MED tube science

30st June Issue 2/2018

Inhibitors of PERK-dependent signaling pathway as a promising therapy for cancer treatment

also

Innovative method of cancer treatment - aerosol chemiotherapy under pressure

How much do we know about healthy tanning without melanoma? A questionnaire study

Non-melanoma skin cancers: perspectives of early diagnosis and therapy

Predictive factors of immediate graft function for living-donor kidney transplant

The correlation between living donor's glomerular filtration rate and early kidney allograft function

Male infertility – causes, diagnosis and management. A review

Evaluation of nutritional status of patients using laboratory tests. A review

Obesity - risk or protection factor? Obesity paradox in cardiovascular diseases

Elicitation and elicitation supported with the phenylpropanoids pathway feeding for the elevation of phenolics content in quinoa sprouts

EDITORIAL BOARD

PUBLISHER

MEDtube Ltd.
Zlota 59 street; 00-120 Warsaw, Poland
Tel.+4822 2402234
Fax. +4822 2224601

US OFFICE

55 Tiemann Place
Suite 29; New York, NY 10027, United States

WEBMASTERING AND IT

Dariusz Calus PhD

DISCLAIMER

Publisher and the Editorial Board accept no liability whatsoever for the consequences of any inaccurate or misleading data, opinions or statements contained in the articles. Every effort is made to ensure that all data and techniques are presented accurately.

CONTACT

science@medtube.net

WEBSITE

http://www.medtube.net/science

MONIKA BIENIASZ MD, PhD

Editor-in-Chief

RAFAL KIESZEK MD, PhD

Editor-in-Chief

ARTUR KWIATKOWSKI MD, PhD

Editor-in-Chief

Dear Colleagues,

We are pleased to present another issue of MEDtube Science - an open access publication distributed online.

MEDtube Science is addressed to all medical professionals - physicians, researchers who are interested in achievements in basic and clinical medical science. MEDtube Science is supposed to satisfy the demand for combining different formats - video and written science in particular. The journal has international scope. It will be published quarterly and exclusively in English. Original articles, reviews, case reports, rapid communications, special articles and letters to the Editor in both basic and clinical medical research are accepted for publication with particular stress on manuscripts enriched with multimedia such as videos or pictures. We have strong confidence that this new project will be quickly recognized in Medline and other medical bases and will become an indexed journal with impact factor.
At the end of our journal you will find information and instructions for authors on how to submit articles.

Kindest Regards,

Monika Bieniasz MD, PhD
Editor-in-Chief

Rafal Kieszek MD, PhD
Editor-in-Chief

Prof. Artur Kwiatkowski MD, PhD
Editor-in-Chief

TABLE OF CONTENTS

Inhibitors of PERK-dependent signaling pathway as a promising therapy for cancer treatment
Wawrzynkiewicz A., Rozpedek W., Pytel D., et al.
PAGES 8-13

Innovative method of cancer treatment - aerosol chemiotherapy under pressure
Kmiecik K., Holynska – Iwan I.
PAGES 14-17

How much do we know about healthy tanning without melanoma? A questionnaire study
Drobek H., Wcislo-Dziadecka D.
PAGES 18-23

Non-melanoma skin cancers: perspectives of early diagnosis and therapy
Kwiatkowski S., Cyranowski S., Osiecka B.J.
PAGES 24-28

Predictive factors of immediate graft function for living-donor kidney transplant
Kwapisz M., Kieszek R., Jedrzejko K., et. al.
PAGES 29-34

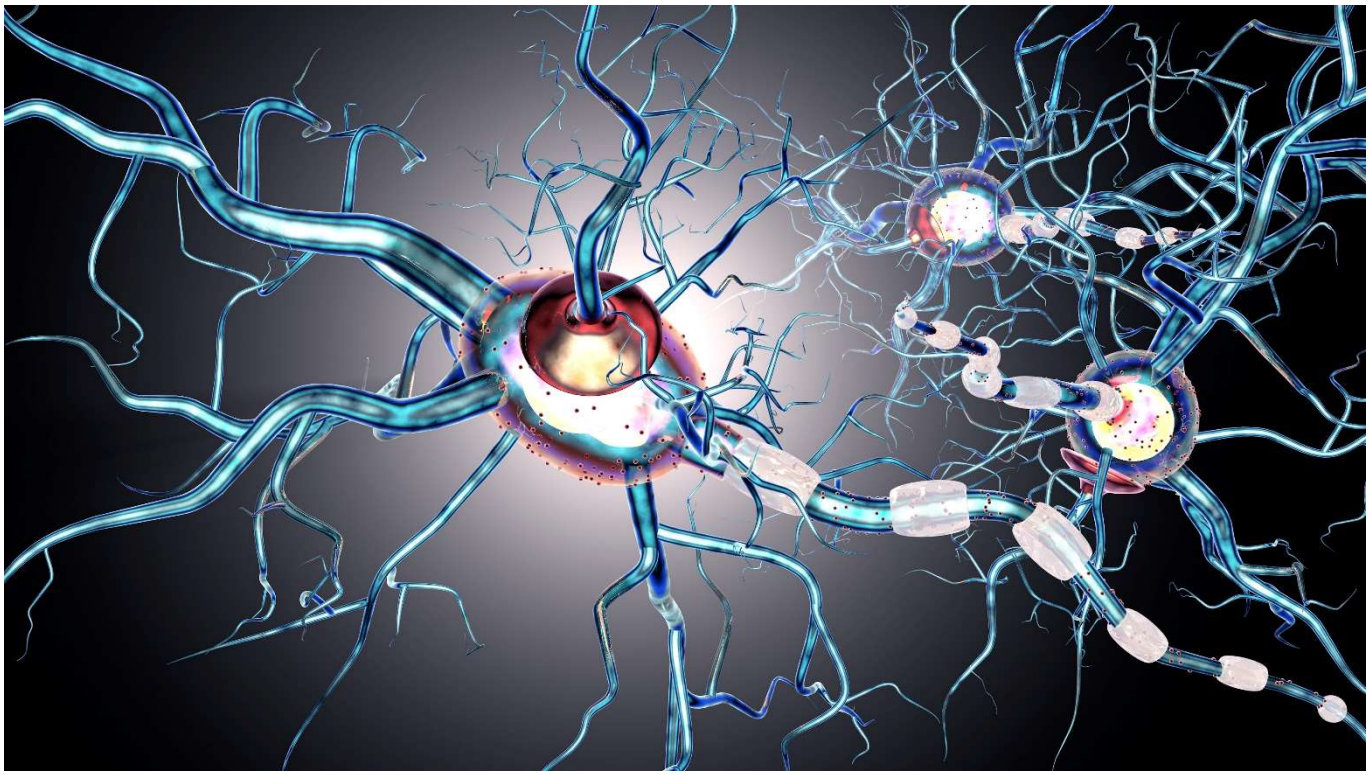
The correlation between living donor's glomerular filtration rate and early kidney allograft function
Kwapisz M., Kieszek R., Bieniasz M., et al.
PAGES 35-40

Male infertility – causes, diagnosis and management. A review
Golik A., Kacperczyk-Bartnik J., Bartnik P.
PAGES 41-46

Evaluation of nutritional status of patients using laboratory tests. A review
Chechla P., Holynska- Iwan I.
PAGES 47-50

Obesity - risk or protection factor? Obesity paradox in cardiovascular diseases
Lesniewska J., Adamek A., Gawrys J., et al.
PAGES 51-54

Elicitation and elicitation supported with the phenylpropanoids pathway feeding for the elevation of phenolics content in quinoa sprouts
Bochnak J., Sikora M., Swieca M.
PAGES 55-60



INHIBITORS OF PERK-DEPENDENT SIGNALING PATHWAY AS A PROMISING THERAPY FOR CANCER TREATMENT

Adam Wawrzynkiewicz[1], Wioletta Rozpedek[1], Dariusz Pytel[2], Adam Dziki[3], Lukasz Dziki[3], Ireneusz Majsterek[1]

1. Department of Clinical Chemistry and Biochemistry, Military-Medical Faculty, Medical University of Lodz, Poland
2. Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Hollings Cancer Center, Charleston, USA
3. Department of General and Colorectal Surgery, Medical University of Lodz, Poland

#Corresponding author: Ireneusz Majsterek, ireneusz.majsterek@umed.lodz.pl, Medical University of Lodz, Hallera 1 St, p. o. box 90-647 Lodz, Poland, phone number: +48 42 639 33 06

RUNNING TITLE	Cancer and PERK Kinase
KEYWORDS	tumour; PERK; eIF2α; Unfolded Protein Response
WORD COUNT	2297
CONFLICT OF INTERESTS	no conflict of interest

ABSTRACT

Currently, cancer constitutes a primary health problem worldwide, since elimination of cancer cells is still inadequate due to insufficient treatment strategy. The newest data has reported that PERK-dependent signaling branches have a significant impact on development and progression of many human diseases including cancer. Hypoxia is the major hallmark of tumour microenvironment, that is strictly associated with rapid cancer progression and induction of metastasis. Low oxygen tension within cancer cells may trigger aggregation of unfolded and misfolded protein within the Endoplasmic Reticulum (ER) lumen and subsequently evoke ER stress condition. As a response to Protein kinase RNA-like endoplasmic reticulum kinase (PERK) oligomerization and trans-autophosphorylation the Unfolded Protein Response (UPR) signaling pathways is activated and regulates their downstream effector such as Eukaryotic Initiation Factor 2 alpha (eIF2α). The eIF2α plays a key role in maintenance of cellular homeostasis via attenuation of global protein synthesis and expression of only selected pro-adaptive genes. Interestingly, UPR has a dual role, since under excessive, long-termed pathological conditions activated PERK contributes to increased translation of CCAAT-enhancer-binding protein homologous protein (CHOP), which may switch on the death signal within cells, that results in apoptotic death of cancer cells. The molecular mechanisms that switch the signal from pro-adaptive into pro-apoptotic is still unknown, but there is an ample of evidence, that utilization of small-molecule PERK inhibitors may lead to the activation of apoptotic cell death and provide an effective elimination of tumour cells. Thereby, potent, highly-selective inhibitors toward PERK may provide a ground-breaking, anti-cancer treatment strategy.

BACKGROUND

Every year more than a million patients are diagnosed with cancer and more than 500,000 patients die of cancer in the United States. Due to above-mentioned statistical data cancer constitutes a major health problem worldwide [1]. In highly developed countries it has become the second cause of human mortality after cardiovascular diseases and it will affect half of men and one third of women during their lifetime [2]. The newest data has reported that, cancer may soon become the main cause of death worldwide due to significant improvement of treatment and prevention of cardiovascular diseases [1, 3].

Tumour is composed of cells, which the characteristic hallmark is uncontrolled proliferation due to their genomic instability and deregulation of cell cycle checkpoint [4, 5]. Moreover, these abnormal cells may rapidly spread in any part of the whole body. We can specify two main types of tumours. The first one is benign tumour, that most commonly grows in a certain location and remains separated from healthy tissues. The latter is known as a malignant tumour. It has an ability to invade surrounding healthy tissues and also to spread from its primary location to other parts of the body, either though lymphatic or vascular system [1]. This process is termed metastasis and it is a major clinical problem due to the fact, that location and time of presenting tumours in other parts of the body is uncertain [6]. There are hundred distinct kinds of human cancer with various behaviour, which vary not only in their areas of body localization, but also on the molecular level including presented mutations and abrogation of numerous signaling pathways, that are directly responsible for maintenance of cellular homeostasis. Due to numerous types of cancer and their unique combinations of genetic alterations elimination of cancer cells by currently used anti-tumour drugs is still inadequate [1, 2].

Cancer development is strongly connected with uncontrolled growth of cells as well as inactivation of apoptosis. The abnormal proliferation of tumour mass contribute to structural disturbances and impaired angiogenesis resulting in hypoxic state. That leads to the occurrence of the ER (Endoplasmic Reticulum) stress conditions, which may directly activate the UPR (Unfolded Protein Response) signaling branches. The subsequent phosphorylation of eIF2α (Eukaryotic Initiation Factor 2 alpha), by activated PERK (Protein kinase R (PKR)-like Endoplasmic Reticulum kinase) triggers rapid downregulation of global protein synthesis and translation of only preferential genes encoding proteins, that play a vital role during adaptation of tumour cells to hypoxic conditions. On the contrary, the long-termed activation of the UPR may lead to the initiation of the apoptotic cell death. There is an ample of evidence, that this dichotomic pathway plays a key role in pathogenesis of many human disease entities such as: atherosclerosis, renal disease, type 2 diabetes, neurodegenerative disease and cancer. Nowadays, development of treatment strategies, which may evoke a pharmacological switch of the UPR signaling pathways from the pro-adaptive into pro-apoptotic has become the main target of numerous studies. Detailed knowledge in this area may contribute to the development of a new, innovative treatment methods, that may overcome current problems of ineffective therapies against various human diseases including cancer [7-9].

ER STRESS AND THE UNFOLDED PROTEIN RESPONSE

The ER is a dynamic membrane system of tubules and sacs. It is made up of different domains such as nuclear envelope (NE), smooth and rough ER as well as the parts contacting with other organelles. The ER plays a key role in synthesis of phospholipids as well as a secretory and membrane proteins [10]. Proteins folding and their subsequent modifications are strictly controlled inside the ER lumen. Misfolded or unfolded proteins are targeted for degradation by the ubiquitin-proteasome pathway [11]. Balance between synthesis and degradation of abrogated proteins results in cellular homeostasis, that may be disturbed by a range of environmental and genetic factors. Hence, redox and calcium homeostasis as well as molecular signaling transduction depend on the proper functioning of the ER [12]. Not only redox, but also intracellular calcium concentration and proper release of calcium ions from the ER lumen are vital in ER-mitochondrion interactions and play the pivotal role during controlling of the cell death by apoptosis [13].

It has been reported, that a range of pathological conditions such as viral infections, toxins, inflammatory cytokines, hypoxia, nutrient deficiency and increased cell proliferation directly evoke perturbation in the ER homeostasis that results in the aggregation of unfolded and misfolded proteins within the ER lumen, and subsequently triggers activation of the UPR branches, which play an important role in restoration of the cellular homeostasis [14]. The UPR is associated with the activation of three ER transmembrane receptors: Activating transcription factor 6 (ATF6), Inositol requiring enzyme 1 (IRE1) and Protein kinase RNA PKR)-like ER kinase (PERK) [15, 16]. ATF6 is classified as a II transmembrane protein kinase, that upon ER stress conditions is translocated from the ER towards the Golgi Apparatus (AG), where undergoes a proteolytic processing to release ATF6 cytosolic fragment that moves towards the nucleus, where plays a key role, as a transcription factor, for various pro-adaptive UPR genes [17]. Adversely to ATF6, IRE1 and PERK belong to the I transmembrane serine/threonine protein kinases and they are activated via oligomerization and trans-autophosphorylation, that lead to the activation of PERK-an IRE1-dependent signaling pathways [18]. As the first response to the ER stress conditions the pro-adaptive branches of the UPR are activated. That inhibits the global protein synthesis within cells, which allows for the reduction of the new protein load in the lumen of the ER. On the other hand, excessive, long-termed ER stress conditions may change the UPR signal towards the pro-apoptotic pathway. The molecular mechanism, that is solely responsible for switch of the UPR signaling

pathways from the pro-adaptive into pro-apoptotic pathway is still unknown [19-21].

PRO-ADAPTATIVE RESPONSE OF THE UPR SIGNALING PATHWAYS IN TUMOR PROGRESSION

Hypoxia, a major hallmark of tumour microenvironment, is strictly associated with rapid cancer progression as well as induction of metastasis. Low oxygen tension within cancer cells may trigger aggregation of unfolded and misfolded protein within the Endoplasmic Reticulum (ER) lumen and subsequently evoke ER stress condition [22]. PERK, under physiological conditions, is associated with the heavy chain binding protein (BiP) also known as glucose regulated protein 78 (GRP78) chaperones. When the unfolded and misfolded proteins accumulate in the ER lumen the BiP/GRP78 are released from the domains of the ER stress receptors. That subsequently leads to oligomerization and trans-autophosphorylation of PERK [23]. Activated PERK phosphorylate its direct substrate such as *α* subunit of the eIF2 in Ser51. As a result, under pathological, hypoxic conditions, the global protein synthesis is significantly arrested [24]. Moreover, translation of only selected proteins like ATF4 (Activating transcription factor 4) is markedly enhanced, that upregulates a board range of cytoprotective genes [25]. Moreover, due to inhibition of global protein synthesis, phosphorylated eIF2α suppresses translation of *cyclin D1*, which causes cell cycle arrest in a G1 phase, thus inhibition of cell proliferation. Thereby, the eIF2α is often referred as a master regulator of cell adaptation to ER stress conditions [26].

PERK-DEPENDENT PRO-APOPTOTIC SIGNALING PATHWYS

During long-termed ER stress conditions, the ATF4, as a transcription factor, promotes expression of *DDIT3* genes encoding protein CCAAT-enhancer-binding protein homologous protein (CHOP), which is commonly known as a major initiator of the pro-apoptotic cascade [27]. Increased expression of CHOP downregulates expression of the anti-apoptotic *Bcl-2* genes and inversely, upregulates expression of genes encoding the pro-apoptotic BH3 domain-only proteins [28]. Additionally, CHOP disrupts the redox homeostasis within the cell, which rapidly evokes cell death via apoptosis [29]. CHOP also acts as a transcription factor of the genes encoding the Growth arrest and DNA damage-inducible protein (GADD34), which promotes dephosphorylation of the eIF2α. Above-mentioned event resumes the global protein translation in stressed cells leading to increased ER proteins load and further promotes ER stress and pro-apoptotic signaling axis of the UPR [30, 31]. The ER oxidoreductin 1α (ERO1α) is the ER membrane enzyme, which requires a molecular oxygen to promote formation of disulphide bonds in newly translated proteins. Expression of the *ERO1α* is enhanced by CHOP under prolonged ER stress conditions, that may lead to excessive production of H_2O_2, resulting in hyperoxidizing environment and apoptotic cell death [32, 33]. High concentration of Reactive oxygen species (ROS) in the ER lumen activates the ER calcium release channel inositol-1, 4, 5-thriphosphate receptor 1 (IP3R1) resulting in calcium leakage into the cell cytoplasm from the ER lumen. Then, calcium/calmodulin-dependent protein kinase II (CaMKII) is activated, which triggers activation of pro-apoptotic signaling branches of the UPR. Activation of the CHOP-ERO1α-IP3R1-CaMKII-dependent signaling pathway leads to induction of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) subunit 2 (NOX2), that subsequently promotes generation of ROS and expression of *DDIT3* genes. Hence, that positive feedback loop is created by ROS, which enhances CaMKII activation and, as a result, translation of pro-apoptotic CHOP protein [20, 34]. The newest data has suggested, that CHOP-mediated cell apoptosis may also be triggered by suppression of cell cycle regulator protein 21 (p21/WAF1). It has a pivotal role in inhibition of the cell cycle in a G1 phase after the interaction with the cyclin-dependant kinase (Cdk) [15, 35]. Expression of WAF1 is closely correlated with tumour suppressor protein p53 (p53). Under stress conditions p53 upregulates WAF1, which results in cell cycle arrest in a G1 phase as a pro-adaptive cellular response [36]. It has been reported that possible crosstalk between CHOP and p21 may be the explanation of transition from pro-adaptive into pro-apoptotic pathway of the UPR under stress conditions. The p21 is controlled by CHOP and while the stress conditions are low to moderate the regulation is stimulated. On the other hand, during chronic or acute stress conditions, the regulation between CHOP and p21 may be suppressed, which leads to apoptotic cell death. Hence, above-mentioned data indicate that the shift of UPR from pro-adaptive into pro-apoptotic pathway in tumour disease may result from the p21 and PERK/eIF2α/ATF4/CHOP pathway interaction [37, 38].

SMALL-MOLECULE PERK INHIBITORS AS A NOVEL TREATMENT STRATEGY

Recent data has confirmed that inhibition of the UPR signaling pathways, activated upon ER stress conditions, may constitute a novel, ground-breaking treatment strategy against various human diseases. There is an ample of evidence, that pathogenesis of several neurodegenerative diseases including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), prion disease and ischaemia lies on the molecular level. Thus, excessive accumulation of misfolded and unfolded proteins in ER lumen triggers disruption of the PERK-dependent signaling pathways [39]. It has been confirmed that deposition of senile plaques and neurofibrillary tangles among the neurons within tissue brain is closely connected not only with genetic factors, but also with overactivation of the ER-stress dependant signaling pathways [40]. Aggregation of amyloid beta (Aβ) plaques in tissue brain activates the PERK-dependant signaling pathways, which is solely implicated in AD development and progression. Interestingly, it has been reported, that phosphorylated eIF2α is present in high levels in brain cells of AD patients leading to the attenuation of global protein synthesis and

promotion of ATF4 synthesis. During excessive, prolonged ER stress conditions ATF4 triggers upregulation of the expression of genes encoding pro-apoptotic CHOP. That results in apoptotic cell death of neuronal cells, thus decreased mass of tissue brain in AD patients. There is an ample of evidence, that inhibition of PERK may decrease the level of phosphorylated eIF2α, which may significantly slow down or completely stop progression of β-amyloidogenesis. Due to the fact, that nowadays only symptomatic treatment against neurodegenerative diseases is available, use of highly-specific, small-molecule inhibitors of PERK may provide a novel, promising therapy against neurodegenerative diseases [41-43].

Moreover, PERK-dependent signaling pathways play a key role in cancer development and progression [20]. The characteristic hallmark of cells in neoplastic disease, due to disturbed angiogenesis and structural malformation, is low oxygen and glucose environment. That evokes activation of the pro-adaptive UPR signaling networks, which enable rapid proliferation of tumour cells [44, 45]. It has been reported that pro-adaptive branches of the UPR has been activated in human glioblastomas, cervical carcinomas, breast and lung cancer [46]. The dichotomic role of PERK-dependent signaling pathway is still poorly understood, but the newest data has reported, that use of small-molecule PERK inhibitors may provide a new, promising anti-cancer strategy, which may evoke the molecular switch of the UPR from the pro-adaptive into pro-apoptotic signaling pathways [47].

GSK2606414 was the first PERK inhibitor synthetized by GlaxoSmithKline. Available data reported that it attenuates subcutaneous pancreatic human tumour xenograft growth in mice [48]. It also attenuated the protein synthesis in prion-infected mice preventing further neurodegeneration [49]. Thereby, it can be concluded, that highly-selective, small-molecule inhibitors of PERK are worthy of further analysis in *in vitro* and *in vivo* models before the clinical trials to gather a detailed knowledge about their impact on whole human organism and potential side effects [50].

CONCLUSIONS

Low oxygen tension within tumour cells is strictly associated with their structurally and functionally abnormal vessels, that directly evokes rapid cancer progression and metastasis. There is an ample of evidence, that hypoxic tumours, compared to better-oxygenated tumours, are characterized by higher resistance to currently used anti-cancer treatment strategies and thereby with poorer overall prognosis. The newest data has reported that tumorigenesis is closely associated with significant perturbation on the molecular level within cells, since hypoxic conditions activate ER stress, and subsequently the UPR signaling pathways, that has a dual pro-adaptive or pro-apoptotic role, which depends on the severity and time of duration of pathological conditions. Currently used anti-cancer therapy evokes numerous side effects in patients, since it target not only cancer, but also normal, healthy cells. Thus, advancing molecular insight into the mechanisms, that are solely responsible for switch of the UPR signaling pathways into its pro-apoptotic branch as well as additional investigations of highly-selective PERK inhibitors are necessary for development an effective, promising anti-cancer therapeutic agents.

ACKNOWLEDGEMENTS

This work was supported by grant PRELUDIUM no. 2015/19/N/NZ3/00055 from the Polish National Science Centre, grant OPUS no. 2016/23/B/NZ5/02630 from the Polish National Science Centre and by grant of Medical University of Lodz, Poland no. 564/1-000-00/564-20-031.

