without the mucin component (AB PAS x200); **D.** GCDFP-15 negative stain for apocrine differentiation (LSAB x200); **E**. CKAE1/AE3 showed strong, diffuse positivity (LSAB x200); **F**. Vimentin stain was negative in tumor cells (LSAB x200).

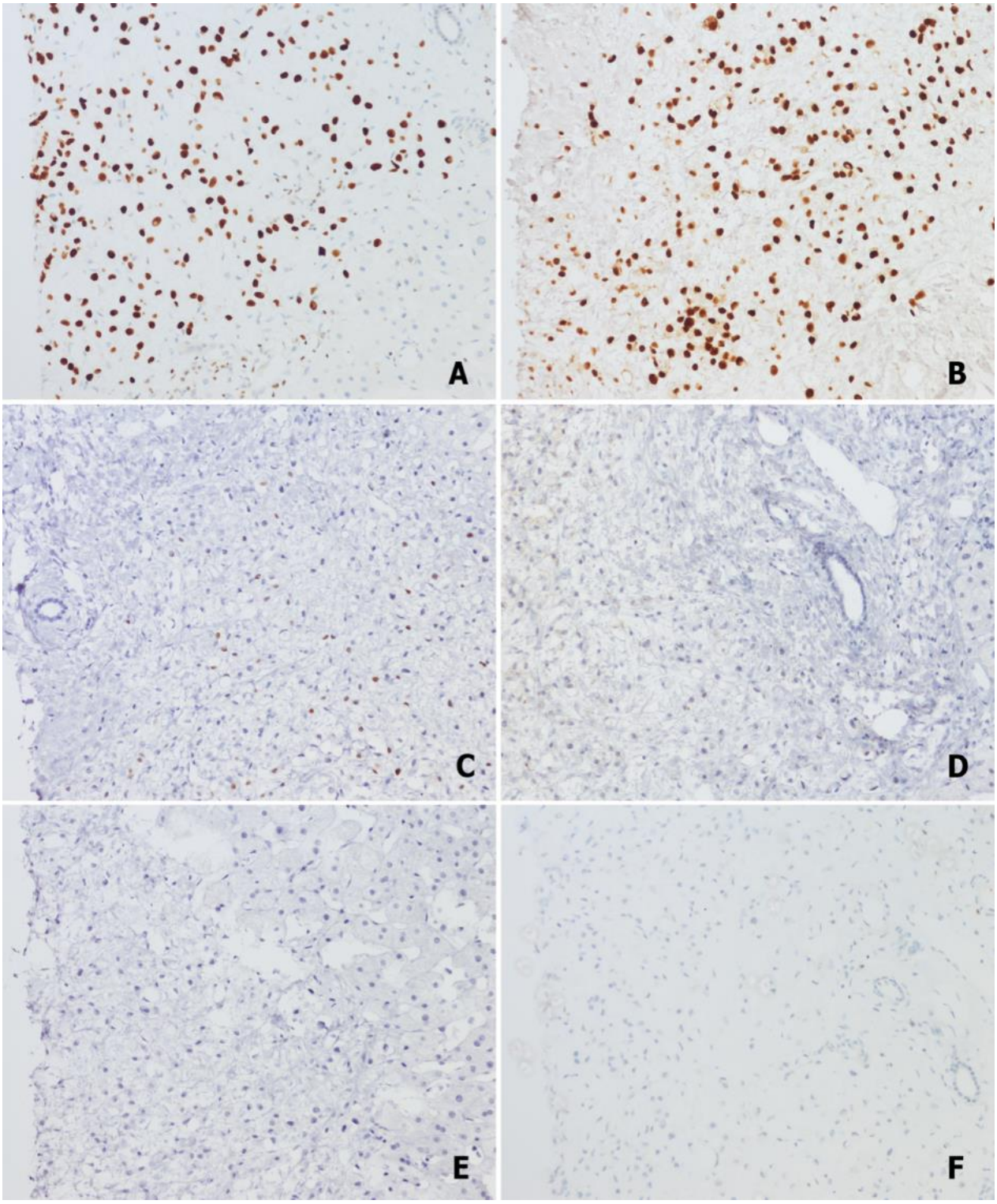


Fig. 2 **A.** GATA3 stain showed strong nuclear positivity in tumor cells (LSAB x 200); **B**. Strong nuclear positivity of ER receptors in all tumor cells (LSAB x200); **C.** Weak nuclear PR receptor positivity in minority of tumor cells (LSAB x200); **D.** Weak membranous stain of HER2 in minority of tumor cells (LSAB x200); **E.** Negative immunoreactivity for CA125 stain (LSAB x200); **F.** Negative immunoreactivity for p16 stain (LSAB x200).