CITE THIS AS

MEDtube Science June, 2018, Vol. VI (2), 8 – 13

ABBREVIATIONS

Aβ – amyloid beta
AD – Alzheimer's disease
ATF4 – activating transcription factor 4
ATF6 – activating transcription factor 6
BiP – binding protein
CaMKII – calcium/calmodulin-dependent protein kinase II
Cdk – cyclin-dependant kinase
CHOP – CCAAT- enhancer-binding protein homologous protein
ER – endoplasmic reticulum
ERO1α – ER oxidoreductin 1alpha
eIF2α – eukaryotic initiation factor 2 alpha
GRP78 – glucose regulated protein 78
GADD34 – growth arrest and DNA damage-inducible protein
HD – Huntington's disease
IP3R1 – inositol-1, 4, 5-thriphosphate receptor 1
IRE1 – inositol requiring enzyme 1
NE – nuclear envelope
NOX2 – nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) subunit 2
PD – Parkinson's disease
PERK – protein kinase RNA-like endoplasmic reticulum kinase
ROS – reactive oxygen species
UPR – unfolded protein response

REFERENCES

1. Cooper GM. The cell : a molecular approach. ASM Press Sinauer Associates 2000: xxiv, 689 p.
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67(1): 7-30.
3. Sudhakar A. History of Cancer, Ancient and Modern Treatment Methods. J Cancer Sci Ther 2009; 1(2): 1-4.
4. Bower JJ, Vance LD, Psioda M, et al. Patterns of cell cycle checkpoint deregulation associated with intrinsic molecular subtypes of human breast cancer cells. NPJ Breast Cancer 2017; 3: 9.

5. Lopez-Saez JF, de la Torre C, Pincheira J, Gimenez-Martin G. Cell proliferation and cancer. Histol Histopathol 1998; 13(4): 1197-1214.
6. Schirrmacher V, Schwartz-Albiez R. Cancer metastasis : molecular and cellular biology, host, immune response, and perspectives for treatment. Springer-Verlag 1989: xiv, 268 p., 264 p. of plates.
7. Ma Y, Hendershot LM. The role of the unfolded protein response in tumor development: friend or foe? Nat Rev Cancer 2004; 4(12): 966-977.
8. Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, et al. PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. Oncogene 2010; 29(27): 3881-3895.
9. Wang WA, Groenendyk J, Michalak M. Endoplasmic reticulum stress associated responses in cancer. Biochim Biophys Acta 2014; 1843(10): 2143-2149.
10. Zhang K, Kaufman RJ. Protein folding in the endoplasmic reticulum and the unfolded protein response. Handb Exp Pharmacol 2006(172): 69-91.
11. Lecker SH, Goldberg AL, Mitch WE. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol 2006; 17(7): 1807-1819.
12. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 2016; 529(7586): 326-335.
13. Gorlach A, Klappa P, Kietzmann T. The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. Antioxid Redox Signal 2006; 8(9-10): 1391-1418.
14. Ozcan L, Tabas I. Role of endoplasmic reticulum stress in metabolic disease and other disorders. Annu Rev Med 2012; 63: 317-328.
15. Mkrtchian S. Targeting unfolded protein response in cancer and diabetes. Endocr Relat Cancer 2015; 22(3): C1-4.
16. Teske BF, Wek SA, Bunpo P, et al. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Mol Biol Cell 2011; 22(22): 4390-4405.
17. Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P. Endoplasmic reticulum stress sensing in the unfolded protein response. Cold Spring Harb Perspect Biol 2013; 5(3): a013169.
18. Liu CY, Schroder M, Kaufman RJ. Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum. J Biol Chem 2000; 275(32): 24881-24885.
19. Bravo R, Parra V, Gatica D, et al. Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration. Int Rev Cell Mol Biol 2013; 301: 215-290.
20. Vandewynckel YP, Laukens D, Geerts A, et al. The paradox of the unfolded protein response in cancer. Anticancer Res 2013; 33(11): 4683-4694.
21. Lai E, Teodoro T, Volchuk A. Endoplasmic reticulum stress: signaling the unfolded protein response. Physiology (Bethesda) 2007; 22: 193-201.
22. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011; 11(6): 393-410.
23. Schwab M. Encyclopedia of cancer. Springer 2011.
24. Rajesh K, Krishnamoorthy J, Kazimierczak U, et al. Phosphorylation of the translation initiation factor eIF2alpha at serine 51 determines the cell fate decisions of Akt in response to oxidative stress. Cell Death Dis 2015; 6: e1591.
25. Blais JD, Filipenko V, Bi M, et al. Activating transcription factor 4 is translationally regulated by hypoxic stress. Mol Cell Biol 2004; 24(17): 7469-7482.
26. Raven JF, Baltzis D, Wang S, et al. PKR and PKR-like endoplasmic reticulum kinase induce the proteasome-dependent degradation of cyclin D1 via a mechanism requiring eukaryotic initiation factor 2alpha phosphorylation. J Biol Chem 2008; 283(6): 3097-3108.
27. Nishitoh H. CHOP is a multifunctional transcription factor in the ER stress response. J Biochem 2012; 151(3): 217-219.
28. Shamas-Din A, Brahmbhatt H, Leber B, Andrews DW. BH3-only proteins: Orchestrators of apoptosis. Biochim Biophys Acta 2011; 1813(4): 508-520.
29. McCullough KD, Martindale JL, Klotz LO, Aw TY, Holbrook NJ. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. Mol Cell Biol 2001; 21(4): 1249-1259.
30. Feldman DE, Chauhan V, Koong AC. The unfolded protein response: a novel component of the hypoxic stress response in tumors. Mol Cancer Res 2005; 3(11): 597-605.
31. Brush MH, Weiser DC, Shenolikar S. Growth arrest and DNA damage-inducible protein GADD34 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. Mol Cell Biol 2003; 23(4): 1292-1303.
32. Sevier CS, Kaiser CA. Ero1 and redox homeostasis in the endoplasmic reticulum. Biochim Biophys Acta 2008; 1783(4): 549-556.
33. Simmen T, Lynes EM, Gesson K, Thomas G. Oxidative protein folding in the endoplasmic reticulum: tight links to the mitochondria-associated membrane (MAM). Biochim Biophys Acta 2010; 1798(8): 1465-1473.
34. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Biol 2011; 13(3): 184-190.
35. Harper JW, Elledge SJ, Keyomarsi K, et al. Inhibition of cyclin-dependent kinases by p21. Mol Biol Cell 1995; 6(4): 387-400.
36. Jeong JH, Kang SS, Park KK, Chang HW, Magae J, Chang YC. p53-independent induction of G1 arrest and p21WAF1/CIP1 expression by ascofuranone, an isoprenoid antibiotic, through downregulation of c-Myc. Mol Cancer Ther 2010; 9(7): 2102-2113.
37. Mihailidou C, Papazian I, Papavassiliou AG, Kiaris H. CHOP-dependent regulation of p21/waf1 during ER stress. Cell Physiol Biochem 2010; 25(6): 761-766.

38. Mihailidou C, Chatzistamou I, Papavassiliou AG, Kiaris H. Improvement of chemotherapeutic drug efficacy by endoplasmic reticulum stress. Endocr Relat Cancer 2015; 22(2): 229-238.
39. Rozpedek W, Markiewicz L, Diehl JA, Pytel D, Majsterek I. Unfolded Protein Response and PERK Kinase as a New Therapeutic Target in the Pathogenesis of Alzheimer's Disease. Curr Med Chem 2015; 22(27): 3169-3184.
40. Schonthal AH. Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. Scientifica (Cairo) 2012; 2012: 857516.
41. O'Brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 2011; 34: 185-204.
42. Irvine GB, El-Agnaf OM, Shankar GM, Walsh DM. Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's diseases. Mol Med 2008; 14(7-8): 451-464.
43. Lee DY, Lee KS, Lee HJ, et al. Activation of PERK signaling attenuates Abeta-mediated ER stress. PLoS One 2010; 5(5): e10489.
44. Yadav RK, Chae SW, Kim HR, Chae HJ. Endoplasmic reticulum stress and cancer. J Cancer Prev 2014; 19(2): 75-88.
45. Lee AS, Hendershot LM. ER stress and cancer. Cancer Biol Ther 2006; 5(7): 721-722.
46. Atkins C, Liu Q, Minthorn E, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res 2013; 73(6): 1993-2002.
47. Wang G, Yang ZQ, Zhang K. Endoplasmic reticulum stress response in cancer: molecular mechanism and therapeutic potential. Am J Transl Res 2010; 2(1): 65-74.
48. Axten JM, Medina JR, Feng Y, et al. Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-p yrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J Med Chem 2012; 55(16): 7193-7207.
49. Moreno JA, Halliday M, Molloy C, et al. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. Sci Transl Med 2013; 5(206): 206ra138.
50. Rozpedek W, Nowak A, Pytel D, Diehl JA, Majsterek I. Molecular Basis of Human Diseases and Targeted Therapy Based on Small-Molecule Inhibitors of ER Stress-Induced Signaling Pathways. Curr Mol Med 2017; 17(2): 118-132.

INNOVATIVE METHOD OF CANCER TREATMENT - AEROSOL CHEMIOTHERAPY UNDER PRESSURE

Katarzyna Kmiecik[1], Iga Holynska–Iwan[2]

1. Student Scientific Group at the Department of Pathobiochemistry and Clinical Chemistry, Faculty of Pharmacy, Collegium Medicum. Ludwika Rydygiera in Bydgoszcz. Nicolaus Copernicus University in Torun, Poland.
2. Department of Pathobiochemistry and Clinical Chemistry, Faculty of Pharmacy, Collegium Medicum. Ludwika Rydygiera in Bydgoszcz. Nicolaus Copernicus University in Torun, Poland.

#Corresponding author: Katarzyna Kmiecik, e-mail: kasia6.k@interia.pl, Department of Pathobiochemistry and Clinical Chemistry, Antoni Jurasz University Hospital No. 1 in Bydgoszcz, M. Curie Sklodowskiej 9 St, p. o. box 85-094 Bydgoszcz, phone number: +48 880 735 563

RUNNING TITLE	Aerosol chemiotherapy under pressure
KEYWORDS	PIPAC; cancer; chemotherapy
WORD COUNT	1403
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

The incidence and death rate due to cancer is constantly increasing. Cancer affects not only the elderly, as one of the effects of an aging population, but also of young people, due to the increased rate of metabolism or genetic predisposition to malignancy of the cancer. The basic method of treatment of malignant tumor is chemotherapy, used in several ways, including intravenously, orally and intraperitoneally. The new method of delivering drugs directly to the abdominal cavity, producing a pressure gradient and spraying the chemotherapeutic directly into neoplastic changes is PIPAC (Pressurized Intraperitoneal Aerosol Chemotherapy), i.e. Intraperitoneal Vacuum Chemotherapy Aerosol. The method was developed by Professor Marc Reymond from the University Hospital in Tübingen. Its aim is to improve the quality and extend the lives of patients in a palliative state. Therapy is used in patients with stomach, ovarian and large intestine cancer, it also aims to induce regression of metastases to the peritoneum. The aim of the work is to present innovative PIPAC therapy in the context of application, course and assessment of the effectiveness of the method based on the available scientific literature.

BACKGROUND

Malignant neoplasms in Poland are the second most common cause of deaths - as a result of these diseases, about 96,000 Poles die annually, which accounts for 25% of all deaths. Colorectal cancer and stomach cancer are among the five most common malignancies in the world [17]. From a clinical point of view, their tendency is to easily spread and metastasize to the peritoneum. This condition is referred to as peritoneal cancer (PC). The average survival time for peritoneal carcinoma is 6-12 months [18]. The main reason for this is the poor penetration of chemotherapeutics used intravenously, which is related to the existence of the blood-peritoneal barrier. PIPAC or Pressure Intraperitoneal Chemotherapy in Aerosol is an innovative combination of laparoscopy techniques with a modern method of drug delivery in the form of properly dispersed drops of aerosol under pressure, inducing regression of tumor metastases to the peritoneum. It is a technique that supports treatment with both intravenous chemotherapeutics and may help support surgery. Peritoneal cancer is a significant clinical problem in contemporary oncology and oncological surgery. The term refers to the phenomenon of metastasis and uncontrolled, rapid tumor growth within the peritoneal cavity, most often cancer: ovary, stomach, large intestine, pancreas, gall bladder and several other tumor subtypes, including primary ones [5,13]. In colon cancer, metastases to the peritoneum can be observed in approximately 15% of cases [15]. Metastasis to the peritoneum is associated with poor prognosis. The pioneering treatment, for the first time in the world, was made in 2011 in Germany by a team led by Prof. Marc Reymond. This technique gives clinical hope for lengthening and increasing the comfort of life for patients who were still low to 30/30 years ago with no major therapeutic options. 800 treatments have been performed all over the world. Poland is in the group of 15 countries that are leaders in the dissemination of the PIPAC method. Currently, the procedure is performed in 20 countries, mainly Western Europe, and for the first time in this part of Europe and for the first time in Poland, it was performed on May 10 at the Oncology Center named after Prof. F. Łukaszczyka in Bydgoszcz at the Department of Cancer Surgery CM UMK a team of doctors composed of: Professor Wojciech Zegarski, PhD MD and Maciej Nowacki, PhD.

CANCER TREATMENT METHODS AND PIPAC

PIPAC treatment is used as a neoadjuvant therapy before CRS and HIPEC. Neoadjuvant treatment - treatment preceding radical surgery or radical radiotherapy. It aims to destroy the alleged metastases or to reduce the tumor mass. HIPEC is the procedure for intra-peritoneal perfusion chemotherapy in conditions of elevated temperature along with the surgical removal of the affected tissue, while the basic idea of CRS is the removal of all macroscopically visible tumor foci [6, 14]. Unfortunately, the CRS and HIPEC procedure as aggressive surgical methods, burdened with the possibility of a wide range of perioperative complications are not the methods of choice in all patients diagnosed with an advanced degree of neoplastic changes within the peritoneum, especially in the elderly, in a poor clinical condition not allowing for full surgical intervention [5, 7, 14]. In addition, both methods carry moderate improvement and often not the best prognosis. Although systemic chemotherapy has made tremendous improvements in the treatment of systemic metastases (especially of the liver and lungs), it appears to be much less effective in peritoneal dissemination, mainly due to pharmacokinetic limitations, probably due to poor peritoneal vasoconstriction that hinders normal drug distribution [6,7]. In a systematic review of literature on complications resulting from the use of both methods, it has been demonstrated that in large specialist centers, the prevalence is 12-52%, while the mortality rate is 0.9-5.8% [16]. In contrast, commonly known tumor markers are ineffective in diagnosing and evaluating response to treatment [3]. PIPAC can be used to improve the results of CRS and HIPEC, to select patients with chemosensitive tumors, to extend the indications of CRS and HIPEC and to reduce the scope of cytoreductive surgery [5]. Considering the pharmacokinetics of HIPEC restriction, PIPAC may be an alternative to patients who are not eligible for radical treatment with HIPEC [7, 9].

PREPARATION OF THE PATIENT

The patient who will be subjected to the PIPAC method of delivering the drug first of all goes through many consultations. Clinical oncologists with surgeons are qualified for this procedure. He comes to the clinic usually one two days before surgery. All basic research and supplementary examinations are performed. The patient is admitted to the surgery clinic. The feeding regime is maintained. Then it is anaesthetized as for a standard surgery. After the surgery, the patient can return home very quickly. Staying in the hospital after applying the procedure is about 3 days. The treatment can also go several times at intervals of six to two weeks, which is a plus of this method [10].

THE COURSE OF THE PROCEDURE

The procedure is performed under general anesthesia in standard operating room conditions. Initially, several biopsies are taken from cancer nodes for further histopathological examination. As a rule, two trocars are used, which, through appropriately made small cuts, with a diameter of 5-12 mm within the abdominal wall, allow to create conditions for insufflation and access to the camera and device spraying therapeutic substances so-called. "Nebulizer" (Figure 1). Then for 30 minutes in the abdominal cavity spray is sprayed under pressure in the process of so-called "Snow storm" in the CAWS system - a closed aerosol discharge system [5, 8, 11]. Spraying causes the drug to reach all corners of the abdomen and directly go to those tissues that are covered with cancer [5]. The pressure in the range of ~ 1500 kPa increases local penetration of tissues and allows to obtain high concentrations of gas molecules within the tumor. Currently recommended doses of aspirated chemotherapeutics are: oxaliplatin, 92 mg / m^2 of body surface area, in colorectal cancer and doxorubicin 1.5 mg

/ m^2, cisplatin 7.5 mg / m^2 in case of cancer with other ethiology [8, 12].

After completing the drug delivery process, the gas from the abdominal cavity is completely aspirated (removed) and the skin incisions for the trocars are closed using standard layered sutures. The entire procedure usually takes about 90 minutes. The PIPAC procedure is also possible to use it as a few-minute supply of medicine between treatments, i.e. supply in the diagram.

THE ADVANTAGES OF THE METHOD

In many scientific studies, a positive therapeutic response and a slowdown in the progression and growth of tumor cells has been demonstrated, which may have a significant impact on the improvement of survival and quality of life [11]. This method, due to its minimally invasive nature, is associated with a much smaller number of complications and therapeutic complications associated, for example, with infections, the possibility of a hernia or adhesions, and intraperitoneal supply reduces systemic toxicity. An important aspect of the interaction is better delivery of anti-cancer compounds and control of peritoneal cancer as part of fully personalized and targeted therapy. It is a technique that supports treatment with both intravenous chemotherapeutics and may allow supportive surgery [2, 4, 9].

POSSIBLE COMPLICATIONS AND SIDE EFFECTS

Most adverse events and perioperative complications do not differ from those associated with standard laparoscopic procedures in oncological surgery. The most common complications, about 50%, include: abdominal pain, vomiting and fever. For relatively rare (1%) intestinal injuries caused by attaching trocars, injuries associated with biopsy retrieval. In contrast, very rare complications (<1%) are: an undesirable skin reaction, metastases within the abdominal integuments, i.e. injection sites. It may also lead to a hernia, intestinal obstruction, hematomas, postoperative bleeding and inflammation of the bladder [8].

CONCLUSION

Although the PIPAC technique is an innovative solution, it should be emphasized that it is not an experimental treatment. PIPAC can have a significant advantage over existing chemotherapy techniques that are painful and debilitating and associated with long-term stay in the clinic and high risk of adverse events. Low-dose intraperitoneal chemotherapy in aerosol (PIPAC) with cisplatin and doxorubicin is a form of intra-abdominal chemotherapy that can be used repeatedly and potentially prevents systemic side effects of chemotherapy. A number of studies are currently underway that bring very positive results.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 14 – 17

ABBREVIATIONS

CRS – Cytoreductive Surgery
HIPEC – Hyperthermic Intraperitoneal Chemotherapy
PIPAC – Pressurized Intraperitoneal Aerosol Chemotherapy

REFERENCES

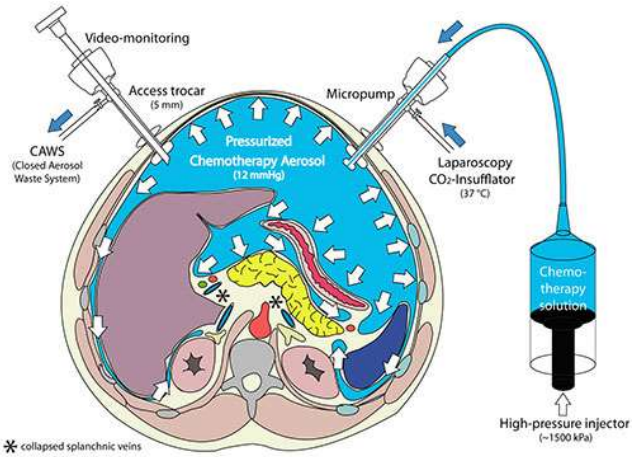
1. Lemmens VE, Klaver YL Verwaal VJ. Predictors and survival of synchronous peritoneal carcinomatosis of colorectal origin: A population-based study. Int J Cancer 2011; 128.
1. Chua TC Yan TD, MorrisDL. Outcomes of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for peritoneal mesothelioma: The Australian experience. J Surg Oncol 2009; 99.
2. Elias D, Honore C, Dumont F. Results of systematic second-look surgery plus HIPEC in asymptomatic patients presenting a high risk of developing colorectal peritoneal carcinomatosis. Ann Surg 2011.
3. Glehen O, Gilly FN, Arvieux C. Peritoneal carcinomatosis from gastric cancer: A multi-institutional study of 159 patients treated by cytoreductive surgery combined with perioperative intraperitoneal chemotherapy. Ann Surg Oncol 2010; 17.
4. Reymond ME, Solass W. PIPAC – Pressurized intraperitoneal aerosol chemotherapy – Cancer under pressure 2014. Tempfer CB, Winnekendonk G, Solass W. Pressurized intraperitoneal aerosol chemotherapy in women with recurrent ovarian cancer: A phase 2 study. Gynecol Oncol 2015; 137.
5. Elias D, Pocard M, Goere D. HIPEC with oxaliplatin in the treatment of peritoneal carcinomatosis of colorectal origin. Cancer Treat Res 2007; 134.
6. Hübner M, Teixeira H, Boussaha T, Cachemaille M, Lehmann K, Demartines N. PIPAC – Chimiothérapie intrapéritonéale vaporisée. Un traitement innovateur de la carcinose péritonéale. Rev Med Suisse 2015; 11: 1325-1330.
7. Klaver YL, de Hingh IH, Boot H, Verwaal VJ. Results of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy after early failure of adjuvant systemic chemotherapy. J Surg Oncol 2011.
8. Hübner M, Grass F, Teixeira-Farinha H, Pache B, Mathevet P, Demartines N. Pressurized IntraPeritoneal Aerosol Chemotherapy – Practical aspects. Eur J Surg Oncol 2017; 17.
9. Giger-Pabst U, Solass W, Buerkle B, Reymond MA, Tempfer CB. Low-dose pressurized intraperitoneal aerosol chemotherapy (PIPAC) as an alternative therapy for ovarian cancer in an octogenarian patient. Anticancer Res 2015; 4.
10. Tempfer CB, Celik I, Solass W et. al. Activity of Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) with cisplatin and doxorubicin in women with recurrent, platinum-resistant ovarian cancer: preliminary clinical experience. Gynecol Oncol 2014; 132(2): 307-11.
11. Demtröder C, Solass W, Zieren J, Strumberg D, Giger-Pabst U, Reymond MA. Pressurized intraperitoneal aerosol chemotherapy with oxaliplatin

in colorectal peritoneal metastasis. Colorectal Dis 2016; 18(4):364-71.
12. Macri`A, Fortugno A, Saladino E. Rationale and techniques ofCRS and peritoneal chemohyperthermia. World J Gastrointest Oncol 2011; 3(12):169-74.
13. Sugarbaker PH, New standard of care for appendiceal epithelial neoplasms and pseudomyxoma peritonei syndrome? Lancet Oncol 2006; 7: 69-76.
14. Chua TC, Yan TD, Saxena A, and Morris DL, Should the treatment of peritoneal carcinomatosis by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy still be regarded as a highly morbid procedure?: a systematic review of morbidity and mortality. Annals of Surgery 2009; 249(6): 900–907.
15. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2008; 2.
16. Teo MCC, Tan GHC, Tham CK, et al. Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy in Asian Patients: 100 Consecutive Patients in a Single Institution, Annals of Surgical Oncology 2013; 20: 2968–2974.
17. Sollas W, Kerb R, Murdter T et al. Peritoneal Carcinomatosis Using Pressurized Aerosol as an Alternative to Liquid Solution: First Evidence for Efficacy, Annals of Surgical Oncology 2013; 21: 553-559.

LIST OF FIGURES

Fig. 1. Diagram of devices used for the PIPAC technique, taking into account the incision size, temperature and pressure under which the procedure is performed [19].

FIG. 1. DIAGRAM OF DEVICES USED FOR THE PIPAC TECHNIQUE, TAKING INTO ACCOUNT THE INCISION SIZE, TEMPERATURE AND PRESSURE UNDER WHICH THE PROCEDURE IS PERFORMED [19].



HOW MUCH DO WE KNOW ABOUT HEALTHY TANNING WITHOUT MELANOMA? A QUESTIONNAIRE STUDY

Hanna Drobek[1] , Dominika Wcislo-Dziadecka[2]

1. STN Students' Association by the Department of Skin Structural Studies, Chair of Cosmetology, School of Pharmacy with Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland
2. Department of Skin Structural Studies, Chair of Cosmetology, School of Pharmacy with Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland

#Corresponding author: Hanna Drobek, e-mail: hdrobek@op.pl, STN Students' Association by the Department of Skin Structural Studies, Chair of Cosmetology, School of Pharmacy with Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poniatowski St 15, 40-055 Katowice, Poland, phone number: +48 32 2591 581

RUNNING TITLE	Melanoma questionnaire study
KEYWORDS	prevention; melanoma; sunscreen; sunburn
WORD COUNT	1581
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

Melanoma develops rapidly from epidermal skin cells known as melanocytes, proceeding in a short time to the advanced stage of disease. Furthermore, it reveals an alarming increase in annual incidence. The essential solution is focused on appropriate prevention and lifestyle. The aim of the following study is the assessment of the prophylaxis use during exposure to detrimental ultraviolet radiation. The research enrolled 208 randomly selected respondents in an online survey, who answered 25 single and multiple-choice questions about melanoma prevention. The questionnaire was conducted in Poland between 29th November 2016 and 10th January 2017. There was no introductory information for the surveyed before responding. The participants were asked about their constant behavior during exposure to UV rays, such as frequency of sunbathing, application of sunscreen products, regularity of check-ups with a dermatologist as well as self-assessment of skin marks. Achieved results were compared in a few groups considering gender, Fitzpatrick skin scale and education. Up to 56% of participants sunbathe during the highest values of UV Index. 86% claim to apply sunscreen before exposure to UV radiation. 86% of respondents have been sunburned at least once in a life. 75% admit to not controlling skin lesions at a dermatologist. More than half of the respondents (54%) have not ever heard about the ABCDEs of melanoma. The awareness of safe tanning is moderate. The majority of respondents apply sunscreen products but to a large extent people disregard the principles of their proper use.

BACKGROUND

Melanoma develops rapidly from epidermal skin cells known as melanocytes, proceeding in a short time to the advanced stage of disease. Furthermore, it reveals an alarming increase in annual incidence. The essential solution is focused on appropriate prevention and lifestyle. The aim of the following study is the assessment of the prophylaxis use on exposure to detrimental ultraviolet radiation [1, 2].

MATERIAL AND METHODS

Online survey with 25 questions about melanoma prevention. The questionnaire was conducted in Poland between 29th November 2016 and 10th January 2017. Statistical results were received in Microsoft Excel by applying a Chi-square test, with the level of significance of 0.05.

RESULTS

The questionnaire enrolled 208 randomly selected respondents with 136 (65%) females and 72 (35%) males. The median age range is estimated to be at 18 - 29 years old (table 1.) with 160 (77%) participants fitting into this bracket. University students constitute the majority of the research participants (60%).

The Fitzpatrick skin scale is based on the appearance and characteristics of a person's skin, including color, exposure to UV radiation, tanning and protective behaviors. It has been successfully applied as a standard for self-assessment of sun sensitivity. The current Fitzpatrick skin type classification is represented by six phototypes (table 2.) [3]. According to the Fitzpatrick scale the Central European phototype constitutes the majority of participants being the phototype of 124 respondents (60%, table 3.). The second most significant phototype is Northern European with 60 (29%) individuals.

The Global Solar UV Index (UVI) describes the level of solar UV radiation at the Earth's surface. The index value indicates the detrimental potential of UV rays to the skin. The risk of occurrence of sun damage is significant for higher values of UVI. The strength of UV radiation and therefore the values of the index vary throughout the day. The maximum level of radiation occurs during the four-hour period around the solar noon [4]. Alarming a majority of respondents decide to sunbathe when values of UVI are at their highest: 10:00 – 12:00 (52%, figure 1.) and 12:00 – 15:00 (56%). At the same time, 42% of participants claim to sunbathe carefully and moderately (table 4.). Furthermore 32% try to avoid the sunlight in case of getting sunburn. This indicates participants' misunderstood approach of proper prophylaxis against the damaging effects of UV radiation (figure 2.). 192 (92%) surveyed people do not apply indoor tanning. Among the 8% frequency of indoor tanning application it is used minimally, a few times a year (44%) and even more rarely (56%).

Additionally, the research reports the most sensitive parts of the body to UV radiation. The surveyed mostly indicate a face (59%, figure 3.), shoulders (55%) and a nape (32%), which are frequently exposed to UV radiation by its revealed localization.

Sunscreens are cosmetic products to protect skin from harmful solar radiation by absorbing or reflecting UV radiation. The American Food and Drug Administration (FDA) requires attaching a Sun Protection Factor (SPF) label on sunscreen products [5]. 179 (86%) respondents apply cosmetics with SPF before sunbathing. 91 (44%) surveyed people claim to use sunscreens before sunbathing and after swimming (figure 4.). 93 (45%) respondents choose cosmetics with SPF 15 – 25 (figure 5). Statistically people with Northern European phototype choose higher value of SPF in sunscreens than respondents with Central European phototype, who prefer lower values of SPF ($p = 0.026$). 147 (71%) respondents admit the cream with UV factor is the best form of cosmetic product (table 5.). The main 3 reasons why people use cosmetics with UV filter are: avoiding sunburn (88%, figure 2.), protecting skin from melanoma development (63%) and prevention of the skin from wrinkles and premature aging (46%). Sun protective clothing is generally evaluated on the basis of clothing indices which is actually a UV protection factor (UPF). Fabric UPF is similar to sunscreen SPF, where sunscreen is replaced by fabric to protect the skin [5]. Popular methods of protection other then sunscreens are: staying in the shade (71%, figure 6.), wearing sunglasses with UV filter (69%), wearing a cap/hat (66%), wearing baggy clothes, covering shoulders (47%). 167 (80%) respondents know that melanoma may develop in a human eyeball. More women are aware of this condition than men ($p = 0.00001519$).

According to researches, an increasing number of sunburns during a whole lifetime is a risk of increased incidence of melanoma. Prevention efforts should focus on reducing all sunburns, regardless of what age they are acquired [6, 7]. Up to 179 (86%) respondents at least once in a life have been sunburned. The survey reveals that 144 (69%) respondents mostly get sunburn during reckless sunbathing (table 6.).

Clinicians can diagnose up to 80% of melanoma cases using the ABCDE rule (asymmetry, border, color, diameter over 6 mm, evolving) [8]. 112 (54%) participants don't know the ABCDEs of melanoma. Nevertheless, 175 (84%, figure 7.) respondents claim that early melanoma may evolve in its structure which means the pigmentary skin mark might change in any way. Positions such as an asymmetric structure, uneven borders and a variety of color have similar frequency. The least popular answer (104 respondents, 50%) is a big size of a skin mark (over 6 mm). Statistically more women know ABCDEs than men ($p = 0.0001$).

Dermoscopy is a simple in vivo technique detecting malignant submicroscopic structures. The diagnostic accuracy of dermoscopy in melanoma is up to 30% better than visual inspection of skin marks [8]. Only 52 (25%) surveyed people admit to control their pigmentary skin nevi at a dermatologist. The majority of respondents (75%) don't check up their moles with a specialist. However, the research reveals that 75% claim to self-assess skin marks. The research reveals that gender is statistically significant and has an impact on the

likelyhood of regular control of pigmentary skin marks at a dermatologist (p = 0.018), as well as in self-assessments (p = 0.00002313). More women tend to control their skin lesions than men.

In the respondents' opinion the prophylaxis against melanoma should include the following statements: avoid sunburns (90%, figure 8.), apply sunscreens (88%) and regular check-ups at a dermatologist (85%). There were also 3 answers intentionally misleading and excluding melanoma prevention: quitting smoking (32%), supplementation of vitamin D (26%), removing every single mole (6%). 66% of respondents would like to improve their knowledge about melanoma prevention. Statistically people who are not familiar with the ABCDEs are more interested in improving their knowledge about melanoma prevention than other respondents (p = 0.002).

DISCUSSION

This study demonstrates objective information about preventive behaviors and habits during exposure to UV radiation. The participants shared their current knowledge with no introductory information before answering.

The alarming amount of respondents who declare to sunbathe during high values of UVI may be reduced by proper education. A similar study (Rodrigues A. M. et al., 2017) enrolled the guidelines of the World Health Organization (WHO) from 2014 during interview about safe tanning. The directions were included in the following sections: seeking shade during strong radiation (between 10 a.m. and 4 p.m.), wearing protective clothing (including hats, sunglasses, loose fitting clothes) and applying a broad-spectrum sunscreen (at least SPF 15) and reapplying every 2 hours or after physical activity (including swimming, walking, outdoor exercising). The participants' knowledge of proper exposure to UV radiation had been checked and then the statements of the WHO followed. The respondents could immediately emend their declarations about proper sunbathing [8].

Another finding confirms that women are apparently more aware of UV prophylaxis than men. The research (Heerfodt I. et al., 2017) reveals that females apply sunscreens 2.24 times more often than males (p < 0.0001) [10].

In the respondents' opinion the face is the most fragile part of the body. According to another study (Pratt et al., 2017) effective application of external parts is crucial and areas that are routinely missed have an increased risk of UV damage. 57 participants were imaged with a UV sensitive camera before and after sunscreen application. Analyses revealed eyelid and periorbital regions to be disproportionately missed during routine sunscreen application (median 14% missed in eyelid region vs 7% in rest of face, p < 0.01) [11].

The occurrence of sunburn is an important short-term marker for excessive sun exposure. Likewise, the risk of melanoma increases with the number of sunburns during one's lifespan, not just in childhood [6, 7, 12]. The use of tanning-facilitating agents can accelerate the process of absorbing solar radiation, causing deeper and more severe damage. The ideal product should contain moisturizing and solar protection agents. As these products are substantially more expensive, poorer and less-educated segments of the population usually improvise with dangerous replacements [12, 13].

Self-examination of the skin may facilitate an early diagnosis of melanoma [8, 14]. Recent finding (Kamińska-Winciorek et al., 2015) indicates that approximately 60% of respondents have been sunburned at least once in their life and only 18.4% declare regular self-assessments of skin marks [14]. Self-control is the result of the following factors: higher education, a sensitive skin phototype, sun-safe tanning rules and a past medical history of surgical excision of naevi [14, 15]. Regular self-assessments are not a common practice, whilst the knowledge about the clinical features of melanoma is varied. Therefore, promotion of regular skin self-examination and education should be performed.

CONCLUSIONS

The awareness of safe tanning is moderate. The majority of respondents notice the need of applying sunscreens. Unfortunately, it is still a rarity to control skin lesions at a dermatologist, as well as knowing the ABCDEs. The dissemination of information about healthy tanning by health care providers should be considered.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 18 – 23

ABBREVIATIONS

FDA – Food and Drug Administration
SPF – Sun Protection Factor
UPF – Ultraviolet Protection Factor
UV – Ultraviolet
UVI – UV Index
WHO – World Health Organization

REFERENCES

1. Drobek H, Bergler-Czop B, Miziołek B. From chemotherapy to oncoviruses – modern methods of treatment in metastatic melanoma. Dermatol Rev/Przegl Dermatol 2017, 104, 396-410.
2. Rutkowski P, Wysocki P, Nasierowska-Guttmejer A, et al. Czerniaki skóry – zasady postępowania diagnostyczno-terapeutycznego w 2016 roku. Przegl Dermatol 2016, 103, 1-18.
3. Sachdeva S. Fitzpatrick skin typing: Applications in dermatology. Indian J Dermatol Venereol Leprol 2009, 75, 93-96.
4. Global Solar UV Index: A Practical Guide. World Health Organization 2002, 1-28.
5. Donglikar M, Deore S. Sunscreens: A review. Pharmacogn. J. 2016, 8, 171-179.
6. Dennis L, VanBeek M, Beane Freeman L, Smith B, Dawson D, Coughlin J. Sunburns and risk of cutaneous melanoma, does age matter: a comprehensive meta-analysis. Ann Epidemiol. 2008, 18, 614-627.
7. Kirk L, Greenfield S. Knowledge and attitudes of UK university students in relation to ultraviolet radiation

(UVR) exposure and their sun-related behaviours: a qualitative study. BMJ Open 2017, 7, 1-11.
8. Troya-Martin M, Blazquez-Sanchez N, Fernandez-Canedo I, Frieyro-Elicegui M, Funez-Liebana R, Rivas-Ruiz F. Dermoscopic Study of Cutaneous Malignant Melanoma: Descriptive Analysis of 45 Cases. Actas Dermosifiliogr. 2008, 99, 44-53.
9. Rodriguesa A, Sniehottaa F, Birch-Machinb M, Araujo-Soaresa V. Aware, motivated and striving for a 'safe tan': an exploratory mixed-method study of sun-protection during holidays. Health Psychology and Behavioral Medicine, 2017, 5, 276-298.
10. Heerfodt I, Philipsen P, Larsen B, Wulf H. Long-term Trend in Sunscreen Use among Beachgoers in Denmark. Acta Derm Venereol 2017, 97, 1202–1205.
11. Pratt H, Hassanin K, Troughton L, et al. UV imaging reveals facial areas that are prone to skin cancer are disproportionately missed during sunscreen application. PLOS ONE 2017, 1-14.
12. Haluza D, Simic S, Moshammer H. Sun Exposure Prevalence and Associated Skin Health Habits: Results from the Austrian Population-Based UVSkinRisk Survey. Int. J. Environ. Res. Public Health 2016, 13, 1-11.
13. Sforza M, Andjelkov K, Zaccheddu R. Severe burn on 81% of body surface after sun tanning. Turkish Journal of Trauma & Emergency Surgery, 2013, 19, 383-384.
14. Kamińska-Winciorek G, Gajda M, Wydmański J, Tukiendorf J. What Do Web Users Know about Skin Self-Examination and Melanoma Symptoms? Asian Pacific Journal of Cancer Prevention, 2015, 16, 3051-3056.
15. Mayer J, Swetter S, Fu T, Geller A. Screening, early detection, education, and trends for melanoma: current status (2007-2013) and future directions: Part I. Epidemiology, high-risk groups, clinical strategies, and diagnostic technology. J Am Acad Dermatol, 2014, 71, 611.

LIST OF TABLES

Tab. 1. The age range of respondents.
Tab. 2. The Fitzpatrick skin type classification.
Tab. 3. Respondents' phototypes according to the Fitzpatrick skin scale.
Tab. 4. The frequency of subathing.
Tab. 5. The sunscreen products.
Tab. 6. The frequency of sunburns.

LIST OF FIGURES

Fig. 1. What time do you sunbathe? (multiple choice).
Fig. 2. Why do you use cosmetics with UV filter? (please choose 3 answers).
Fig. 3. What is you're the most sensitive part of the body to UV radiation? (multiple choice).
Fig. 4. How often do you use sunscreens?
Fig. 5. What is the value of SPF in sunscreen products, which you usually apply during sunbathing?
Fig. 6. What are the other methods of protection except sunscreens that you use during exposure to sunlight? (multiple choice).
Fig. 7. A question of multiple choice: How in your opinion may a skin mark with an early stage of melanoma look like?
Fig. 8. What in your opinion should melanoma prevention include? (multiple choice).

TAB. 1. THE AGE RANGE OF RESPONDENTS.

Age range	Respondents
< 18	1
18 – 29	160
30 – 39	18
40 – 49	7
50 – 59	8
60 – 69	13
70 <	1

TAB. 2. THE FITZPATRICK SKIN TYPE CLASSIFICATION.

Phototype	Characterisctics
Celtic (I)	Very fair, burns easily, never tans
Northern European (II)	Fair, burns easily, tans with difficulty
Central European (III)	White, burns moderately, tans moderately
Southern European (IV)	Olive, burns minimally, tans easily
Middle Eastern (V)	Moderate brown, burns rarely, tans easily and profusely
African (VI)	Dark brown or very dark, never burns, tans profusely

TAB. 3. RESPONDENTS' PHOTOTYPES ACCORDING TO THE FITZPATRICK SKIN SCALE.

Phototype	Respondents
Celtic	5
Northern European	60
Central European	124
Southern European	18
Middle Eastern	1
African	0

TAB. 4. THE FREQUENCY OF SUBATHING.

How often do you sunbathe?	Respondents
Very often, every sunny weather.	5
Often, usually when it's sunny.	33
I sunbathe carefully and moderately.	87
Rarely, I hide in the shadow and don't want to get a sunburn.	66
I don’t sunbathe.	17

TAB. 5. THE SUNSCREEN PRODUCTS.

Cosmetic products with SPF	Respondents
Cream	147
Oil	29
Spray	23
Different	9

TAB. 6. THE FREQUENCY OF SUNBURNS.

The frequency of sunburns.	Respondents
Very often, usually before first sunbathing in a year and even later	10
Often, usually before first sunbathing in a year	28
Rarely, generally during reckless sunbathing	144
It doesn’t sunburn	26

FIG. 1. WHAT TIME DO YOU SUNBATHE? (MULTIPLE CHOICE).

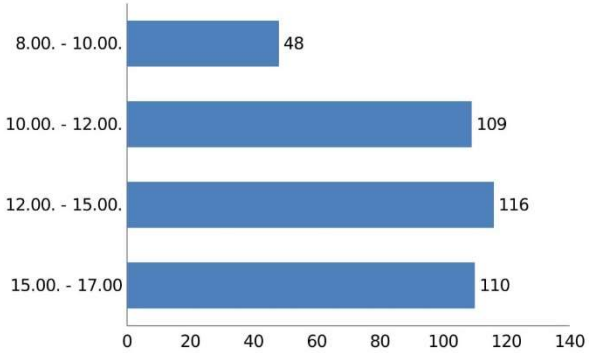


FIG. 2. WHY DO YOU USE COSMETICS WITH UV FILTER? (PLEASE CHOOSE 3 ANSWERS).

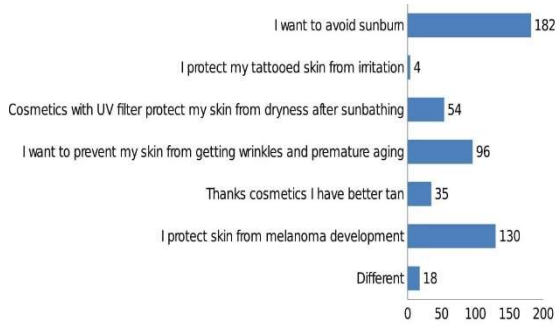


FIG. 3. WHAT IS YOU’RE THE MOST SENSITIVE PART OF THE BODY TO UV RADIATION? (MULTIPLE CHOICE).

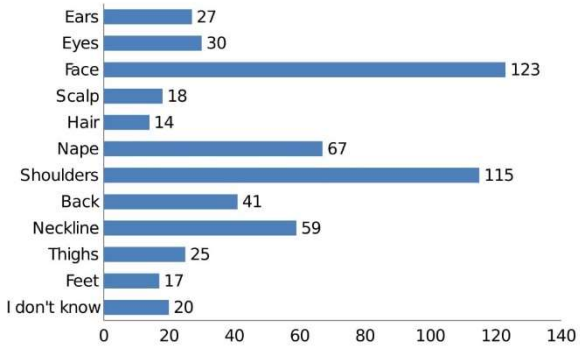


FIG. 4. HOW OFTEN DO YOU USE SUNSCREENS?.

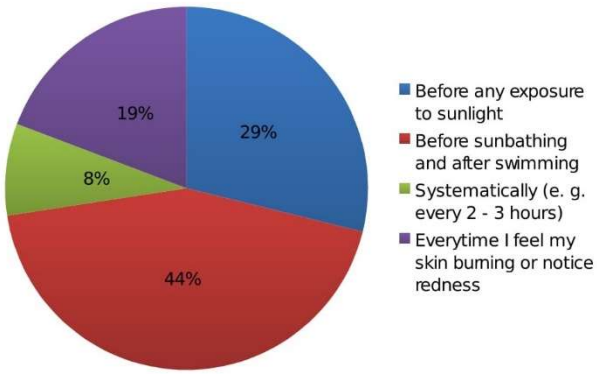


FIG. 5. WHAT IS THE VALUE OF SPF IN SUNSCREEN PRODUCTS, WHICH YOU USUALLY APPLY DURING SUNBATHING?.

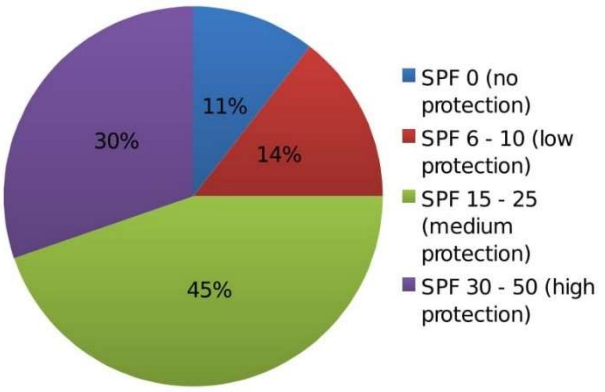


FIG. 6. WHAT ARE THE OTHER METHODS OF PROTECTION EXCEPT SUNSCREENS THAT YOU USE DURING EXPOSURE TO SUNLIGHT? (MULTIPLE CHOICE).

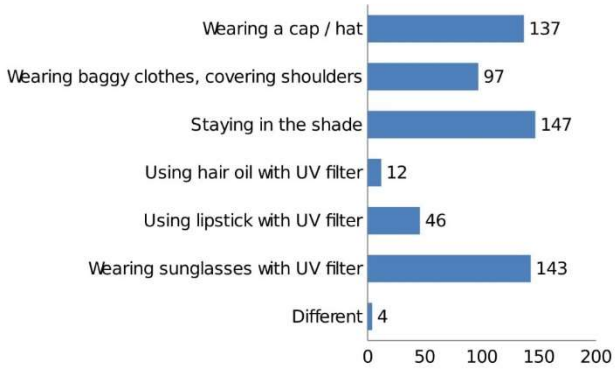


FIG. 7. A QUESTION OF MULTIPLE CHOICE: HOW IN YOUR OPINION MAY A SKIN MARK WITH AN EARLY STAGE OF MELANOMA LOOK LIKE?.

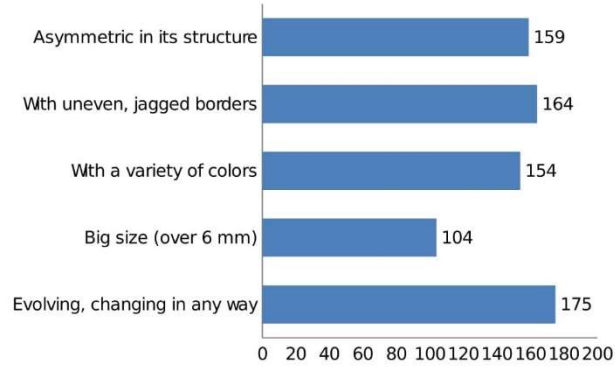
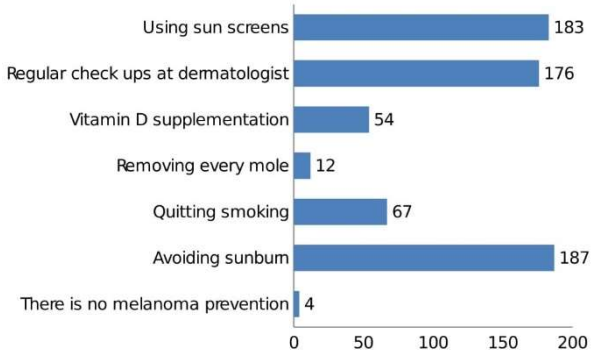


FIG. 8. WHAT IN YOUR OPINION SHOULD MELANOMA PREVENTION INCLUDE? (MULTIPLE CHOICE).