Invasive lobular carcinoma is the second most common histologic type of breast carcinoma after ductal invasive carcinoma (NST type in the majority of cases). Morphologically, in most cases, this histologic type is characterized by infiltration of single-cell file type or discohesive epithelial cells, which consist of monotonous nuclei and inconspicuous nucleoli [11]. In the case of metastasing breast cancer confirmation of metastatic disease and identification of its primary origin is further complicated by the existence of multiple (two or more) primary malignancies, such as synchronous/ metachronous breast cancer and gynecological malignant tumors with an estrogen receptor-positive/cytokeratin 7-positive (ER+/CK7+) and mammaglobin-positive immunohistochemical profile. GATA3 expression has been reported as a useful marker for differential diagnosis between malignancies from other localisations and breast carcinomas (both primary and metastatic) [6].

A comparison of hormone receptors and HER2 receptor immunohistochemical expression between primary and metastatic breast carcinoma in the liver was also useful in establishing the differential diagnosis. In addition, immunohistochemical studies for tissue-specific markers, such as gross cystic disease fluid protein 15 (GCDFP-15) and mammaglobin, will be helpful [11]. Considering the aforementioned differential diagnoses immunohistochemical panel that showed CKAE1/E3, GATA3, ER positivity and CA125, GCDFP-15, p16, PR, HER2, and vimentin negativity was used. Our data (ER+, PR-,HER-2-) matches the result of the study [3] in terms that ER+ or PR+/HER-2- (luminal A) subtypes were predominant in the sites of liver metastases (75.0%).

In our case, tumor cells were partially hormone- sensitive and HER-2 negative.

Other factors besides the hormonal (tamoxifen therapy and other causes of hypestrogenism) might be at play, such as: HER2- negative status, age, body weight, genetic predisposition, etc.

Conclusion

This case report highlights a late manifestation of liver metastasis from breast cancer, 20 years after the primary diagnosis. Considering that our patient had a previous history of breast malignancy, clinical suspicion for metastatic disease was justified. Also, clinical and radiological features may be non-specific, sometimes misleading, so the definitive diagnosis represents a challenge for the pathologist.

Late metastasis may be attributed to its hormonal profile which also resulted in the formation of metachronous endometrial cancer a year before the liver metastasis. Immunohistochemistry was vital for the diagnosis on the liver biopsy, in particular GATA3 positivity and vimentin negative staining, which helped us exclude endometrial cancer metastasis. GATA 3(+)/vimentin(-)panel proved to be superior to GCDFP-15 and mammaglobin in proving the breast origin of the secondary tumor deposit.

Generally, liver metastasis from breast cancer may occur many years after the primary diagnosis,which shows the importance and necessity for long -term follow-up of these patients.

References

1. Yazdani A. Risk factor of liver metastases in breast cancer. Arch Cancer Sci Ther 2019; 3:63–65.
2. Hess KR, Varadhachary GR, Taylor SH, Wei W, Raber MN, Lenzi R, et al. Metastatic patterns in adenocarcinoma. Cancer 2006; 106:1624–1633.
3. Koo JS, Jung W, Jeong J. Metastatic breast cancer shows different immunohistochemical phenotype according to metastatic site. Tumori 2010; 96: 424–432.
4. Sangoi AR, Shrestha B, Yang G, Mego O, Beck AH. The novel marker GATA3 is significantly more sensitive than traditional markers mammaglobin and GCDFP15 for identifying breast cancer in surgical and cytology specimens of metastatic and matched primary tumors. Appl Immunohistochem Mol Morphol 2016; 24:229–237.
5. Takeshita T, Yan Li, Asaoka M, Rashid O, Takabe K. Late recurrence of breast cancer is associated with pro-cancerous immune microenviroment in the primary tumor. Sci Rep 2019; 15:16942.
6. De Lara S, Parris TZ, Ronnerman EW, Helou K, Kovacs A. GATA3 as a putative marker of breast cancer metastasis – a retrospective immunohistochemical study. Breast J 2018; 24: 184–188.
7. Schenker JG, Levinsky R, Ohel G. Multiple primary malignant neoplasms in breast cancer patients in Israel. Cancer 1984; 54:145–150.
8. Kirova YM, Rycke De, Gambotti L, Pierga JY, Asselain B, Fourquet A. Second malignancies after breast cancer: the impact of different treatment modalities. Br J Cancer 2008; 98:870–874.
9. Mellemkjaer L, Friis S, Olsen JH, Scelo G, Hemminki K, Tracey E, et al. Risk of second cancer among women with breast cancer. Int J Cancer 2006; 118:2285–2292.
10. Kim JY, Song HS. Metachronous double primary cancer after treatment of breast cancer. Cancer Res Treat 2015; 47:64–71.
11. Park JH, Kim JH. Pathologic differential diagnosis of metastatic carcinoma in the liver. Clin Mol Hepatol 2019; 25:12–20.

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. 22, N° 2, 2020

Contents

Funded by the
Erasmus+ Programme
of the European Union