NON-MELANOMA SKIN CANCERS: PERSPECTIVES OF EARLY DIAGNOSIS AND THERAPY

Stanislaw Kwiatkowski[1], Salwador Cyranowski[2], Beata Joanna Osiecka[1]

1. Department of Pathology, Wroclaw Medical University, Wroclaw, Poland
2. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

#Corresponding author: Stanislaw Kwiatkowski, e-mail: stkwiatek@gmail.com, Department of Pathology, Wroclaw Medical University, Marcinkowskiego 1 St, p. o. box 50-368 Wroclaw, Poland, phone number: +48 889 217 311

RUNNING TITLE	Early diagnosis and therapy of non-melanoma skin cancers
KEYWORDS	non-melanoma skin cancers; basal cell carcinoma; squamous cell carcinoma
WORD COUNT	3220
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

Non-melanoma skin cancers (NMSC) belong to the group of most frequent malignant cancers among Caucasian race. Similar to other cancer types, NMSC progression is an outcome of environmental factors and genetic background. The predominating risk factor for NMSC is the prolonged exposure to ultraviolet (UV) light. The origin place for non-melanoma skin cancers is the epidermis. There are two main types: basal cell carcinoma (BSC) and squamous cell carcinoma (SCC). Basal cell carcinoma origins from the basal layer of epidermis. BCC progresses relatively slowly but exerts a substantial damaging impact on adjacent tissues. SCC, in contrast to basal cell carcinoma, metastasise more frequently, typically to local lymph nodes. The observed rise of NMSC incidence in recent years prompts to move forward to a more effective prophylactic approach and comprehensive treatment. Ultimately, the actual diagnosis of all subtypes of NMSCs is based on the result of a histopathological examination of an excised skin fragment. The therapeutic process depends on several features that account for the clinical image of the lesion: the primary focus, the presence of metastases to the local lymph nodes and to distant parts of the body. Photodynamic diagnosis (PDD) and photodynamic therapy (PDT) are promising, non-invasive methods which may be helpful in early diagnosis and treatment of superficially growing NMSCs.

BACKGROUND

Non-melanoma skin cancers (NMSC) belong to the group of most frequent malignant cancers among Caucasian race [1]. Similar to other cancer types, NMSC progression is an outcome of environmental factors and genetic background [2]. The predominating risk factor for NMSC is the prolonged exposure to ultraviolet (UV) light, particularly that of type B (UVB). Another significant etiological factor is light skin phenotype (commonly referred to as phenotypes I-III). Despite the expanding awareness of the harmfulness of UV radiation (UVR), the number of patients suffering from NMSC is on a steady rise [3]. There is a positive correlation between the NMSC incidence and the geographical latitude – it is substantially higher in the equatorial zone [4].

SKIN CANCERS

Skin cancers can be classified into two groups based on their origin: melanoma and NMSC. Melanoma is a highly metastatic cancer that arises from neuroectodermal melanocytes. In contrast to melanoma, NMSC are of epithelial origin, do not contain any pigment (melanin) and are thus colourless [5].

NON-MELANOMA SKIN CANCERS

The origin place for non-melanoma skin cancers is the epidermis. There are two main types: basal cell carcinoma (BSC) and squamous cell carcinoma (SCC) [6].

Basal cell carcinoma origins from the basal layer of epidermis. BCC progresses relatively slowly but exerts a substantial damaging impact on adjacent tissues. It is malignant only locally as the long-distance metastases are described in the literature as individual cases (<0.1%).

BCC accounts for 70-80% of all diagnosed non-melanoma skin cancers. The average age for the first diagnosis of NMSC is 60 years, but the incidence has been reported to be increasing in the younger groups of patients. Typically, a professional dermatologic help is sought by patients when a slowly growing lesion, bleeding upon mechanical disturbance, appears [7].

Clinical classification of basal cell carcinoma is based primarily on its morphological features and includes the following subtypes: superficial (*BCC superficiale*), nodulous (*BCC nodosum*), ulcerating (*BCC exulcerans, ulcus rodens*), cystic (*BCC cysticum*), pigmented (*BCC pigmentosum*) and morphoeic or sclerosing (*BCC morpheiforme*) [8].

BCC lesions localise predominantly within the facial skin (upper-middle part) that has been damaged by prolonged exposure to light radiation. Superficial subtypes usually localise on the truncal areas of skin [9].

Squamous cell carcinoma is a malignant cancer that originates from the stratum spinosum of epidermis. SCC, in contrast to basal cell carcinoma, metastasise more frequently, typically to local lymph nodes in 2.5-50% of reported cases [10]. Immunocompromised patients are at the upmost risk of SCC, thus SCC has 60-100 times higher incidence in post-transplantation patients who are receiving immunosuppressive drugs as compared with healthy population [11].

There are two subtypes of squamous cell carcinoma based on the morphological features of the lesion: *SCC exulcerans* and *SCC vegetans*. Progression and invasiveness of SCC are dependent on the localisation of the tumour and its malignancy. Squamous cell carcinoma most typically localises on the edge of skin and mucosa – on lower lip, auricular (high metastatic potential), areas adjacent to nostrils and reproductive organs.

Squamous cell carcinoma arises from the areas of skin damaged by UVR, at the spots of chronic inflammation, irritation (mechanical or chemical) or at the sites of actinic keratosis (AK). SCC that emerges *de novo* is characterised with a higher malignancy than that of AK origin [12].

ACTINIC KERATOSIS

In the context of the most recent molecular and genetic studies, AK is considered as squamous cell carcinoma *in situ*. Emergence of AK is induced by prolonged exposure to ultraviolet radiation, UVB in particular. Lesions occur typically in the areas exposed to sunlight, i.e. the skin of face, head, auricular and upper limbs. These lesions take a form of erythema spots, most frequently with superficial hyperkeratosis. Statistically, around 20% of untreated AK develop into metastatic squamous cell carcinoma [13].

The recommended method of AK treatment is a therapy of both clinically evident keratosis together with broader areas of sunlight damaged skin, the so-called field cancerisation. These areas appear as unaffected epithelium but are actually fields of photocancerisation. Within such fields, there are keratinocytes that harbour genetic alterations predisposing for skin cancer development. They can potentially give rise to AK, and to SCC as a consequence [14].

The consideration of field cancerisations in patient's treatment delivers preventive and therapeutic outcomes. It leads to the remission of the already emerged AK lesions and prevents the formation of potential new changes with cancer promotion included.

DIAGNOSIS OF NON-MELANOMA SKIN CANCERS

The diagnosis of typical forms of NMSC can be conducted based on the morphological features of the lesion. The majority of NMSCs (excluding superficial BCC) localise at the field of UVR damage. However, an examination of the whole skin is necessary as the disease might occur in multiple lesion spots.

The most frequent form of basal cell carcinoma, nodulous BCC, appears as a convex nodule surrounded by a pearly edge. Pigmented BCC can be recognised as a form of nodulous BCC with accommodation of melanin. Superficial BCC has a particularly chronic progression and localises in the shallow layers of truncal skin. Lesions are usually numerous, flat and well defined by a slightly protruding edge. *BCC exulcerans* exhibits an ulcerating focus surrounded by a rigid and infiltrated edge. This subtype can damage the adjacent tissues deeply, affecting muscles and bones (*ulcus rodens*). Lesions of

BCC morpheiforme subtype have a porcelain-like appearance and typically do not break apart. *BCC cysticum* takes a form of petit, transparent nodules located usually on eyelids. Dermoscopy might be a helpful tool in the diagnosis of certain types of BCC, such as pigmented BCC [15].

Squamous cell carcinoma typically appears with an infiltrated base and often with protruding, flipped edges but are devoid of pearly-like edge observed in BCC.

Upon examination of a patient diagnosed with cancer, it is recommended to always assess local lymph nodes of the head and neck. If the lesions are advanced, it is recommended to prescribe scans, i.e. abdominal USG, chest radiograph, to exclude long-distance metastases.

Ultimately, the actual diagnosis of all subtypes of NMSCs is based on the result of a histopathological examination of an excised skin fragment. In addition to diagnosis, tumour biopsy allows practitioners to assess the lesion in accordance to TNM classification (tumour, nodes, metastasis) [16].

PHOTODYNAMIC DIAGNOSIS

Photodynamic diagnosis (PDD) is a tool that allows for an early diagnosis of cancer and the assessment of the margins of the lesion. This method is based on the fluorescence of locally administered compound that sensitizes the skin against light, the so-called photosensitizer. Due to the fact that the photosensitizer accumulates selectively in cancerous cells, PDD enables the visualisation of pathological foci.

The mechanisms of PDD is based on the co-operation of two components:

1. the photosensitizer, a pigment that selectively accumulated in atypical tissue and sensitizes it against light,
2. the source of light of a specific wavelength [17].

The most frequently used photosensitizers are porphyrin compounds. In order to induce fluorescence, it is necessary to shine a light of a wavelength corresponding with the peak absorption of the photosensitizer. In the case of protoporphyrin IX (PpIX), a compound that arises from a precursor – aminolevulinic acid, the required wavelength is ca. 408 nm. PpIX becomes excited and upon return to its base energy state it emits red fluorescent light within the pathologically altered tissue that it accumulated in [18].

The 'shining tumour' image is usually visible by a naked eye. To further analyse the emission spectrum, it is possible to capture it as an image and then process it digitally.

Photodynamic method does not exclude the necessity to conduct histopathological examination but it facilities the determination of the biopsy site. It thus provides a kind of "optical biopsy" [19]. Such approach allows for the visualisation of the tumour together with the margin of dysplastic cells surrounding it. The latter are the main reason of cancer reappearance after imprecise surgical excision.

THERAPY OF NMSCs

The therapeutic process depends on several features that account for the clinical image of the lesion: the primary focus (dimensions, depth of invasion), the presence of metastases to the local lymph nodes and to distant parts of the body [20].

In order to absolutely eradicate an NMSCs lesion, it is crucial to use the most efficient therapy and at the same time, take necessary measures to ensure positive aesthetical effect as the lesions concern predominantly easily visible areas of patient's skin. Surgical measures oftentimes lead to deforming scars, and thus are not welcome by the patients [21]. That is why, provided that prophylactic examinations are conducted properly, a preferred way of treatment is a local operation, especially if the lesion is superficial or unlikely to metastasize. In a case of cancer reappearance or failure to eradicate it in the first attempt, a surgical operation is recommended (given that a margin of 4-6 mm is possible). If a radical surgical operation is not possible, it is necessary to apply radiotherapy [22].

Local ways of NMSC treatment include cryotherapy and photodynamic therapy (PDT), as well as pharmacological methods: 5-fluoruracyl and imiquimod [23].

Cryotherapy is a simple, cheap and quick method that can be applied to erase individual, non-invasive foci of NMSC. There are, however, unwelcome side effects: pain, the risk of pigmentation and peeling of the adjacent skin [24].

5-fluoruracil (5-FU) is a chemotherapeutic designed for a local administration that inhibits DNA synthesis and alters RNA function. The main disadvantage of 5-FU is the long treatment time and certain side effects: itching, erythema, pain and secondary infections and depigmentation.

Imiquimod is a immunomodulatory compound that affects immune response by stimulating monocytes and macrophages. Its anti-cancer effect is based on the enhanced cell-mediated immunity through the stimulation of immunocompetent cells and the release of pro-inflammatory cytokines. The most frequently observed side effects of imiquimod are excessive immune response of the skin [25].

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) enables a selective destruction of pathologically altered cells without the risk of damaging healthy tissues. The mechanism of action of photodynamic therapy requires presence of three components: a photosensitizer that localizes in pathologically altered tissue, a light source of specific wavelength that activates the photosensitizer and oxygen molecules dissolved within the tissue. The photosensitizer, administered locally, accumulates selectively in cancerous cells. The reason behind this phenomenon lies in the property of the photosensitizer – it tends to be retained by cells of pathologically high metabolic rate and proliferating in an uncontrollable manner, such as cancerous cells. Due to the radiation of light of specific wavelength adjusted to the absorptive properties of the photosensitizer, the latter becomes activated and passes the energy acquired from light to

surrounding molecules. This photodynamic reaction results in excitation of oxygen molecules and thus production of free radicals (*ROS – reactive oxygen species*) that lead to the cell death [26].

Among all ways of NMSCs treatment, PDT is reported as a non-invasive, selective and effective method [27].

Numerous clinical studies confirm the efficacy of PDT in basal cell carcinoma treatment, particularly the superficial and nodulous subtype of relatively small dimensions. Rhodes *et al.* observed a complete response of nodulous basal cell carcinoma after PDT, almost as high as surgical excision (91% and 98% in 3 months after treatment, respectively), however, the first yielded outstanding cosmetic results in comparison to the latter [28]. In the study of Peng *et al.* the application of ALA-PDT resulted in the treatment of 87% of lesions of superficial BCC subtype and 53% in the case of nodulous subtype [29]. Cosgarea *et al.* reported a similar therapeutic response in case of PDT treatment and surgical operation of the lesions (95.83% and 95.65%, respectively) [30].

Considering the limitations of light penetration into the tissues and bioavailability of photosensitizers, photodynamic therapy is not recommended as a monotherapy for deeply infiltrating squamous cell carcinomas. Early surgical operation is recommended to prevent the spread of SCC to local lymph nodes. It is noteworthy, however, that PDT is an effective method for the treatment of *in situ* SCC (carcinoma *in situ*, ca *in situ*) [31].

Photodynamic therapy plays an important role in the treatment of lesions of actinic keratosis, especially that of milder hyperkeratosis. In the study conducted by Piacquadio *et al.* out of 243 patients diagnosed with numerous multiple lesions, PDT yielded therapeutic effects in 77% of the examined group – already after the first light exposure [32]. It is worth to mention that PDT results in much better aesthetical effects than cryotherapy that is routinely practiced in the treatment of AK (taking into account comparable therapy outcomes).

CONCLUSIONS

Non-melanoma skin cancers are a challenge of modern oncological dermatology, both in terms of diagnosis and therapy. The observed rise of NMSC incidence in recent years prompts to move forward to a more effective prophylactic approach and comprehensive treatment. Photodynamic method, that encompasses both diagnostic and therapeutic tools, plays an important role in the treatment of non-melanoma skin cancers. Its selectivity and non-invasiveness makes it a preferred method for the treatment of easily visible areas of the body. Its aesthetical effectiveness makes it an anticipated therapy for patients who would otherwise have to face an invasive surgical operation followed by tissue damage, scars and no guarantee of absolute eradication of the cancerous cells.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 24 – 28

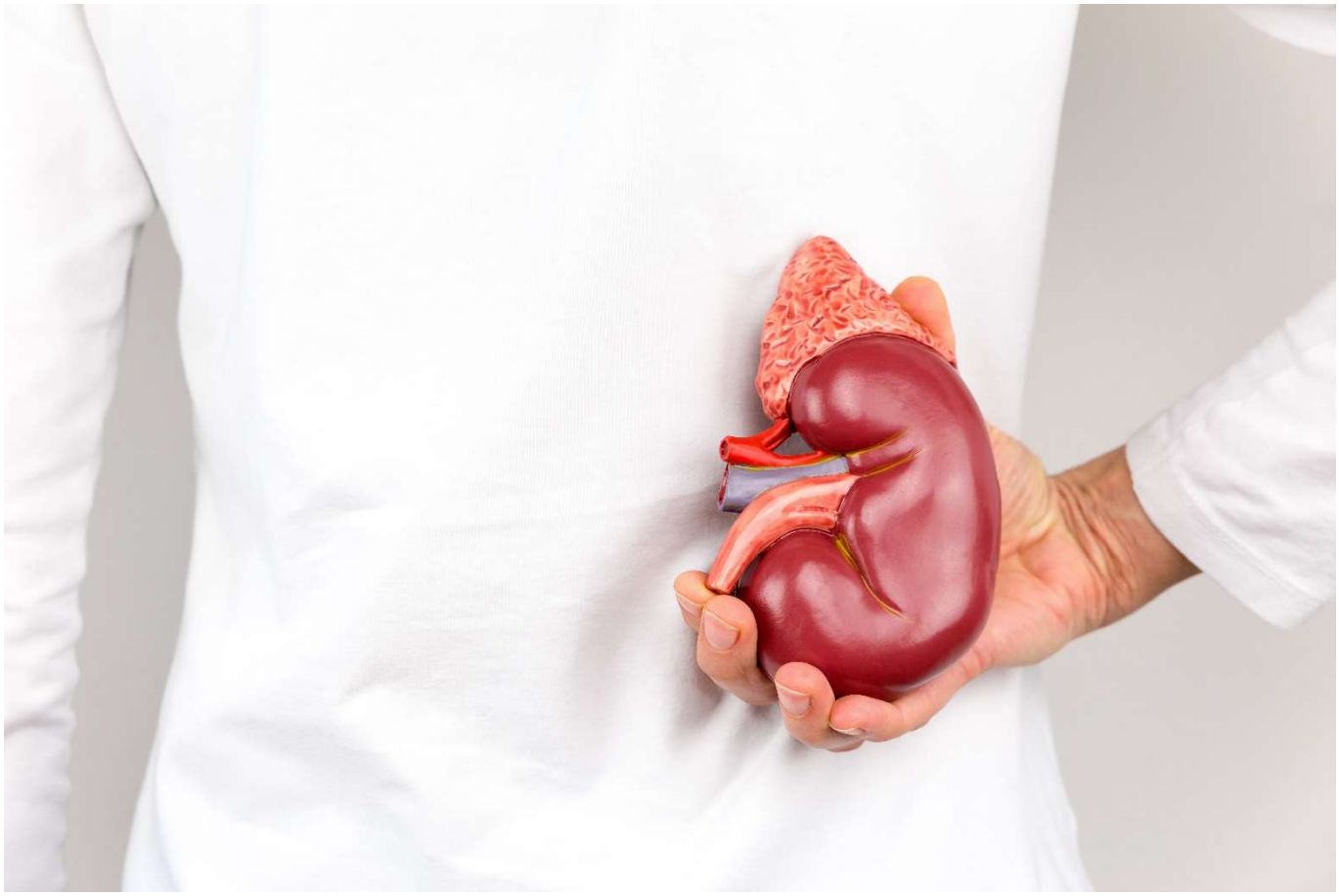
ABBREVIATIONS

5-FU – 5-fluoruracil
AK – actinic keratosis
BSC – basal cell carcinoma
NMSC – non-melanoma skin cancers
PDD – photodynamic diagnosis
PDT – photodynamic therapy
PpIX – protoporphyrin IX
SCC – squamous cell carcinoma
UVR – UV radiation

REFERENCES

1. Samarasinghe V, Madan V. Nonmelanoma Skin Cancer. J Cutan Aesthet Surg 2012; 5 (1): 3-10.
2. Saladi RN, Persaud AN. The causes of skin cancer: a comprehensive review. Drugs Today 2005; 41(1): 37-53.
3. Zak-Prelich M, Narbutt J, Sysa-Jedrzejowska A. Environmental risk factors predisposing to the development of basal cell carcinoma. Dermatol Surg 2004; 30: 248-252.
4. Tran H, Chen K, Shumack S. Epidemiology and aetiology of basal cell carcinoma. Br J Dermatol 2003; 149: 50-52.
5. Mueller CS, Reichrath J. Histology of melanoma and nonmelanoma skin cancer. Adv Exp Med Biol 2008; 624: 215-226.
6. Paolino G, Donati M, Didona D, Mercuri SR, Cantisani C. Histology of Non-Melanoma Skin Cancers: An Update. Biomedicines 2017; 5(4): 71-83.
7. Lear W, Dahlke E, Murray CA. Basal cell carcinoma: review of epidemiology, pathogenesis, and associated risk factors. J Cutan Med Surg 2007; 11(1): 19-30.
8. Dourmishev LA, Rusinova D, Botev I. Clinical variants, stages, and management of basal cell carcinoma. Indian Dermatol Online J 2013; 4(1): 12-17.
9. Chung S. Basal Cell Carcinoma. Arch Plast Surg 2012; 39(2): 166-170.
10. Kallini JR, Hamed N, Khachemoune A. Squamous cell carcinoma of the skin: epidemiology, classification, management, and novel trends. Int J Dermatol 2015; 54(2): 130-140.
11. Chockalingam R, Downing C, Tyring SK. Cutaneous Squamous Cell Carcinomas in Organ Transplant Recipients. J Clin Med. 2015; 4(6): 1229-1239.
12. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. J Cutan Pathol 2006; 33(3): 191-206.
13. Ko, CJ. Actinic keratosis: facts and controversies. Clin Dermatol 2010; 28(3): 249-253.
14. Torezan LAR, Festa-Neto C. Cutaneous field cancerization: clinical, histopathological and therapeutic aspects. An Bras Dermatol 2013; 88(5): 775-786.
15. Fargnoli MC, Kostaki D, Piccioni A, Micantonio T, Peris K. Dermoscopy in the diagnosis and management of non-melanoma skin cancers. Eur J Dermatol 2012; 22(4): 456-463.

16. Talmi YP. Problems in the current TNM staging of nonmelanoma skin cancer of the head and neck. Head Neck 2007; 29(6): 525-527.
17. Fritsch C, Ruzicka T. Fluorescence diagnosis and photodynamic therapy in dermatology from experimental state to clinic standard methods. J Environ Pathol Toxicol Oncol 2006; 25(1-2): 425-39.
18. Fritsch C, Lang K, Neuse W, Ruzicka T, Lehmann P., Photodynamic diagnosis and therapy in dermatology. Skin Pharmacol Appl Skin Physiol 1998; 11(6): 358-373.
19. de Leeuw J, van der Beek N, Neugebauer WD, Bjerring P, Neumann HA. Fluorescence detection and diagnosis of non-melanoma skin cancer at an early stage. Lasers Surg Med. 2009; 41(2): 96-103.
20. Lazareth V. Management of non-melanoma skin cancer. Semin Oncol Nurs 2013; 29(3): 182-194.
21. Goh CL. The Need for Evidence-Based Aesthetic Dermatology Practice. J Cutan Aesthet Surg 2009; 2(2): 65-71.
22. McPartlin AJ, Slevin NJ, Sykes AJ, Rembielak A. Radiotherapy treatment of non-melanoma skin cancer: a survey of current UK practice and commentary. Br J Radiol 2014; 87(1043): 1-7.
23. Amini S, Viera MH, Valins W, Berman B. Nonsurgical Innovations in the Treatment of Nonmelanoma Skin Cancer. J Clin Aesthet Dermatol 2010; 3(6): 20-34.
24. Chiriac A, Mihaila D, Foia L, Solovan C. Basal cell carcinomas in elderly patients treated by cryotherapy. Clin Interv Aging 2013; 8: 341-345.
25. Savoia P, Cremona O, Fava P. New Perspectives in the Pharmacological Treatment of Non-Melanoma Skin Cancer. Curr Drug Targets 2016; 17(3): 353-374.
26. Allison RR, Moghissi K. Photodynamic Therapy (PDT): PDT Mechanisms. Clin Endosc 2013; 46(1): 24–29.
27. Cohen DK, Lee PK. Photodynamic Therapy for Non-Melanoma Skin Cancers. Cancers (Basel) 2016; 8(10): 90.
28. Rhodes LE, de Rie M, Enström Y, et al. Photodynamic therapy using topical methyl aminolevulinate vs surgery for nodular basal cell carcinoma: results of a multicenter randomized prospective trial. Arch Dermatol 2004; 140(1): 17-23.
29. Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky KE, Nesland JM. 5-aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. Cancer 1997; 79(12): 2282-2308.
30. Cosgarea R, Susan M, Crisan M, Senila S. Photodynamic therapy using topical 5-aminolaevulinic acid vs. surgery for basal cell carcinoma. J Eur Acad Dermatol Venereol 2013; 27(8): 980-984.
31. Morton CA, McKenna KE, Rhodes LE. Guidelines for topical photodynamic therapy: update. Br J Dermatol 2008; 159(6): 1245-1266.
32. Piacquadio DJ, Chen DM, Farber HF. Photodynamic therapy with aminolevulinic acid topical solution and visible blue light in the treatment of multiple actinic keratoses of the face and scalp: investigator-blinded, Phase 3, multicenter trials. Arch Dermatol 2004; 140(1): 41-46.



PREDICTIVE FACTORS OF IMMEDIATE GRAFT FUNCTION FOR LIVING-DONOR KIDNEY TRANSPLANT

Magdalena Kwapisz, Rafal Kieszek, Kalina Jedrzejko, Monika Bieniasz, Andrzej Chmura, Artur Kwiatkowski

Department of General and Transplantation Surgery, Orlowski Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

#Corresponding author: Rafal Kieszek, email: rafal.kieszek@gmail.com, Department of General and Transplantation Surgery, Orlowski Transplantation Institute, Medical University of Warsaw, Infant Jesus Teaching Hospital, ul. Nowogrodzka 59, 02-006 Warsaw, phone number +48 22 5021470

RUNNING TITLE	Predictors of immediate graft function
KEYWORDS	immediate graft function; predictive factors; predictors of IGF; kidney transplant; living kidney donor
WORD COUNT	2200
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

Favorable outcome of kidney transplantation is particularly expected in the case of living donation. Satisfactory result can be referred as immediate graft function, defined by fast postoperative recovery of renal function with satisfactory diuresis and no further need for dialysis. Prospective analysis of 40 living-donor renal transplants was performed to assess whether there are any predictive factor of immediate graft function. Patients were compared in two groups in accordance with their initial graft function (immediate vs. slow or delayed). Clinical data relevant to the recipients, their donors and harvested organs (kidney weight and dimension) were assessed. No statistically significant differences were found between the groups. Further long-sampled studies are required to investigate the predictors of successful outcome of living-donor kidney transplantation.

BACKGROUND

Nephroureterectomy for living kidney donation is an unique procedure, seeing that highly invasive surgical intervention is performed on a totally healthy person who does not receive any direct benefits to itself. It should be noted that an altruistic sense of accomplishment is the only reward for a donor, while the risk of postoperative ailments and complications, although determined as being marginal, exists. That is the reason, inter alia, that the successful outcome of living donor transplantation is generally expected. Three groups of kidney transplant recipients can be specified in accordance with the initial graft function. Those requiring dialysis therapy within the first week after transplant form the delayed graft function (DGF) group. The others, who are nondialyzed and show a fast recovery of renal function with satisfactory diuresis, can be determined as immediate graft function (IGF) group. An intermediate ones, defined as a slow graft function (SGF) group, do not have IGF, but their graft dysfunction is not sufficient to be classified as DGF. Risk factors of SGF and DGF for deceased-donor (DD) grafts are well known. The purpose of our study was to investigate the predictive factors for IGF after living-donor (LD) kidney transplantation, which have not been well defined yet in the literature.

MATERIAL AND METHODS

The assessment of adult kidney transplants from living donor performed between August 20, 2014 and December 01, 2016 included 40 cases. The subjects with inadequate data or graft loss during the first week after transplant (caused by vascular thrombosis or other) were excluded. Also those with surgical complications, that may affect the initial graft function (e.g. urinary leakage, artery stenosis, folding of the artery), have not been admitted to evaluate. Both donation and transplantation procedures were held at the Department of General and Transplant Surgery, Medical University of Warsaw. Recipients were compared in two groups based on their initial graft function. IGF group was defined as the serum creatinine concentration level (SCr) lower than 3 mg/dl by 5th postoperative day (POD). SGF/DGF group included slow graft function patients defined as SCr above 3 mg/dl on 5th POD with no need for dialysis and those referred as delayed graft function, meaning first-week dialyzed or preemptively transplanted with 2th POD SCr higher than 0.9 of its pretransplant value. Maintenance immunosuppression included steroids, mycophenolate mofetil (2g/day initially) and tacrolimus (0.1 mg/kg/day) for all subjects. Induction treatment was delivered in all cases using basiliximab or thymoglobulin. Data of harvested kidney's weight and dimensions (pole-to-pole length, thickness and width measured through the middle of the hilum) after its cold preparation and perfusion were prospectively collected. Clinical evidence of age, sex, body mass index (BMI) and body surface area (BSA) were also gathered, in reference both to the donors and the recipients. Variables were compared between groups using t-Student, Cochran's C, Mann-Whitney and Chi-Square Pearson tests. A p value of 0.05 was considered significant. The analysis was specified at predictors for IGF, as compared with factors for SGF or DGF. All analyses were performed using StatSoft, Inc. (2014). STATISTICA (data analysis software system), version 12. www.statsoft.com.

RESULTS

Recipient characteristics

Of the 40 renal transplant recipients, IGF was observed in 24 cases (60%; with a male-to-female ratio of 13:11, [n.s.]) at the mean age of 34.9, ranged 20 – 60.3 years old. 16 patients (40%; with a male-to-female ratio of 12:4, [n.s.]) at the mean age of 36.6, ranged 22.3 – 65.7 years old were classified as SGF (12 cases in all) or DGF (4 cases). The difference noticed in average age value between the groups revealed no statistical significance (Mann – Whitney U=183, p=0.81). Mean recipient BMI was 22.37 kg/m^2 in IGF group, whilst 23.82 kg/m^2 in SGF/DGF group (Mann-Whitney U=149, p=0.235, [n.s.]). Average BSA (based on the Moesteller formula) was estimated on 1.78 m^2 vs. 1.85 m^2 consecutively for IGF and SGF/DGF (t-Student test, t(38)= -0.96, p=0.36, [n.s.]). Demographic characteristics of the recipients are shown in Table 1. 37 patients received left-sided organ (23 vs. 14 for IGF and SGF/DGF, respectively, [n.s.]). Right-sided organ was transplanted in 3 cases only (1 vs. 2 for IGF and SGF/DGF, respectively [n.s.]). No significant differences were observed in human leukocyte antigen (HLA) matching between the groups (Mann-Whitney U=137.5, p=0.13 [n.s.]). More than 3 HLA mismatches were found in 14 cases (58.3%) in IGF group, while in 5 cases (31.3%) in SGF/DGF group.

Donor characteristics

Renal transplantations were performed from 12 unrelated (7 vs. 5 for IGF vs. SGF/DGF) and 28 related (with the IGF-to-SGF/DGF ratio of 17:11) living donors. There were no significant difference observed in IGF vs. SGF/DGF cases between patients who received organ from related vs. genetically unrelated living donor (Chi-squared Pearson =0.020, p=0.89, [n.s.]). Mean donor age reached 44.96 y.o. (from 24.84 up to 72.49) and mean donor BMI was 24.23 kg/m^2 (ranged 19.16 - 32.63) for the recipients of IGF group. Similarly, donor age averaged 48.16 y.o. (in the range of 31.36 - 59.79) and mean donor BMI was 24.13 (ranged 20.57 - 29.04) for SGF/DGF recipients. No significant differences between groups were observed in terms of donors age (t-Student test, t(38)= -0.981, p=0.378) and donors BMI (t-Student test, t(27)= 0.095, p=0.93) as well.

Transplant characteristics

The analysis of impact of the organ-related factors for initial graft function was specially performed. The results are summarized in the Table 2. Mean cold ischemia time (CIT) was 48.4 minutes for IGF recipients vs. 66.8 minutes for SGF/DGF recipients (Mann-Whitney U=7.0, p=0.296, [n.s.]) and mean anastomosis time (AT) was 33.3 minutes vs. 36.88 minutes for IGF and SGF/DGF group consecutively (t-Student test, t(14)= -0.69, p=0.5, [n.s.]). There were no notable differences in terms of harvested organ's dimensions between compared groups. Mean length of the kidney transplanted with the IGF result was 115.2 mm and its averaged width and thickness were 58.3 mm and 41.9 mm, respectively. At

the same time, organs donated for SGF/DGF group were on mean 111.1mm-length, 56.3mm-width and they had an average of 42.3 mm of thick. It means that average volume of the donated organ (based on ellipsoid to approximate) was estimated at 145.8 cm^3 for IGF and 138.2 cm^3 for SGF/DGF consecutively (t-Student test, t(38)=0.60, p=0.55, [n.s.]). All harvested kidneys were weighed after their preparation and perfusion on the cold table, just before being transplanted. The accuracy of mensuration was 0.001 kg. Although the mean weight in IGF group was determined on 0.163 kg (in the range of 0.112 – 0.228) vs. 0.153 kg (in the range of 0.120 – 0.242) for SGF/DGF, no statistical significance was observed (Mann-Whitney, U =139, p=0.242). As it follows, the mass of 1cm3 of transplanted kidney is estimated at 1.17g in average for IGF recipient, while it is 1.16g for SGF/DGF group (Mann-Whitney, U=175, p=0.90, [n.s.]).

We also compared the impact of Kidney Weight/Recipient Weight Ratio (Kw/Rw), Kidney Weight/Recipient BMI Ratio (Kw/BMI) and Kidney Weight/Recipient BSA Ratio (Kw/BSA) on initial graft function in both recipient subgroups, as multiple prior studies have described this direction of association [1–3]. No significant differences were observed between the groups compared in current study (Table 3).

DISCUSSION

Delayed graft function is a common complication after kidney transplantation, that affects the allograft in the immediate post-transplant period and impacts on the long-term results of the procedure. IGF patients are observed to have a better long-term outcome of organ transplantation than SGF or DGF patients [4, 5]. SGF and DGF recipients have a lower renal function with serum creatinine concentration significantly worse at 12 months and higher rate of acute rejection (AR) episodes than IGF group [4]. Also worse graft survival for SGF and DGF is showed by some authors [4, 6]. However, studies on graft survival among recipients with SGF in comparison to DGF are conflicting, as Zeraati et al. observed in their study a similar impact of IGF and SGF on kidney graft survival and showed it being better than those of DGF [5]. At the same time, there is no differences observed in the incidence of AR among the SGF and DGF patients [4]. It means that kidney transplant recipients with SGF show a worse outcome than those with IGF, similar to DGF patients, despite not needing dialysis [4]. Narayanan et al. reported in their study consisted of 44630 adult US living transplant recipients, that death with graft function is more prevalent in patients classified as DGF [7]. DGF also has negative implications in terms of economic, because of additional costs related with prolonged hospitalization after surgery and possibly needed hemodialysis [6,8].

The reported frequency of DGF after DD kidney transplantation are extremely variable worldwide [6, 9, 10]. According to UNOS data, 23% of DD renal transplants in US, and even up to 30% in some centers in Europe, manifest an early dysfunction leading to the clinical syndrome of DGF [5]. At the same time, an average annual rate of DGF for LD kidney transplantations is estimated at about 3.5% in United States [11, 12]. Although the enumeration of its incidence suggests that the occurrence of DGF is statistically much less frequent after LD transplantation, its impact for the long-term outcome of the procedure is severe enough to warrant a strict monitoring to reduce a risk for individuals. Recognizing, a patients with higher risk of worse initial graft function is justified due to possibility of suitable and timely posttransplant intervention. It should be emphasized that any negative outcomes of living kidney donation affects not only the recipient, but may also be associated with psychological and emotional distress in the donor. And this may further tends to discourage other potential altruists from kidney donation.

The risk factors of slow or delayed graft function in deceased-donor kidney transplants are well-understood as a result of the large amount of evidence focused on this aspect. Also an effects of DGF and SGF on health outcomes for DD graft recipients have been well reported. Unfortunately, the same cannot be said for the living kidney transplants. There is a lack of evidence on this clinical issue and it seems to be a lack of awareness about it. Despite the lack of a large-scale studies on initial graft function factors in LD kidney transplants, those currently available studies show clearly the negative impact of DGF in terms of acute rejection and patient or graft survival in them likewise [5, 7, 11]. Some demographical and clinical risk factors for DGF in LD kidney recipients have been already described by Otaibi et al. [11]. Older donor age was assessed to be associated with initial DGF in LD transplantation by Lee et al. [13], however Lan et al. reported, that there were no significant difference in the incidence of DGF in patients who undergone LD kidney transplant from donors older than 60 y.o. in comparison to those with younger donor. However, it should be noted that a small size of older donor group in their study limits the interpretation of statistical significance [14]. Some other studies have also obtained the female gender of donor, allograft multiple arteries, previous transplantation as a DGF risk factors upon univariate analysis models, but it has been not confirmed by multivariate analysis yet [11, 15]. Molnar et al. reported in their study that recipient higher body weight and higher body mass index are associated with a higher risk of DGF [16]. Also an inflammatory markers, diabetes mellitus, ischemia and vascular anastomosis time, donor-recipient relatedness, duration of dialysis treatment, HLA mismatch and AB0 compatibility have been already investigated for possible association with the incidence of DGF after LD kidney transplantation [10, 11]. Factors mostly considered to impact the early outcome after DD renal transplantation are cold ischemia time (CIT), retransplantation, warm ischemia time (WIT), donor creatinine, recipient age and HLA-match. Transplantation of LD organ generally provides closer immunological match than DD grafts [11]. The incidence of DGF among DD transplants remains high due to the expansion of acceptable donors criteria (more frequent accepting marginal and older donors) in order to reduce the organ shortage [8]. It can be also caused by the qualification of recipients with greater predispositions to the development of DGF. As Redfield et al. identified, that LD kidney recipient with DGF were more often male,

diabetic, more HLA mismatched, highly sensitized and had higher BMI and longer CIT, what remains similar to DD kidney recipients [12]. Determination of the importance of specific factors in current study groups requires further research. However, planning a large-sample study is timely-limited, as annual total number of LD kidney transplantation in Poland still remains about 50 – 60 [17].

CONCLUSIONS

Conventionally, the perception of DGF predominant association with deceased-donor kidney transplant causes the scarcity of studies focused on DGF in living-donor kidney transplants. With an increasing number of LD kidney transplantations, detailed understanding of determinants of posttransplant results seems to be essential. Although there were a slight differences, leading toward those being reported worldwide, noticed between compared groups in our current analysis, no statistical significance was observed. The influence of a small sample cannot be excluded. Further studies, with a greater number of cases included, investigating a predictors of LD kidney transplant immediate function are urgently needed. Minimizing the risks associated with negative outcomes after living-donor graft transplantation will not only improve the direct recipient results, but will also have a positive impact on an incentive for other altruists to living kidney donation.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 29 – 34

ABBREVIATIONS

AT – Anastomosis time
BMI – Body mass index
BSA – Body surface area
CIT – Cold ischemia time
DD – Deceased donor
DGF – Delayed graft function
HLA – Human leukocyte antigen
IGF – Immediate graft function
LD – Living donor
POD – Postoperative day
SCr – Serum creatinine level
SGF – Slow graft function

REFERENCES

1. Wszoła M, Kwiatkowski A, Bednarska K, et al. The impact of kidney weight to recipient weight ratio (Kw/Rw) on kidney graft function after transplantation. MEDtube Science 2014; 2 (3): 13-17.
2. Kim Y, Moon J, Kim D, Kim S I. Ratio of donor kidney weight to recipient bodyweight as an index of graft function Germline SDHD mutation in familial phaeochromocytoma. Lancet 2001; 357: 1180–1181.
3. Hwang J, Kim Y, Kim S, et al. Does Donor Kidney to Recipient Body Weight Ratio Influence Long- Term Outcomes of Living-Donor Kidney Transplantation? Transpl Proc 2011; 44: 276–280.
4. Rodrigo E, Ruiz J, Pinera C, et al. Similar Impact of Slow and Delayed Graft Function on Renal Allograft Outcome and Function. Transpl Proc 2005; 37: 1431–1432.
5. Zeraati A, Naghibi M, Kianoosh S, Ashraf H. Impact of Slow and Delayed Graft Function on Kidney Graft Survival Between Various Subgroups Among Renal Transplant Patients. Transpl Proc 2009; 41: 2777-2780.
6. Martínez E, Mateu L, Calabuig A, et al. Delayed Graft Function After Renal Transplantation: An Unresolved Problem. Transpl Proc 2011; 43: 2171-2173.
7. Narayanan R, Cardella C, Cattran D, et al. Delayed Graft Function and the Risk of Death With Graft Function in Living Donor Kidney Transplant Recipients. Am J Kidney Dis 2010; 56: 961-970.
8. Ounissi M, Cherif M, Abdallah T, et al. Risk Factors and Consequences of Delayed Graft Function. Saudi J Kidney Dis Transpl 2013; 24: 243–246.
9. Perico N, Cattaneo D, Sayegh M, Remuzzi G. Delayed graft function in kidney transplantation. Lancet 2004; 364: 1814-1827.
10. Weissenbacher A, Jara M, Ulmer H, et al. Recipient and Donor Body Mass Index as Important Risk Factors for Delayed Kidney Graft Function. Transplantation 2012; 93: 524–529.
11. Otaibi T, Ahmadpoor P, Abdulmajid A, et al. Delayed Graft Function in Living-Donor Kidney Transplant: A Middle Eastern Perspective. Exp Clin Transplant 2016; 1: 1–11.
12. Redfield R, Scalea J, Zens T, et al. Predictors and outcomes of delayed graft function after living-donor kidney transplantation. Transpl Int 2016; 29: 81-87.
13. Lee S, Chung B, Piao S, et al. Clinical significance of slow recovery of graft function in living donor kidney transplantation. Transplantation 2015; 90(1): 38-43.
14. Lan G, Yang L, Peng L, et al. Long-term Results of Renal Transplant From Living Donors Aged Over 60 Years. Exp Clin Transpl 2012; 5: 471–474.
15. Ghods A, Savaj S, Abbasi M, Heidari H, Rokhsatyazdi H. The incidence and risk factors of delayed graft function in 689 consecutive living unrelated donor renal transplantation. Transpl Proc 2007; 39: 846-847.
16. Molnar M, Kovesdy C, Mucsi I, et al. Higher recipient body mass index is associated with post-transplant delayed kidney graft function. Kidney Int 2011; 80(2): 218-224.
17. Organizacyjno-koordynacyjne Centrum do Spraw Transplantacji POLTRANSPLANT. Biuletyn informacyjny 2016; 1(24): 30.

LIST OF TABLES

Tab. 1. Clinical features of study groups.
Tab. 2. Clinical details of transplanted organs.
Tab. 3. Comparison of the analysis results between study groups.

TAB. 1. CLINICAL FEATURES OF STUDY GROUPS.

	IGF Group	SGF/DGF Group	P value
No. of patients	24	16	-
Sex Male Female	 13 11	 12 4	0.182 [n.s.]
Mean age [years]	34.9 (20.0 – 60.3)	36.6 (22.3 – 65.7)	0.81 [n.s.]
Mean BMI [kg/m^2]	22.37	23.82	0.235 [n.s.]
Mean BSA [m^2]	1.78	1.85	0.36 [n.s.]
Donors characteristics			
Donor-recipient relatedness Related Unrelated	 17 7	 11 5	0.89 [n.s.]
HLA mismatches: average no.	3.0	2.13	0.13 [n.s.]
0 of 6	1	3	
1 of 6	3	0	
2 of 6	6	8	
3 of 6	8	3	
4 of 6	1	1	
5 of 6	1	1	
6 of 6	4	0	
Mean donor age [years]	44.96 (24.84 – 72.49)	48.16 (31.36 – 59.79)	0.378 [n.s.]
Mean donor BMI [kg/m^2]	24.23	24.13	0.93 [n.s.]

n.s. – non-significant

TAB. 2. CLINICAL DETAILS OF TRANSPLANTED ORGANS.

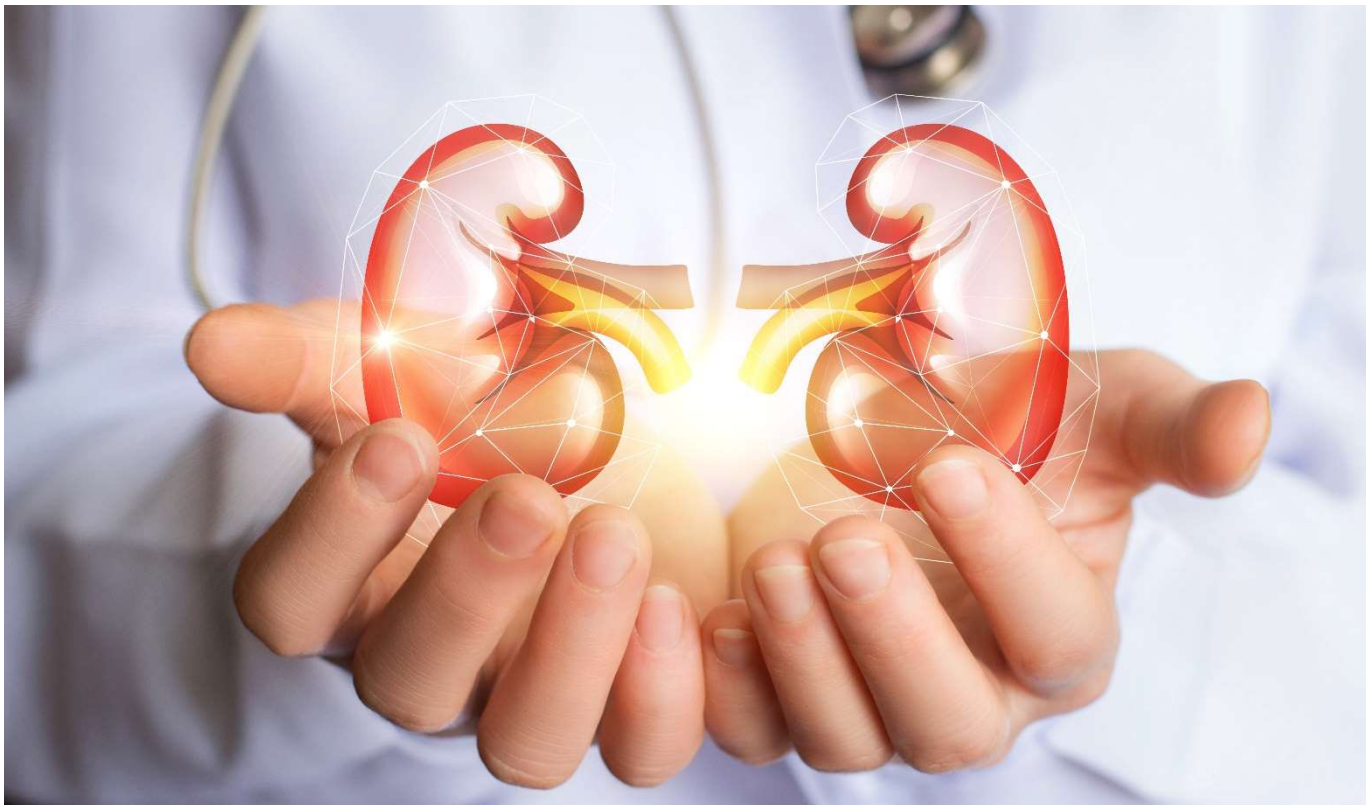
	IGF Group	SGF/DGF Group	P value
Mean length [mm]	115.2 (95.0-135.0)	111.1 (95.0-130.0)	0.233 [n.s.]
Mean width [mm]	58.3 (30.0 – 75.0)	56.3 (40.0 – 75.0)	0.57 [n.s.]
Mean thickness [mm]	41.9 (30.0 – 60.0)	42.3 (30.0 – 55.0)	0.90 [n.s.]
Mean volume [cm^3]	145.8 (83.65 – 238.1)	138.2 (78.5 – 197.2)	0.55 [n.s.]
Mean weight [kg]	0.163 (0.112 – 0.228)	0.153 (0.120 – 0.242)	0.242 [n.s.]
Mass of $1cm^3$ [g]	1.17 (0.76 – 1.95)	1.16 (0.78 – 1.73)	0.9 [n.s.]
Mean CIT [min]	48.4 (27.0 – 75.0)	66.8 (45.0 – 120.0)	0.296 [n.s.]
Mean AT [min]	33.3 (23.0 – 45.0)	36.88 (23.0 – 58.0)	0.5 [n.s.]

n.s. – non-significant

TAB. 3. COMPARISON OF THE ANALYSIS RESULTS BETWEEN STUDY GROUPS.

	IGF Group	SGF/DGF Group	P value
Mean Kidney Weight/Recipient Weight Ratio (Kw/Rw), g/kg	2.5 (1.64 – 4.3)	2.3 (1.35 – 4.2)	0.116 [n.s.]
Mean Kidney Weight/Recipient BMI Ratio (Kw/BMI), gm2/kg	7.4 (4.65 – 10.5)	6.7 (4.1 – 14.0)	0.145 [n.s.]
Mean Kidney Weight/Recipient BSA Ratio (Kw/BSA) g/m2	93.0 (66.4 – 141.0)	85.5 (57.4 – 140.9)	0.137 [n.s.]

n.s. – non-significant



THE CORRELATION BETWEEN LIVING DONOR'S GLOMERULAR FILTRATION RATE AND EARLY KIDNEY ALLOGRAFT FUNCTION

Kwapisz Magdalena, Kieszek Rafal, Bieniasz Monika, Jedrzejko Kalina, Kwiatkowski Artur

Department of General and Transplantation Surgery, Orlowski Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

#Corresponding author: Rafal Kieszek, email: rafal.kieszek@gmail.com, Department of General and Transplantation Surgery, Orlowski Transplantation Institute, Medical University of Warsaw, Infant Jesus Teaching Hospital, ul. Nowogrodzka 59, 02-006 Warsaw, phone number +48 22 5021470

RUNNING TITLE	The correlation between GFR and IGF
KEYWORDS	living kidney donor; glomerular filtration rate; immediate graft function; delayed graft function; kidney transplant result
WORD COUNT	2212
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

Glomerular filtration rate of living kidney donor candidate must be appropriate to provide him sufficient filtration after unilateral nephrectomy and to ensure satisfactory renal graft function to recipient as well. Predictors for immediate function of living donor kidney transplant have not been well defined yet in the literature. Meanwhile, immediate graft function is well-known as closely related with better long-term outcome of transplantation procedure. The aim of the study was to analyse the impact of donor's pre-donation estimated glomerular filtration rate on initial function of living donor kidney allograft. Analysed set consisted of 129 living kidney donors. Comparative analysis was performed between IGF Group (including cases with immediate graft function defined as recipient's serum creatinine concentration below 3 mg/dl in 5th postoperative day) and Non-IGF Group (consisted of all other cases). GFR was estimated before nephroureterectomy procedure with using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. There were 82 cases in the mean age of 44.8±10.54 years (55 were female) included to IGF Group and 47 cases in the mean age of 47.22±11.04 years (35 were female) included to Non-IGF Group. Mean GFR was estimated at 97.66±14.64 ml/min/1.73m^2 (ranged, 63.07 – 123.40 ml/min/1.73 m^2) in the IGF Group vs. 90.82±16.24 ml/min/1.73 m^2 (ranged, 50.6 – 125.24 ml/min/1.73 m^2) in Non-IGF Group. The finding was statistically significant (p=0.018). The optimum GFR cut-off point was calculated at 97.04 ml/min/1.73 m^2 in analysed cohort. Higher donor's glomerular filtration rate correlates with the incidence of immediate graft function in living donor kidney transplantation, thus also affects long-term outcome.

BACKGROUND

Proper determination of living-donor kidney function guarantees the longevity of post-transplantation donor renal function and recipient graft function. Ensuring the safety and well-being to every living kidney donor is a priority value for any transplant centre conducting the program of living-donor kidney transplants. The influence of unilateral nephroureterectomy procedure on the risk of post-donation renal failure development has been repeatedly studied so far. The follow-up systems provide the proper care of donors residual kidney function. However, it should be noted that acceptable glomerular filtration rate (GFR) in a living kidney donor candidate is that which can be predicted to provide adequate GFR for both donor and recipient after nephrectomy/transplantation procedure. Most studies of living kidney donor (LKD) GFR analyse its correlation with post-donation residual kidney function in this healthy volunteer. The number of reports on the significance of GFR values for a recipient of a kidney transplant from LKD is insufficient. However, it is well established that the initial kidney graft function has a significant impact on long-term outcomes. The aim of this study was to analyse the impact of estimated glomerular filtration rate on initial graft function in living donor kidney transplantation.

MATERIAL AND METHODS

Retrospective cohort analysis of 129 LKDs, who underwent unilateral nephroureterectomy for organ donation were performed. All the procedures were carried out at the Department of General and Transplant Surgery, Medical University of Warsaw, Poland, from March 2009 to May 2018. Estimated GFR before nephroureterectomy procedure was evaluated. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used for the estimation (according to the formula as followed [1]: GFR = 141 * $\min(SCr/\kappa,1)^{\alpha}$ * $\max(SCr/\kappa, 1)^{-1.209}$ * 0.993^{Age} * 1.018 [if female] * 1.159 [if black]; in which SCr is serum creatinine (mg/dl), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1). Comparative analysis was performed between two subgroups. IGF Group consisted of a cases with immediate graft function (IGF) defined as recipient's serum creatinine concentration below 3 mg/dl in 5th postoperative day. All other cases were included to Non-IGF Group. There were both delayed graft function (DGF) and slow graft function (SGF) cases.

Analyses were performed using Dell Inc. (2016). Dell Statistica (data analysis software system), version 13. software.dell.com. The examined variables were compared with the use of the t-Student and Mann-Whitney tests (for continuous variables) or the Chi-Square Pearson test (for categorical variables). Statistical significance was defined as p-value of less than 0.05. Youden's index in conjunction with receiver operating characteristic (ROC) was used for calculating the optimum GFR cut-off point.

RESULTS

Immediate graft function was diagnosed in 63.57% of evaluated cases. There were 82 LKDs in the mean age of 44.8±10.54 years (with the male-to-female ratio 27:55) included to IGF Group. Non-IGF Group consisted of 47 LKDs in the mean age of 47.22±11.04 years (with the male-to-female ratio 12:35). Nephrectomies were mostly left-sided in both groups (84.15% in IGF Group vs. 80.85% in Non-IGF Group; p=0.632; n.s.). Demographic characteristic (age-sex structure) of organ recipients were also comparable [Table 1]. Mean pre-donation GFR was observed higher in IGF cases. It was estimated at 97.66±14.64 ml/min/1.73m^2 (ranged, 63.07 – 123.40 ml/min/1.73m^2) in IGF Group vs. 90.82±16.24 ml/min/1.73m^2 (ranged, 50.6 – 125.24 ml/min/1.73m^2) in Non-IGF Group. The finding was statistically significant (Mann-Whitney U=1444, p=0.018) [Figure 1]. The optimum threshold GFR value was evaluated at 97.04 ml/min/1.73m^2 in analysed set (Youden's index = 0.25). The trade-off between clinical sensitivity and specificity for every possible cut-off point was presented on the ROC Chart (the area under the ROC curve (AUC) = 0.625) [Figure 2].

DISCUSSION

A shortage of organs for transplantation caused interest in broadening the acceptance range for the organ donors. The growing gap between demand and supply for kidney transplants has led to the use of expanded criteria deceased-donors (ECD) in an effort to increase the donor pool. Most studies of the results of kidney transplantation confirm the generally worse outcomes with a lower survival rates of ECD allografts when compared to the standard criteria donor kidneys [2]. However, even ECD recipients have a survival rates improved than wait-listed dialysis patients [3]. Also the living donor kidney transplantations have been already extended for marginal organs. However, in case of living kidney donation, not only an increased risk of graft failure, but mostly the possibility of accelerated loss of donors renal function after nephrectomy should be taken into consideration during decision making to accept or decline living kidney donor candidates. Comprehensive assessment of the candidate's general health condition is always necessary. Any abnormalities related with the increased risk of renal failure must be mandatory excluded. A complete medical evaluation of kidney function prior to donation is constantly the most important qualification stage. Glomerular filtration rate (GFR) of living kidney donor candidate must be appropriate to ensure not only satisfactory renal graft function to recipient, but also to provide donor sufficient filtration after unilateral nephrectomy as well.

The acceptable level of renal function has been the subject of numerous discussions so far. Some literature report that pre-donation GFR < 80 ml/min/1.73 m^2 cannot be expected to provide an optimal kidney function after nephrectomy and an estimated GFR > 80 ml/min (or 2 standard deviations below normal, based on age, gender, and BSA corrected to 1.73/m^2) is commonly acceptable. Lower values generally precludes donation, but any candidacies should be considered individually on a case

by case. The european guidelines of ERBP (European Renal Best Practice) on the lowest value of glomerular filtration rate that exclude kidney donation are very unprecise. It is only recommended that all potential living kidney donors have a GFR value that provide satisfactory renal function after donation [4]. It must be sufficient to ensure an effective GFR to the anticipated end of life, independently of the age at which he or she donated. According to KDIGO (Kidney Disease: Improving Global Outcomes) recommendations [5], GFR ≥90 ml/min/1.73m^2 is acceptable for kidney donation and GFR <60 ml/min/1.73m^2 is a lower threshold value to routinely decline donor candidate, whereas GFR of 60–89 ml/min/1.73 m^2 is an intermediate range in which transplant centres can individualize decisions on the basis of clinical risk factors and age, as the renal function deteriorates over the years of life. As many as 20% of U.S. transplant centres accept a creatinine clearance even as low as 60 ml/min [6–8]. Cohen et al. noted a decrease in GFR of approximately 1 ml/min/1.73m^2 per 1 year [9]. If so, definition of "normal" GFR also changes over time. On this basis, the acceptable GFR values by donor age prior to donation were compiled by a Joint Working Party of The British Transplantation Society and The Renal Association. According to the Third edition (May 2011) of United Kingdom Guidelines For Living Donor Kidney Transplantation, the safe threshold value for pre-operative GFR oscillates at 80 ml/min/1.73m^2 for adults up the age of 46 years and declines to 50 ml/min/1.73 m^2 at age of 80 [8]. It is estimated that it will ensure a GFR of the remaining kidney greater than 37.5 ml/min/1.73 m^2 at age 80 after unilateral nephroureterectomy (given the reduction in GFR due to donation and a cautious estimate of the rate of annual decline). In the transplant centre represented by authors of current study, the original requirement to qualify for donation is GFR > 80 ml/min/1.73m^2. Therefore, the majority of individuals (70%) had a creatinine clearance of greater than 60 ml/min/1.73m^2 on the long-term follow-up, and none were below 33 ml/min/1.73m^2 so far [10]. It must be emphasized, that unilateral nephrectomy in healthy volunteers leads to the reduction of a half of the renal mass, not a half of the renal function. The phenomenon of the compensatory adaptation mechanism of the residual kidney after contralateral nephrectomy causes that creatinine clearance of LKD decreases approximately by 30 – 35% of its pre-donation value [10, 11, 12].

"Normal" renal function should be determined by GFR assessment. However, the unified criteria of GFR evaluation methods for living kidney donors have not been established. Variety of methods are available, including estimating equations and clearance measurements, but the accuracy of them is not known with sufficient certainty to define specific threshold values for each one. Some guidelines recommend that GFR should be measured by using a clearance of an exogenous filtration markers, rather than estimated, however measurement methods are difficult, expensive and time-consuming [13]. Huang et al. demonstrated that measured GFR may not be necessary in a large portion of donor candidates as estimated GFR is high enough to ensure > 95% probability that measured GFR would be greater than 80 ml/min/1.73m^2 [14]. 24-hour urine creatinine clearance with creatinine-based GFR estimation equations as the primary method of GFR evaluation are mostly used in U.S. transplant centres. Less than 10% of transplant units use GFR measurement for donor kidney function evaluation [15]. Methods using the clearance of an exogenous filtration markers are required only if there are doubts about the accuracy of the GFR values determined by estimation methods [16]. The authors of current study use 2-stage GFR estimation with CKD-EPI equation in their transplant program as serum creatinine level is considered inadequate because of its interdependence of muscle mass, age, gender and body weight. Transplant candidates commonly undergo magnetic resonance imaging to characterize the renal anatomy before surgery, which additionally allow for the opportunity to measure GFR via contrast media clearance. Within the evaluation process of living kidney donors, split renal function is usually evaluated by renal scintigraphy. The KDIGO 2012 Evaluation and Management of Chronic Kidney Disease Guidelines recommend implementation of The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula as a method of glomerular filtration rate estimation. Both CKD-EPI and Modification of Diet in Renal Disease (MDRD) equations were found to be equally accurate in patients with estimated GFR lower than 60 ml/min/1.73m2, however the CKD-EPI equation is more precise and less biased than the MDRD formula in subgroup with GFR values between 60 and 120 ml/min/1.73 m^2 [1].

The accurate evaluation of kidney function in living donor candidates provides the prediction of long-term risk of end-stage renal disease (in the absence of and after donation) based on level of pre-donation GFR and other factors. As can be found in numerous literature reports, GFR loss to less than 60 ml/min/1.73m^2 is rated as 15 - 30% of cases of a large LKDs cohorts, depending on the transplant centre and measurement method [10, 17] - [19]. ESRD was noticed in only 0.3% - 0.5% cases [17], [20, 21]. However, it should be emphasized that the long-term impact of GFR lower than 60 ml/min after unilateral nephrectomy in healthy donors is still poorly understood and it cannot be excluded that the implications may not be the same as chronic kidney disease stage 3 in other health conditions. As current study has shown, the value of pre-donation donor's GFR is important not only for organ donor but for the transplant recipient as well. It allows to predict the immediate graft function, which is a factor affecting the long-term outcome. Some previous reports identified deceased donor final serum creatinine level as an independent risk factor for development of DGF, after both cardiac and brain death donation, though the cut-off values of terminal serum creatinine levels differ from centre to centre [22–26]. The association of the incidence of IGF with the creatinine clearance of the living kidney donor has not been reported in an indisputable way so far. In the case of living donor organ transplantation, the higher expectations of positive results are justified, not only due to the donor's altruistic attitude, but also because of the often emotional relationship between the donor and his/her recipient. It is well established, that transplantation from LKD is not only

related with better long-term outcome than deceased donor (DD) transplant, but also may be the only chance for transplant for some highly immunized patients waiting in the National Waiting List. Therefore, each potential donor candidate should be considered individually, as justified by the cases of living donor marginal organs that were successfully transplanted [27–30]**.** Volunteers should not be disqualified too hastily. The lack of organs for transplantation justifies the clinicist's temptations to qualify donors with GFR as low as possible. However, a strict definition of a threshold value, that will be not only safe for the donor, but also favourable for the recipient, is still missing. The analysis of our collected data showed that GFR value of 97.04 ml/min/1.73m^2 is the optimum threshold for the recipient. Donor's pre-donative GFR estimated above 97 ml/min/1.73m^2 revealed as a predictive factor of immediate graft function and thus as a predictor of better long-term outcome.

CONCLUSION

Pre-donative renal function of living kidney donor not only determines his/her postoperative remaining kidney function, but also significantly affects allograft function in the recipient. Higher donor's glomerular filtration rate correlates with the higher incidence of immediate graft function, thus also affects long-term outcome of living donor kidney transplantation. However, each donor candidacy should be considered individually on the basis of many clinical factors. Development of the criteria for an optimal donor selection should be further continued.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 35 – 40

ABBREVIATIONS

CKD EPI – The Chronic Kidney Disease Epidemiology Collaboration
DD – deceased donor
DGF – delayed graft function
ECD – expanded criteria donor
ERBP – European Renal Best Practice
IGF – immediate graft function
GFR – glomerular filtration rate
KDIGO – Kidney Disease: Improving Global Outcomes
LKD – living kidney donor
MDRD – Modification of Diet in Renal Disease
ROC – receiver operating characteristic
SGF – slow graft function

REFERENCES

1. Levey A, Stevens L, Schmid Ch, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150 (9): 604–612.
2. Rao P, Ojo A. The alphabet soup of kidney transplantation: SCD, DCD, ECD - Fundamentals for the practicing nephrologist. Clin J Am Soc Nephrol 2009; 4 (11): 1827–1831.
3. Filiopoulos V, Boletis JN. Renal transplantation with expanded criteria donors: Which is the optimal immunosuppression? World J Transplant 2016; 6 (1): 103-114.
4. Abramowicz D, Cochat P, Claas F, et al. European Renal Best Practice Guideline on kidney donor and recipient evaluation and perioperative care. Nephrol Dial Transplant 2015; 30 (11): 1790–1797.
5. Lentine KL, Kasiske BL, Levey AS, et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. Transplantation 2017; 101 (8): 1-109.
6. Bertolatus J, Goddard L. Evaluation of renal function in potential living kidney donors. Transplantation 2001; 71 (2): 256–260.
7. Delmonico F. A report of the Amsterdam forum on the care of the live kidney donor: Data and medical guidelines. Transplantation 2005; 79 (6): 491–492.
8. Bia M, Ramos E, Danovitch G, et al. Evaluation of living renal donors. The current practice of US transplant centers. Transplantation 1995; 60 (4): 322-327.
9. Cohen E, Nardi Y, Krause I, et al. A longitudinal assessment of the natural rate of decline in renal function with age. J Nephrol 2014; 27 (6): 635–641.
10. Kwapisz M, Kieszek R, Jędrzejko K, Bieniasz M, Gozdowska J, Kwiatkowski A. Efficacy of follow-up care system of living kidney donor in monitoring of residual kidney function. Transplant Proc 2018; 50 (6): 1590-1596.
11. Wetzels J, Kiemeney L, Swinkels D, Willems H, Heijer M. Age- and gender-specific reference values of estimated GFR in Caucasians: The Nijmegen Biomedical Study. Kidney Int 2007; 72 (5): 632–637.
12. Katz A, Epstein F. Relation of glomerular filtration rate and sodium reabsorption to kidney size in compensatory renal hypertrophy. Yale J Biol Med 1967; 40 (3): 222–230.
13. Stevens L, Levey A. Measured GFR as a Confirmatory Test for Estimated GFR. J Am Soc Nephrol 2009; 20 (11): 2305–2313.
14. Huang N, Foster M, Lentine K, et al. Estimated GFR for living kidney donor evaluation. Am J Transplant 2016; 16 (1): 171–180.
15. Mandelbrot D, Pavlakis M, Danovitch G, et al. The medical evaluation of living kidney donors: a survey of US transplant centers. Am J Transplant 2007; 7: 2333-2343.
16. Davis C. Evaluation of the living kidney donor: current perspectives. Am J Kidney Dis 2004; 43: 508.
17. Ibrahin M, Foley R, Tan L, et al. Long-term consequences of kidney donation. N Eng J Med 2009; 360 (5): 459–469.
18. Cherikh W, Young C, Kramer B, Taranto, Randall H, Fan P. Ethnic and gender related differences in the risk of end-stage renal disease after living kidney donation. Am J Transplant 2011; 11 (8): 1650–1655.
19. Inker L, Astor B, Fox C, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. Am J Kidney Dis 2014; 63 (5): 713–735.

20. Fehrman-Ekholm I, Norden G, Lennerling A, et al. Incidence of end-stage renal disease among live kidney donors. Transplantation 2006; 82 (12): 1646–1648.
21. Wafa E, Refaie A, Abbas T, et al. End-stage renal disease among living-kidney donors: Single- center experience. Exp Clin Transplant 2011; 9 (1): 14-19.
22. Jung G, Yoon M, Kim S, et al. The risk factors of delayed graft function and comparison of clinical outcomes after deceased donor kidney transplantation: Single-center study. Transplant Proc 2010; 42 (3): 705–709.
23. Helfer M, Vicari A, Spuldaro F, Gonçalves L, Manfro R. Incidence, risk factors, and outcomes of delayed graft function in deceased donor kidney transplantation in a brazilian center. Transplant Proc 2014; 46 (6): 1727–1729.
24. Patel S, Duhart B, Krauss A, et al. Risk factors and consequences of delayed graft function in deceased donor renal transplant patients receiving antithymocyte globulin induction. Transplantation 2008; 86 (2): 313–320.
25. Quiroga I, McShane P, Koo D, et al. Major effects of delayed graft function and cold ischaemia time on renal allograft survival. Nephrol Dial Transplant 2006; 21 (6): 1689–1696.
26. Shao M, Ye Q, Ming Y, et al. Delayed graft function after DCD kidney transplantation: risk factors for and impact on transplantation. J Cent South Univ (Med Sci) 2012; 37 (10): 1045-1049.
27. Li Z, Wang Y, Ma L, et al. Marginal living donor in kidney transplantation: experience in a Chinese single center. Chin Med J (Engl) 2013; 126 (15): 2810-2804.
28. Oikawa O, Hatakeyama S, Narita T, et al. Safety and effectiveness of marginal donor in living kidney transplantation. Transplant Proc 2016; 48 (3): 701-705.
29. Soboniak M, Kieszek R, Jedrzejko K, Gozdowska J, Kwapisz M, Kwiatkowski A. Living kidney donor with expanded criteria for qualification – a case report. MEDTube Science 2016; 2 (4): 29-31.
30. Kwapisz M, Kieszek R, Jedrzejko K, et al. Can transplantation of kidney from living related donor with both-sided double renal arteries to ADPKD patient allergic to muscle relaxant succeed? – a case study. MEDtube Science 2017; 5 (3): 34 – 37.

LIST OF TABLES

Tab. 1. Clinical and demographic features of living kidney donors.

LIST OF FIGURES

Fig. 1. Pre-donative value of living kidney donors glomerular filtration rate.
Fig. 2. ROC chart.

TAB. 1. CLINICAL AND DEMOGRAPHIC FEATURES OF LIVING KIDNEY DONORS.

	IGF Group	Non-IGF Group	p-value
No. of cases	82	47	-
Male-to-female ratio	27:55	12:35	0.379
Mean age ±SD [years]	44.8±10.54 [range 23.53 – 72.49]	47.22±11.04 [range 27.85 – 70.09]	0.225
Nephrectomy side [left : right]	69 : 13	38 : 9	0.632
Mean GFR ±SD [ml/min/1.73m^2]	97.66±14.64 [range 63.07 – 123.4]	90.82±16.24 [range 50.6 – 125.24]	**0.018**
Recipient's age ±SD [years]	35.74±11.23 [range 19.08 – 60.31]	35.97±11.27 [range 16.4 – 65.73]	0.877
Recipient's gender [M:F]	55:27	38:9	0.093

FIG. 1. PRE-DONATIVE VALUE OF LIVING KIDNEY DONORS GLOMERULAR FILTRATION RATE.

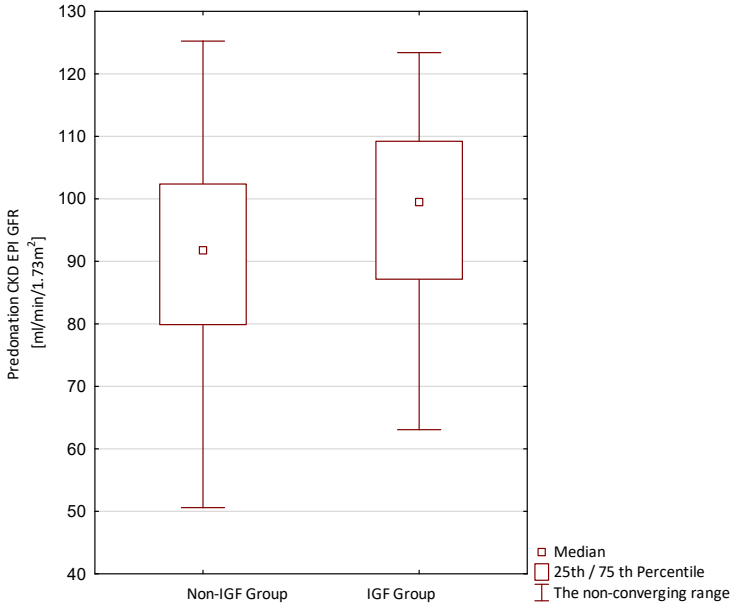
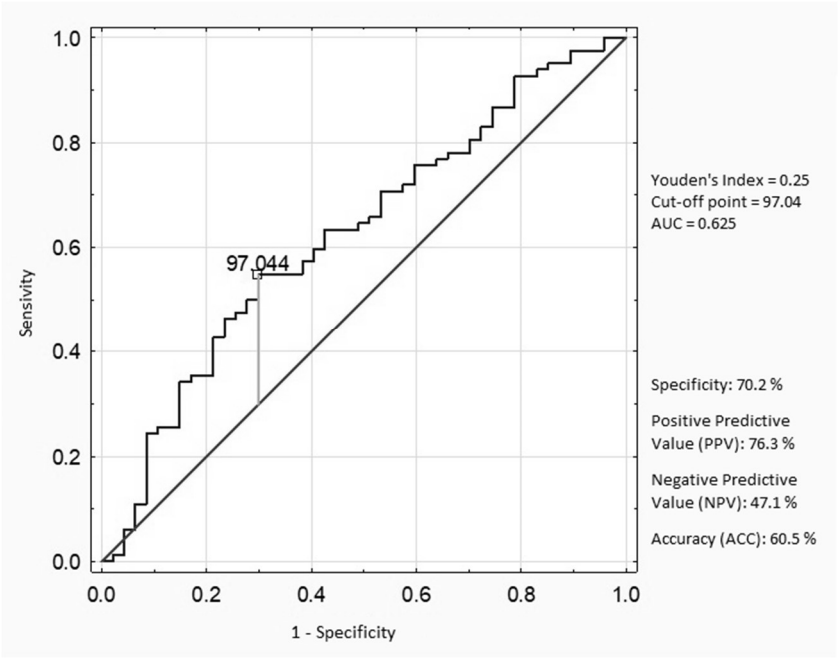
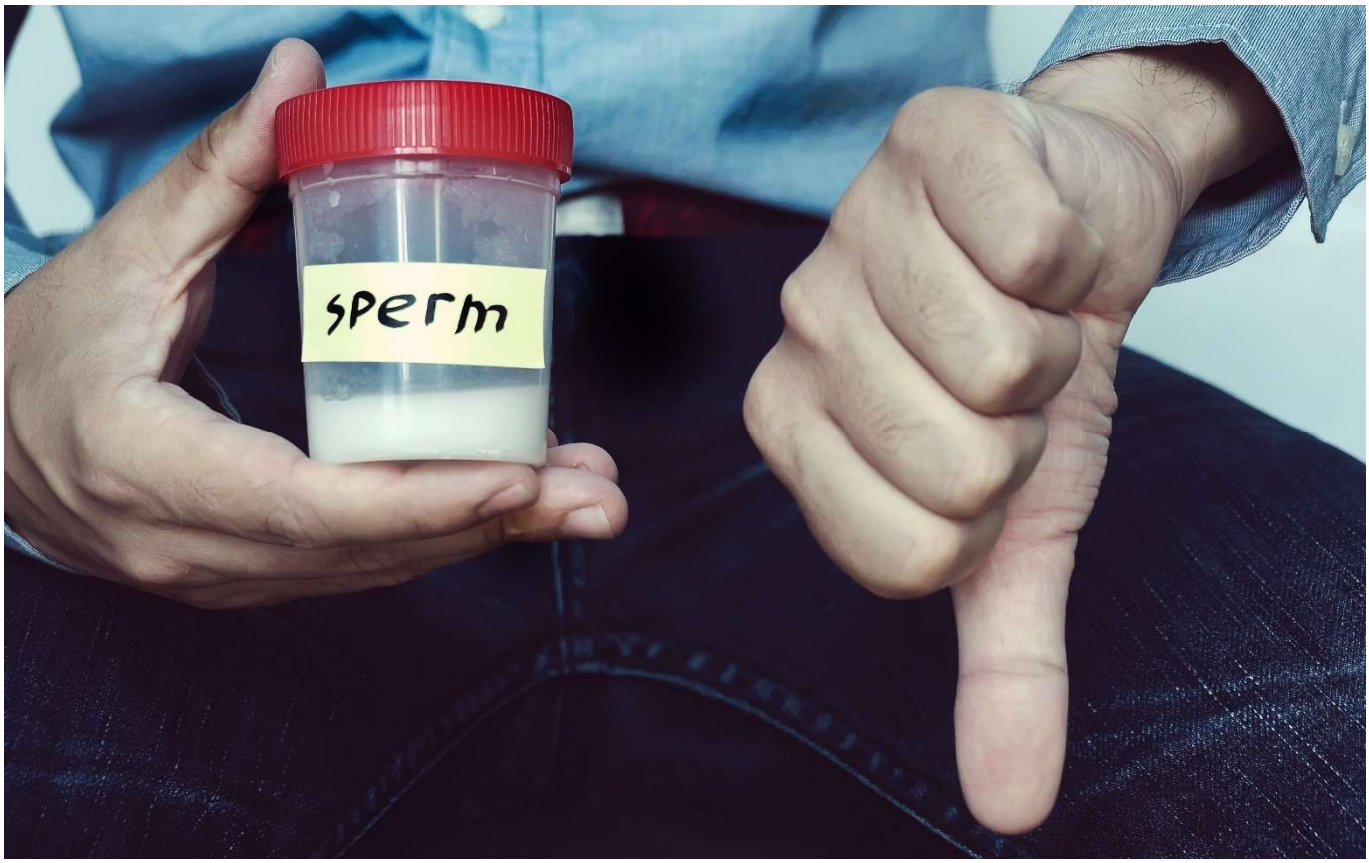


FIG. 2. ROC CHART.





MALE INFERTILITY – CAUSES, DIAGNOSIS AND MANAGEMENT. A REVIEW

Agata Golik[1], Joanna Kacperczyk-Bartnik[2], Pawel Bartnik[2], Agnieszka Dobrowolska-Redo[2], Ewa Romejko-Wolniewicz[2]

1. Students' Scientific Group affiliated to 2nd Department of Obstetrics and Gynecology, Medical University of Warsaw
2. 2nd Department of Obstetrics and Gynecology, Medical University of Warsaw

#Corresponding author: Pawel Bartnik, e-mail: bartnik.pawel@gmail.com, 2nd Department of Obstetrics and Gynecology, Medical University of Warsaw, Karowa 2 St, p. o. box 00-315 Warsaw, Poland, phone number: +48 22 5966421

RUNNING TITLE	Male infertility
KEYWORDS	male infertility; male urogenital diseases; reproductive health
WORD COUNT	2729
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

The World Health Organization defines infertility as an inability to get pregnant after a year of regular intercourse, 3-4 times a week, without use of preventive methods. The problem of infertility concerns about 15% of the population, in 20-30% of cases the cause is on the male side, in 20-35% couples struggle with female infertility, and in 25-40% of cases fertility problems concern both partners. Male infertility is defined as the inability of a man to cause pregnancy in a fertile woman. Male infertility is caused by change in sperm concentration and/or motility and/or morphology in at least one sample of two semen analyses. The reasons for male infertility can be divided into four main groups: primary testicular defects in spermatogenesis, endocrine and systemic disorders, sperm transport disorders, idiopathic male infertility. There are also many reasons that worsen semen quality, resulting in difficulty in conceiving offspring, we can distinguish neurological disorders, obesity, smoking, autoimmune diseases or intensive sports. The causes of infertility can lie on both the male and female side, therefore it is important to examine both partners. Diagnosis of male infertility usually includes: medical history, physical examination, semen analysis, scrotal and transrectal ultrasound, hormonal tests, testicular biopsy. Further diagnosis and treatment depends on the results of semen analysis. Much of the causes of male infertility are idiopathic, however if the cause is determined, treatment is targeted and results in high success rate. Both conservative methods and surgical procedures are used in therapy. It should also be borne in mind that male infertility is often a multifaceted problem; supportive therapy is necessary in addition to targeted therapy.

BACKGROUND

Infertility is a common condition with significant psychological, economic, demographic and medical consequences. Male infertility is defined as the incapacity of a man to cause pregnancy in a fertile woman. Male infertility is caused by change in sperm concentration and/or motility and/or morphology in at least one sample of two semen analyses, collected at an interval of 1 and 4 weeks. It affects about 7% of all men [1, 2].

Men, whose semen parameters are below the normal values, defined by the World Health Organization (WHO), are infertile. The most common disturbances in the quantity and quality of semen include: oligospermia (<15 million spermatozoa/mL), azoospermia (no sperm count in the ejaculate), asthenospermia (<40% motile spermatozoa) or teratozoospermia (<4% normal forms). The origin of male infertility can arise from genetic disorders, disturbances in sperm transport, may have autoimmune background or other, often idiopathic cause [1, 2].

This article presents the most common causes of male infertility, methods of their diagnosis, as well as the treatment options.

CAUSES OF MALE INFERTILITY

The reasons for male infertility can be divided into four main groups: primary testicular defects in spermatogenesis – 65-80%, endocrine and systemic disorders – 2-5%, sperm transport disorders – 5%, idiopathic male infertility – 10-20% [3].

PRIMARY TESTICULAR DEFECTS IN SPERMATOGENESIS

The causes of primary testicular defects are usually unknown, defined as idiopathic. A significant group of infertile men have congenital and developmental disorders resulting in primary testicular defects in spermatogenesis. The most common congenital reasons are described below.

Klinefelter syndrome (KS) (47, XXY) is the most common chromosomal disorder in men (1: 650 born boys). It involves the presence of at least one additional X chromosome. Patients often have small testes and male gonads are unable to produce sperm (azoospermia) or produce them in a reduced amount (oligospermia) [4].

Cryptorchidism, undescended testes, are characterized by the absence of one or both testes in the scrotum. Men with the history of cryptorchidism have oligozoospermia or azoospermia and lower sperm quality [5]. Infertility is much more common in cases of bilateral undescended testes, in unilateral cases fertility is affected less often. The study of Kobayashi et al. found that sperm density was decreased in about 30% of men with unilateral cryptorchidism, in cases of bilateral undescended testicles about 50% of patients presented reduced sperm density [6]. Patients with unilateral cryptorchidism were characterized by rates of paternity within the normal ranges and showed normal sperm concentration in comparison to only 2 out of 15 men with bilateral cryptorchidism in the past, managing to obtain offspring [6].

Miotonic dystrophy is an autosomal dominant disease, one of most common of human muscular dystrophies. This condition involves progressive myopathy and myotonia, which are often associated with hypogonadism. Men may have small testicles and sperm production may be reduced [7].

Androgen biosynthesis disorders are relatively rare causes of sexual ambiguity in men (46, XY). The inherited decrease in testosterone synthesis and secretion is caused by a mutation in genes that encode the enzymes of the testosterone synthesis pathway (StAR) and the steroidogenic enzymes (P450scc, P450c17, 3betaHSDII, 17betaHSDIII, and 5alpha-reductase). For each of these enzymes, abnormalities lead to male pseudohermaphroditism [8]. Many acquired causes such as varicocele, infections, drugs, environmental toxins, testicular torsion can cause primary testicular defects in spermatogenesis. Genetic disorders (Y chromosome microdeletions, autosomal and X chromosome abnormalities, mutations leading to severe spermatogenesis defects) are responsible for approximately 5% of infertility cases. Abnormalities in spermatogenesis may also be caused by systemic diseases such as renal failure, hepatic cirrhosis, cancer, and sickle cell disease [8].

ENDOCRINE AND SYSTEMIC DISORDERS

Hypothalamus and pituitary gland disorders may cause hypogonadotrophic hypogonadism, a deficiency in gonadotrophin releasing hormone (GnRH) or gonadotropin deficiency, and thus cause infertility. The most frequent causes of hypogonadotropic hypogonadism are presented below.

Congenital hypogonadotrophic hypogonadism (CHH) is a rare disease characterized by the delay or lack of sexual maturity and infertility. This condition is caused by insufficient production, secretion or activity of gonadotrophin releasing hormone (GnRH). In the majority of cases, the disease is accompanied by anosmia or hyposmia. This presentation is called Kalmann's syndrome [9]. Mutations of the gonadotropin subunit may also cause hypogonadotropic hypogonadism. M. Grigorowa et al. in their study showed that genetically conditioned, low levels of follicle-stimulating hormone (FSH) can have a significant impact on testicular function, reduce testicular hormone levels, and thus reduce male reproductive potential [10]. Male infertility may also result from acquired disorders of the hypothalamus and pituitary gland such as tumors, sarcoidosis, histiocytosis, injuries, surgeries or intracranial radiation. These diseases can cause damage to GnRH neurons in the hypothalamus, gonadotrophic cells of the pituitary gland and lead also to the disruption of hypothalamic-pituitary circulation [3].

Endocrine disorders which can lead to hypogonadism include hyperprolactinemia, estrogen excess, glycocorticosteroids and androgens excess as well as hypo and hyperthyroidism [11]. Hyperprolactinemia is a relatively common endocrine disorder resulting in hypogonadotropic hypogonadism. Prolactin excess leads

to disturbances in lutropin and follicle stimulating hormone secretion, which results in spermatozoa and testicles endocrine function suppression [11]. Excessive secretion or exogenous administration of androgens or steroids results in negative hypothalamic-pituitary axis feedback and inhibits the secretion of FSH and luteinizing hormone (LH). Infertility associated with excess of these hormones results in oligozoospermia or azoospermia [12]. Thyroid disease can decrease libido, cause erectile dysfunction or delay ejaculation [13].

SPERM TRANSPORT DISORDERS

Infertility may result from the absence or bilateral obstruction of semen pathways. It may be congenital (cystic fibrosis, vascular aplasia, Yong's syndrome) or acquired (epididymitis, seminal vesicles). Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population due to mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The intravital section of the vas is hypoplastic in men or may not develop. There is also a disturbance in the structure of seminal vesicles - hypoplasia and cystic changes, which lead to low sperm volume. Congenital absence of the vas defects is also possible [14]. Young's syndrome is a rare disease characterized by infertility as a result of obstructive azoospermia and chronic bronchitis. Azoospermia results from blocking the epididymis with protein masses. In males, there are disturbances in the structure of centriole and dynein proteins, which causes disturbances of motility of sperm and cilia in the epididymis and respiratory tracts [15]. The primary ciliary dyskinesia (PCD) is also responsible for disturbances in semen transport. It is a congenital disorder characterized by functional or structural ciliary disorders. Patients with PCD have frequent infections of the upper and lower respiratory tract and about 50% have sperm immunities [16]. Obstruction of the semen leading pathways may occur in congenital diseases, e.g. Muller's cyst, it may also be the result of estrogen excess, toxins or antenatal infections also infections of the male reproductive system (e.g., gonorrhea, chlamydiasis, infections by Mycoplasma or Ureaplasma) [17,18]. Iatrogenic obstruction may be the result of accidental intersection of the semen leading pathways during surgery and endoscopic procedures. It may also be caused by intentional vasectomy for contraception purposes [19].

IDIOPATHIC MALE INFERTILITY

Idiopathic male infertility refers to men in whom repeated attempts to analyse semen do not show sperm abnormalities, but they cannot achieve pregnancy with a fertile female partner despite the assessment of all possible mechanisms of male infertility.

CONDITIONS WORSENING THE SEMEN QUALITY

When discussing the causes of infertility, it is worth mentioning factors which worsen semen quality, and thus hinder the conception of the offspring.

Neurological mechanisms play an important role in proper reproductive system functioning. Their disturbances may lead to infertility due to erectile dysfunction, ejaculatory dysfunction and semen abnormalities. Disorders that cause these problems include congenital spinal defects, diabetes, pelvic and retroperitoneal surgery, multiple sclerosis and spinal cord injury [20]. Obesity which affects an increasing number of men in the reproductive age is associated with spermatogenesis defects, erectile dysfunction and decreased libido. The concentration of sex hormone binding globulin (SHBG) is lower which results in higher serum free testosterone concentration and higher free estrogen level [21,22].

The negative impact of smoking on semen quality has also been proved. Many studies have shown the decrease in sperm quality, disturbances in spermatogenesis and sperm function disorders. Disorders result from DNA damage, increased oxidative stress or apoptosis of cells due to ingredients contained in tobacco [23].

Sport is an important element of a healthy lifestyle. Sport practiced recreationally has a positive effect on the quality of semen. It reduces the risk of obesity, diabetes or cardiovascular diseases which leads to a secondary semen quality improvement. However, too much effort, professional sports, have an adverse effect on male fertility. Safarinejad et al. showed the adverse effects of intense exercises on the quality of semen along with the decrease in testosterone levels, LH and FSH hormones and the increase in prolactin and SHBG [24]. Cycling is a popular sport but it can worsen male fertility. It can cause pudendal compression syndrome and erectile dysfunction. During cycling, there is also the pressure on the vessels, which may cause hypoxia. Chronic hypoxia causes connective tissue proliferation, resulting in erectile dysfunction [25]. It is also worth mentioning that this discipline is associated with frequent injuries of the genitourinary system which may cause numerous pathological changes in the testes. Testicular injuries are also common in other athletes, especially horse riders and contact sports [26].

Autoimmune infertility is due to the presence of antibodies against sperm that may be present in serum, semen or on the sperm surface. Antibodies may cause immobilization or agglutination of sperm, as well as impaired implantation of a fertilized egg. Typical causes of autoimmune infertility include previous genital infection, testicular biopsy, testicular injury, testicular turn, and vasectomy [27].

DIAGNOSIS

Ineffective waiting for a pregnancy for one year is an indication for diagnostic process, which should always apply to both partners [27, 28].

INITIAL DIAGNOSIS

MEDICAL HISTORY

It is important to collect full medical history. Attention should be paid to history of sexual development, chronic systemic illnesses, previous infections, especially

mumps and genitourinary tract infections, which are often linked to male infertility. History of previous surgeries, especially those involving pelvic and inguinal regions and genitalia, as well as radiations covering the groin and scrotum are important. The physician should ask about medications intake, environmental exposures and sexual life (libido, erections) [27, 28].

PHYSICAL EXAMINATION

Physical examination should include a general medical examination and the results of basic laboratory tests in order to examine general health, diagnose obesity or overt signs of endocrinopathies, which may be the cause of infertility. During the examination, special attention should be paid to skin discoloration, which may occur in many congenital conditions. Particular attention is devoted to the examination of the urogenital system, especially for the presence of inflammation and varicocele, which are common causes of male infertility [27-29].

SEMEN ANALYSIS

Semen analysis is the basis for further diagnosis and treatment. Semen testing should be performed after 2-5 days of sexual abstinence. The test should be carried out in a laboratory in accordance with national quality control standards. During sperm analysis, it is important to distinguish asthenozoospermia, oligozoospermia and teratozoospermia. Sometimes these pathologies can occur together, which is known as oligo-asteno-teratozoospermia. Determination of these parameters is important for further management. If the semen parameters are in accordance with the WHO criteria, the result of one test is enough. If the test results are incorrect, semen analysis should be repeated. If semen abnormalities are present in subsequent tests, further diagnosis is necessary [27-30].

ADDITIONAL TESTS

HORMONAL TESTS

Hormonal disorders are a rare cause of male infertility. Standard tests, in case of the incorrect semen analysis result include FSH, LH and testosterone levels. Decreased testosterone levels and increased FSH levels suggest primary hypogonadism, while low testosterone levels and low levels of FSH, secondary hypogonadism. Low levels of testosterone along with low levels of LH usually justify the study of serum prolactin levels [27-30].

SCROTAL AND TRANSRECTAL ULTRASOUND

This examination enables the diagnosis of venous changes in spermatic fusions, assessment of the prostate gland and other structures of the genital system. It allows detection of obstructive azoospermia, which should be suspected especially in men with normal testicular volume, normal testosterone, FSH, LH level and azoospermia [27-30].

GENETIC TESTS

Genetic tests allow detection of hereditary and innate syndromes. These may include karyotyping, testing for Y-chromosome microdeletion, detection of mutations in the CFTR gene responsible for cystic fibrosis. These tests also allow assessment of the risk of transferring genetic abnormalities to future offspring [28, 29].

TESTICULAR BIOPSY

If the result of testicular biopsy shows that sperm production is normal, it indicates that infertility problems are associated with blockage or disturbances in semen transport [28, 29].

TREATMENT

Many cases of male infertility are idiopathic, however some of them can be detected. Depending on the cause, the most appropriate therapeutic treatment is selected, individually for each patient. It should be remembered that male infertility is often a multifaceted problem, additional therapy is needed apart from targeted therapy. This includes the control of chronic diseases (hypertension, diabetes), quitting bad habits such as smoking or alcohol abuse, and implementing regular physical activity. Treatment is a long-term process, often lasting many months, requiring patience from the patient.

MEDICAL TREATMENT

Various studies proved the positive effect of antioxidant treatment (folic acid, vitamin E, zinc, selenium) on the quality of sperm, which resulted in the improvement of spontaneous pregnancies [31,32]. None of the clinical trials showed any improvement, in case of unexplained infertility, in men after gonadotropin treatment (FSH, hMG, hCG), androgens, antiestrogens (clomifene, tamoxifen), dopamine D2 receptor agonists or steroids. Therefore, in case of patients with idiopathic infertility this type of treatment is not recommended [31,32]. Improvement in fertility after medical treatment can be obtained in men with low testosterone level (clomiphene citrate or tamoxifen), hypogonadotropic hypogonadism (treatment with gonadotropins and hCG), hyperprolactinemia (dopamine agonists) [31, 32].

SURGICAL TREATMENT

VARICOCELE

Treatment of varicocele is controversial. According to current data, varicocele causes damage to the testicles beginning in adolescence, resulting in male fertility. According to some research, the quality of semen after treatment is improved. Varicose veins treatment may be effective in adolescents. In adults with subclinical varicocelic signs, no treatment benefit was observed. The surgical treatment may be effective for men with oligozoospermia and clinical venous degeneration [31-33].

MICROSURGERY/VASOVASOSTOMY AND EPIDIDYMOVASOSTOMY

Vasectomy reversal procedure involves the removal of the scar changed section of the vas and fusion of the end to the end of the vas deferens (vasovasostomy) or the implantation of the ventral section of the vas deferens into the epididymitis head (vasoepididymostomy). These micro-surgical procedures should only be performed by experienced urologists. The chance of having a descendant is inversely proportional to the obstruction interval, it is less than 50% after 8 years. Other important factors are the quality of the sperm after the procedure and partner's age. In some cases, epididymal obstruction coexists, which is an indication for vasoepididymostomy surgery. Since this procedure has a limited effect on pregnancy rates, these procedures should be combined with microsurgical epididymal sperm aspiration (MESA), and cryopreservation of harvested spermatozoa for intracytoplasmic sperm injection (ICSI) [31, 34].

IN VITRO FERTILIZATION

If other techniques fail, in vitro fertilization (IVF) is possible. This treatment begins with stimulating ovulation in a woman. Next, under the control of USG, oocytes are collected, which is combined with selection and preparation in the laboratory sperm. The embryos obtained are then cultured for 2-5 days and introduced into the uterus via a catheter. The in vitro method is used in the case of low semen quality, unsuccessful insemination attempts, complete obstruction of the vas deferens, fallopian tubes and when the cause of infertility was not determined [35].

MESA/TESE

If in men with obstructive azoospermia vasovasostomy, vaso-epididymostomy cannot be performed or is ineffective, MESA in combination with ICSI is recommended. Percutaneous aspiration of spermatozoa from the caput epididymium (PESA) can be performed as an alternative method. If these methods fail, testicular sperm extraction (TESE) can be advised. In this method semen is prepared in the laboratory conditions with selection of the most viable and mobile sperm. Semen is injected into the uterus during natural or hormonally-stimulated ovulation. As a rule, the treatment must be repeated several times. If patient suffers from azoospermia and sperm cannot be obtained, intrauterine inseminations are performed using donor sperm [31-33]. Donor sperm is used if patient has azoospermia, significant semen pathology and lack of pregnancy despite numerous ICSI attempts. It can be also considered if there are contraindications to perform ICSI or in case of high risk of genetic disease transfer [31].

CITE THIS AS

MEDtube Science Jun, 2018, Vol. V (2), 41 – 46

ABBREVIATIONS

CHH – Congenital Hypogonadotrophic Hypogonadism
CF – Cystic Fibrosis
CFTR – Cystic Fibrosis Transmembrane Conductance Regulator
FSH – Follicle-stimulating Hormone
GnRH – Gonadotrophin Releasing Hormone
ICSI – Intracytoplasmic Sperm Injection
IVF – In Vitro Fertilization
KS – Klineferter Syndrome
LH – Luteinizing Hormone
MESA – Microsurgical Epididymal Sperm Aspiration
PCD – Primary Ciliary Dyskinesia
SHBP – Sex Hormone Binding Globulin
TESA – Testicular Sperm Extraction

REFERENCES

1. Cooper TG, Noonan E, Von Eckardstein S et al. World Health Organization reference values for human semen characteristics. Human Reproduction Update 2009; 16(3): 231-245.
2. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature, J Hum Reprod Sci. 2015; 8(4): 191-196.
3. Jungwirth A, Giwercman A, Tournaye H, et al. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol 2012; 62: 324-332.
4. Kanakis GA, Nieschlag E, Klinefelter syndrome: more than hypogonadism, Metabolism. 2018; pii: S0026-0495(18).
5. Fawzy F, Hussein A, Eid MM et al. Cryptorchidism and Fertility. Clin Med Insights Reprod Health. 2015; 9: 39-43.
6. Kobayashi H, Nagao K, Nakajima K. Therapeutic Advances in the Field of Male Infertility: Stem Cell Research. Advanced Studies in Medical Sciences. 2013; 1: 39-54.
7. Meola G. Clinical aspects, molecular pathomechanisms and management of myotonic dystrophies. Acta Myol. 2013; 32(3): 154-165.
8. Miller WL. Disorders of androgen biosynthesis. Semin Reprod Med. 2002; 20(3): 205-216.
9. Vezzoli V, Duminuco P, Bassi I, Guizzardi F, Persani L, Bonomi M. The complex genetic basis of congenital hypogonadotropic hypogonadism. Minerva Endocrinol. 2016; 41(2): 223-239.
10. Grigorova M, Punab M, Zilaitiene B, et al. Genetically determined dosage of follicle-stimulating hormone (FSH) affects male reproductive parameters. J Clin Endocrinol Metab 2011; 96: E1534.
11. McClure RD. Endocrinology of Male Infertility, Office Andrology, pp 11-37.
12. Dohle GR, Smit M, Weber RF. Androgens and male fertility. World J Urol. 2003; 21: 341-345.
13. Carani C, Isidori AM, Granata A, et al. Multicenter study on the prevalence of sexual symptoms in male hypo- and hyperthyroid patients. J Clin Endocrinol Metab 2005; 90: 6472-6479.
14. Wilschanski M, Corey M, Durie P, et al. Diversity of reproductive tract abnormalities in men with cystic fibrosis, JAMA. 1996; 276(8): 607-608.

15. Arya AK, Beer HL, Benton J, Lewis-Jones I, Swift AC. Does Young's syndrome exist? J Laryngol Otol. 2009;123(5): 477-81.
16. Bush A. Primary ciliary dyskinesia. Acta Oto-rhino-laryngologica Belgica 2000; 54(3): 317-324.
17. Stillman RJ. In utero exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance and male and female offspring, Am J Obstet Gynecol. 1982; 142(7): 905-921.
18. Priebe Jr CJ. Holahan JA, Ziring PR. Abnormalities of the vas deferens and epididymis in cryptorchid boys with congenital rubella. Journal of Pediatric Surgery. 1979; 14: 834-838.
19. De Francesco MA, Negrini R, Ravizzola G, Galli P, Manca N. Bacterial species present in the lower male genital tract: a five-year retrospective study. Eur J Contracept Reprod Health Care. 2011; 16(1): 47-53.
20. Fode M, Krogh-Jespersen S, Brackett NL, Ohl DA, Lynne CM, Sonksen J. Male sexual dysfunction and infertility associated with neurological disorders. Asian J Androl. 2012; 14(1): 61-68.
21. Craig JR, Jenkins TG, Carrell DT, Hotaling JM. Obesity, male infertility, and the sperm epigenome. Fertil Steril. 2017; 107(4): 848-859.
22. Wu FC, Tajar A, Pye SR, et al, Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab. 2008; 93: 2737-2745.
23. Blanco-Munoz J, Lacasana M, Aguilar-Garduno C. Effect of current tobacco consumption on the male reproductive hormone profile. Sci Total Environ. 2012; 426: 100-105.
24. Safarinejad MR, Azma K, Kolahi AA. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus-pituitary-testis axis, and semen quality: a randomized controlled study. J Endocrinol. 2009; 3: 259–271.
25. Sommer F, Goldstein I, Korda JB. Bicycle riding and erectile dysfunction: a review. J Sex Med. 2010; 7: 2346-2358.
26. Bagga HS, Fisher PB, Tasian GE, et al. Sports-related genitourinary injuries presenting to United States emergency departments. Urology 2015; 1: 239-244.
27. Jungwirth A, Diemer T, Dohle GR, et al. EAU Guidelines on male infertility; European Association of Urology 2016; 12-46.
28. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. Clinics 2011; 66(4): 691-700.
29. Crosignani P, Rubin B. Optimal use of infertility diagnostic tests and treatments. The ESHRE Capri Workshop Group. Hum Reprod. 2000;15: 723–732.
30. World Health Organization Department of Reproductive Health and Research. World Health Organization Laboratory Manual for the Examination and Processing of Human Semen, 5th ed, World Health Organization, Geneva, Switzerland 2010.
31. Jungwirth A, Diemer T, Dohle GR et al. Guidelines for the investigation and treatment of male infertility. Eur Urol 2012; 61(1): 159-163.
32. Practice Committee of the American Society for Reproductive Medicine: Optimal evaluation of the infertile female. Fertil. Steril. 2006; 86: 264-267.
33. Practice Committee of American Society for Reproductive Medicine, Society for Male Reproduction and Urology. Evaluation of the azoospermic male. Fertil Steril. 2008; 90:5 Supl.74–77.
34. Lopushnyan NA, Walsh TJ. Surgical techniques for the management of male infertility. Asian Journal of Andrology. 2012; 14: 94–102.
35. Esteves SC, Miyaoka R, Orosz JE, Agarwal A. An update on sperm retrieval techniques for azoospermic males. Clinics (Sao Paulo). 2013; 68: 99–110.

EVALUATION OF NUTRITIONAL STATUS OF PATIENTS USING LABORATORY TESTS. REVIEW

Paula Chechla[1], Iga Holynska-Iwan[2]

1. Students' Scientific Group at the Department of Pathobiochemistry and Clinical Chemistry, Nicolaus Copernicus University in Torun Collegium Medicum in Bydgoszcz
2. Department of Pathobiochemistry and Clinical Chemistry, Nicolaus Copernicus University in Torun Collegium Medicum in Bydgoszcz

#Corresponding author: Paula Chechla, e-mail: qaula16822@gmail.com, Nicolaus Copernicus University in Torun Collegium Medicum in Bydgoszcz, Iwiec 1 St, p. o. box 89-512 Iwiec, Poland, phone number: +48 509 910 454

RUNNING TITLE	Evaluation of nutritional status of patients using laboratory tests
KEYWORDS	nutritional status; markers; laboratory test; nutritional index
WORD COUNT	1461
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

Malnutrition is a condition resulting from the lack of absorption or consumption of nutritional substances, leading to changes in body composition, physical and mental impairment of bodily functions, and adverse effects on the treatment of any underlying disease. In Poland, since 2012, assessing the nutritional status of each patient admitted to hospital is required, the exception of hospital emergency departments. However, there is no gold standard for this assessment. Currently, the main diagnostic criteria are anthropometric measurements and an interview with questions related to nutrition. There are some situations in which checking biochemical markers of malnutrition would be extremely helpful for correct diagnosis.

BACKGROUND

Malnutrition is not only an independent disease, but it is also a very important problem in people suffering from other comorbid diseases. Numerous studies have shown that patient malnutrition may be associated with a longer stay in hospital, more readmission and increased mortality. Longer treatment and hospital stays result in the increase in expenses for these patients. Bearing in mind the importance of health in the patient and economic factors, it is clear, that early diagnosis of the patient's nutritional status is extremely important [1].

In 2015, the European Society for Clinical Nutrition and Metabolism (ESPEN) presented new consensus criteria for the diagnosis of malnutrition. Three variables were selected to reflect malnutrition, i.e. weight loss, reduction in BMI and reduction in FFMI. Although weight loss can depend on the clinical condition of the patient, unintentional weight loss >5% in the last three months, with acute illness, or >10% of normal weight over an undetermined time, indicates the possibility of malnutrition. According to WHO recommendations, a BMI value <18.5 kg/m^2 can be used as a general determiner for underweight. In the case of FFMI, <15 kg/m^2 for women and <17 kg/m^2 for men can be used as an indicator of malnutrition [2].

These criteria will not always be useful. For example, in patients on peritoneal dialysis, the content of lean body mass decreases and chronic inflammation develops. These are symptoms of malnutrition- inflammation complex syndrome (MICS) or malnutrition- inflammation-atherosclerosis (MIA). Weight loss occurs in these patients, but as they are very often obese, malnutrition will not be reflected in their BMI and FFMI values [3]. In such situations, it would be helpful to check biochemical markers of malnutrition in order to diagnose malnutrition correctly and monitor the effectiveness of any nutritional treatment undertaken.

LABORATORY TESTS

Albumin

The usefulness of albumin determinations is increasingly questioned because of its long biological half-life of about 21 days. For this reason, it may be important in the diagnosis of chronic malnutrition, but it is of no use in diagnosing the development of malnutrition or in the assessment of any introduced nutritional intervention. In addition, hydration status affects the concentration of albumin, so results from people, who are dehydrated or who have severe burns will be unreliable. Maintaining the stability of albumin levels in the blood is influenced by its large pool in the intravascular space and the ease of mobilization of extravascular resources [4].

Prealbumin

Prealbumin is mainly synthesized in the liver. It is a transport protein that, by forming a complex with a retinol binding protein, mediates the transport of vitamin A. It also has the ability to bind and transfer thyroid hormones and certain drugs. A small pool of prealbumin in circulation (concentration 0.20-0.40 g/l in healthy people) and a short biological half-life of about two days make the determination of this protein useful in monitoring the effectiveness of nutritional treatment. The value of protein supply in pregnant women can be assessed based on prealbumin concentration, which in the case of shortages falls below the norm in the mother's blood and umbilical cord blood, correlating with low birth weight [5].

Transferrin

The main role of transferrin is the binding and transport of iron from enterocytes and liver cells to all cells of the body. The transfer of iron to the cells is mediated by specific membrane receptors- transferrin receptors (TfR). Compared to albumin, transferrin has a relatively short biological half-life of about 8 days, and its concentration in the blood serum of healthy people is 2.0-3.8 g/l. Although the level of transferrin is highly related to a person's nutritional status, due to its influence on the concentration of iron levels, some people question the usefulness of using this protein as a marker of malnutrition [6].

Retinol binding protein (RBP)

The main biological function of RBP is the binding and transport of vitamin A. It is synthesized in the liver as apoRBP, and after the attachment of retinol it is secreted into the circulation, where it forms a complex with transthyretin. Recent studies have shown that the ability to synthesize retinol-binding protein also has adipocytes (RBP4). It is believed that only about 4% of RBP circulates in free form, unrelated to prealbumin. Malnutrition, especially vitamin A deficiency, is associated with a reduction in the production and secretion of RBP from the liver. RBP concentration in the serum of healthy people ranges from 0.03-0.06 g/l, and the biological half-life is about 12 hours. Determinations of this protein, like prealbumin, have been used to monitor the effectiveness of nutritional treatment, in particular parenteral nutrition [7].

Insulin-like growth factor (IGF-1)

Insulin-like growth factor-1 is synthesized in most body tissues. Diet has a significant influence on the synthesis of IGF-1 along with growth hormone. A reduction in protein supply is associated with a decrease in the concentration of not only IGF-1, but also its main IGFBP-3 carrier protein. Over 90% of IGF-1 circulate in a form bound to this protein. Binding to IGFBP-3 prolongs the plasma half-life of IGF-1. The half-life of the IGF-1 / IGFBP-3 complex is about 12-15 hours, while the half-life of insulin, which has no carrier proteins, only 10 minutes. In healthy adults, the plasma concentration of IGF-1 is approximately 200 ng / ml. Determinations of IGF-1 have proved useful not only in assessing nutritional status, but also for monitoring nutritional treatment [8].

Calculated indicators of malnutrition and inflammation

The Nutrition Risk Index (NRI) has been identified as one of the most useful tools for assessing malnutrition in patients. NRI is based on two parameters: the concentration of serum albumin and weight loss. However, a normal body weight is required in order to weight loss to be assessed. This is a limitation for elderly patients because it is difficult to identify their correct weight. For this reason, the Geriatric Nutritional Risk Index (GNRI) has been developed as a new indicator for

assessing at-risk older medical patients. GNRI is based on two parameters: the concentration of serum albumin and the ideal body weight calculated using Lorentz or BMI equations. The formula is as follows: GNRI = 1.487 × serum albumin concentration (g/L) + 41.7 × pre-operative body weight / ideal body weight (kg). Previous studies have supported the use of GNRI due to its significant association with most nutritional parameters and short and long-term results. In addition, GNRI can explain both acute and chronic causes of malnutrition associated with underlying diseases and age-related factors [9].

The Prognostic Inflammatory and Nutritional Index (PINI) is a formula developed by Ingenbleek and Carpentier to assess nutritional status and prognosis in critically ill patients. It has been found that conventional nutritional assessments are insufficient for such patients and it has been proposed that inflammation may also affect nutritional status. PINI was created to take this into account. Ingenbleek also suggested that the PINI result can be used to track most pathological conditions. PINI has been measured in several settings and it has been found that it is a reliable indicator of both nutritional status and prognosis in injuries, burns, infected patients and patients with heart problems [10].

Prognostic results based on inflammation, such as the Glasgow Prognostic Score (GPS), have been predictive in patients with several types of advanced cancers. Although GPS is based only on C-reactive protein (CRP) serum and serum albumin levels (Alb), it may reflect systemic inflammation and nutritional status. CRP is the main indicator of inflammation, and hypoalbuminemia is related to malnutrition. These factors are associated with immunosuppression in cancer patients [11].

The control feeding result (CONUT) is calculated using serum albumin, total cholesterol and number of lymphocytes [13]. Serum albumin reflects the protein reserve, total cholesterol reflects the loss of calories, and the number of lymphocytes reflects immune defence. A higher CONUT indicates an inferior nutritional status. In patients with heart failure, the three-year survival with a CONUT score of 0-1, 2 and ≥3 was 95.5%, 92.3% and 73.2%, respectively ($p < 0.001$) [12].

CONCLUSION

Malnutrition is a problem not only as a separate disease, but also as a disorder that accompanies other diseases. It affects the duration of the other diseases, the effectiveness of their treatment and the survival of patients. For this reason, diagnosing malnutrition or its risk is very important because it determines the quality of subsequent treatment. In Poland, every person who goes to hospital is examined for malnutrition, excluding hospital emergency departments. Determination of biochemical markers of malnutrition may be helpful in diagnosing malnutrition in patients, especially when used in conjunction with other criteria, such as anthropometric measurements and BMI and FFMI values. These biological markers will be of greater use in the monitoring of nutritional treatment, since the values of some of them, e.g. prealbumin and transferrin, will quickly respond to any nutritional intervention due to their short half-life.

ACKNOWLEDGEMENTS

The authors are grateful to the medical staff from Department of Neurology at Medical University of Lublin for providing an opportunity to do this scientific work.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 47 – 50

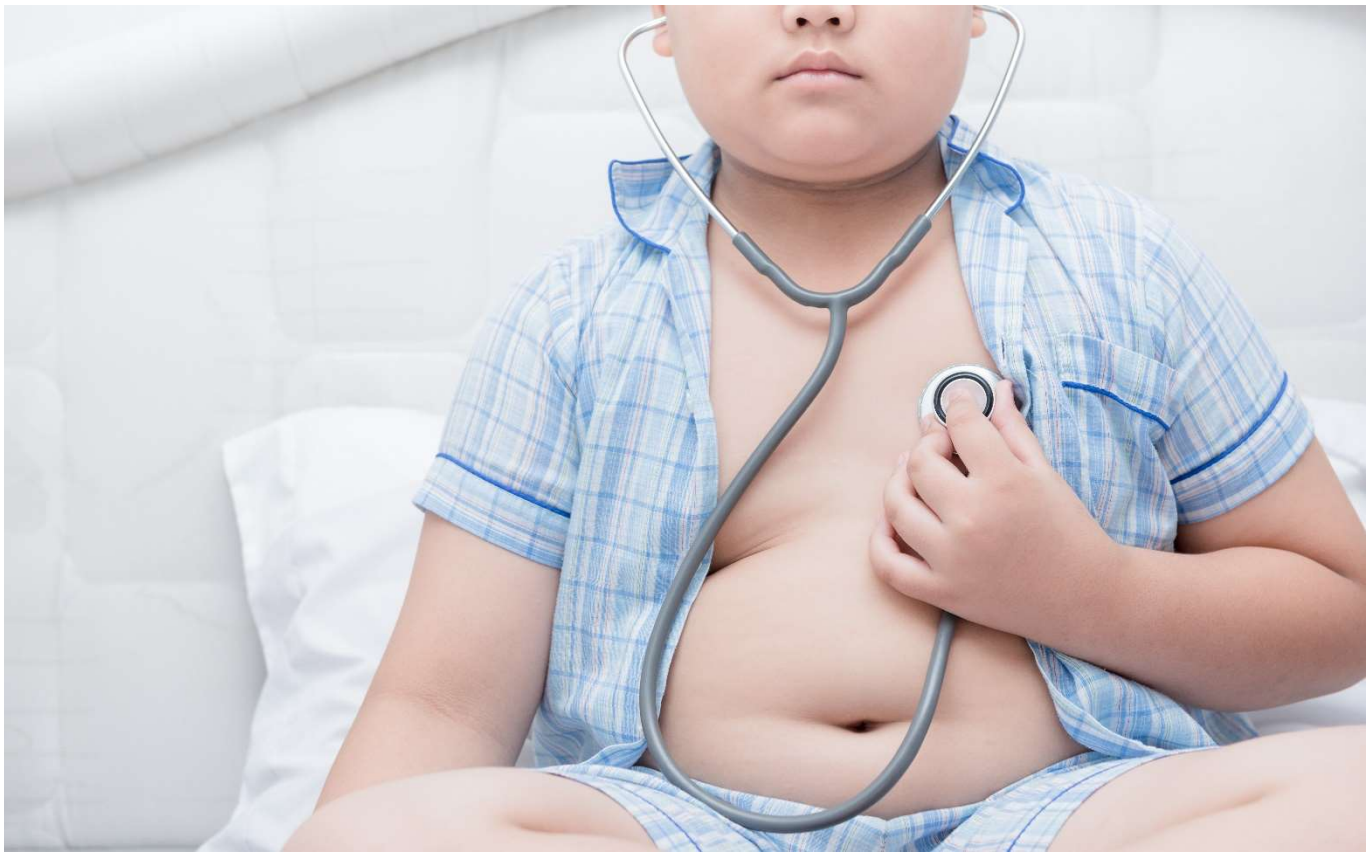
ABBREVIATIONS

Alb – Albumin
BMI – Body Mass Index
CONUT – Control Feeding Result
CRP-C – reactive Protein
ESPEN – European Society for Clinical Nutrition and Metabolism
FFMI – Fat Free Mass Index
GNRI – Geriatric Nutritional Risk Index
GPS – Glasgow Prognostic Score
IGF–1 – Insulin- like Growth Factor-1
IGFBP-3 – Insulin- like Growth Factor Binding Protein- 3
MIA – Malnutrition- Inflamation- Atherosclerosis
MICS – Malnutrition- Infalmation Complex Syndrome
NRI – Nutrition Risk Index
PINI – Prognostic Inflammatory and Nutritional Index
RBP – Retinol Binding Protein
TfR – Transferrin receptor
WHO – World Health Organization

REFERENCES

1. Agarwal E, Ferguson M, Banks M, et al. Malnutrition and poor food intake are associated with prolonged hospital stay, frequent readmissions, and greater in-hospital mortality: Results from the Nutrition Care Day Survey 2010. Clin Nutr 2013; 32: 737-745.
2. Cederholm T, Bosaeus I, Barazzoni R, et al. Diagnostic criteria for malnutrition- An ESPEN Consensus Statement. Clin Nutr 2015; 34: 335-340.
3. Małgorzewicz S, Lichodziejska- Niemierko M. Niedożywienie i otyłość u chorych dializowanych otrzewnowo. Forum Nefrologiczne 2009; 4 (2): 216-221.
4. Akirov A, Masri-Iraqi H, Atamna A. Low Albumin Levels Are Associated with Mortality Risk in Hospitalized Patients. Am J Med 2017; 130 (12): 1465.e11-1465.e19.
5. Delliere S, Cynobera L. Is transthyretin a good marker of nutritional status? Clin Nutr 2017; 36 (2): 364-370.
6. Boshuizen M, van der Ploeg K, von Bonsdorff L. Therapeutic use of transferrin to modulate anemia and conditions of iron toxicity. Blood Rev 2017; 31 (6): 400-405.
7. Villaça Chaves G, Arantes W, Peres F, et al. Vitamin A and retinol-binding protein deficiency among chronic liver disease patients. Nutrition 2015; 31 (5): 664-668.
8. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocrine Rev 1994; 15(1): 80-101.
9. Shoji F, Matsubara T, Kozuma Y, et al. Preoperative Geriatric Nutritional Risk Index: A predictive and prognostic factor in patients with pathological stage I

non-small cell lung cancer. Surg Oncol 2017; 26 (4): 483-488.
10. Nelson KA, Walsh D. The Cancer Anorexia–Cachexia Syndrome: A Survey of the Prognostic Inflammatory and Nutritional Index (PINI) in Advanced Disease. J Pain Symptom Menage 2002; 24 (4): 424-428.
11. Okimoto S, Kobayashi T. Significance of the Glasgow Prognostic Score for patients with colorectal liver metastasis. Int J Surg 2017; 42: 209-214.
12. Horiuchi Y, Tanimoto S, Okuno T. Hemodynamic correlates of nutritional indexes in heart failure. J Cardiol 2018; 71(6): 557-563.



OBESITY - RISK OR PROTECTION FACTOR? OBESITY PARADOX IN CARDIOVASCULAR DISEASES

Julia Lesniewska[1], Anna Adamek[1], Jakub Gawrys[2], Karolina Gawrys[3], Magdalena Piotrowska[1]

1. Student Scientific Organization for Internal Diseases and Hypertension at the Department and Clinic of Internal and Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Poland
2. Department and Clinic of Internal and Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Poland
3. Clinical Department of Endocrinology, Diabetology and Metabolic Diseases, 4th Military Hospital, Wroclaw, Poland

#Corresponding author: Julia Lesniewska, e-mail: juliia.lesniewska@gmail.com, Wroclaw Medical University, Wybrzeże Pasteura 1 St, p. o. box 50-367 Wroclaw, Poland, phone number: +48506277136

RUNNING TITLE	Obesity paradox in cardiovascular diseases
KEYWORDS	obesity; cardiovascular disease; obesity paradox
WORD COUNT	1981
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

Obesity, by definition, is a chronic disease which is the result of excessive accumulation of fatty tissue and characterized by increasing body mass index (BMI) ≥ 30 kg/m^2. It is classified as a civilization disease. According to World Health Organization (WHO) in 2016 worldwide, there were 650 million obese people. As evidenced, obesity increases the probability of developing cardiovascular diseases such as hypertension, heart failure and coronary artery disease. Interestingly, a lot of studies have been carried out and results of these studies indicate that obesity, which is an important risk factor for cardiovascular disease, may also act as a favorable prognostic factor. Obese patients with chronic diseases, for instance heart failure or patients with coronary artery disease, who underwent percutaneous coronary intervention, have better results of treatment compared to patients with normal body weight. This phenomenon, which is based on the paradoxical relationship of increased body mass with lower mortality, is defined as the obesity paradox. We present a review of the literature, which will allow understanding the problem of this phenomenon, its pathophysiology and results of the patients with cardiovascular disease connected with obesity paradox.

BACKGROUND

Obesity is defined by World Health Organisation (WHO) as an excessive accumulation of fatty tissue that may influence health condition. It can be found in International Statistical Classification of Diseases and Related Health Problems (ICD-10). One of the methods of classification of obesity is Body Mass Index (BMI). It equals body mass in kilograms divided by square of height in meters. For overweight, it has value over 25 and for obesity, it is bigger than 30. In the years 1980-2008 number of obese people in the world has doubled [1]. High BMI is an essential risk factor for many non-communicable diseases like cardiovascular diseases, diabetes mellitus, musculoskeletal disorders and some of the neoplasms [2]. High BMI relates to over 100 million years of healthy life lost due to premature death or disability (DALYs – disability-adjusted life years) [3]. However higher value of BMI does not combine with higher mortality in every group. Analysis of some patients with cardiovascular diseases has revealed that those with overweight or moderate obesity had a longer time of survival compared to patients with normal weight. Diagrams of survival depending on BMI among them form a "U" shape with the most favorable score for 25kg/m^2, which is a lower threshold for overweight. This phenomenon is called an obesity paradox [4, 5].

OBESITY PARADOX IN CORONARY ARTERY DISEASE AND ACUTE CORONARY SYNDROME

Coronary artery disease (CAD) is a disorder of blood flow to cardiomyocytes due to changes in the coronary arteries. Progressing CAD may lead to the occurrence of acute coronary syndrome when part of heart muscle dies. In many types of research overweight and obesity were proved to be modifying risk factors for CAD [6]. Therefore observation of longer survival of the overweight among patients suffering from CAD may seem paradoxical. This conclusion was submitted by Romero-Corral et al. after meta-analysis of 40 studies including 250, 152 people. Patients after the percutaneous coronary intervention, coronary artery bypass graft or myocardial infarction were taken into consideration. Then they were divided into groups according to their BMI. The highest risk of death occurred among patients with underweight and class II obesity. It had intermediate value for a class I obesity and was the lowest for overweight [7]. In studies conducted by Levi et al. [8] 570 patients suffering from CAD were split due to body fat (BF) and lean mass index (LMI). Patients with lower BF (men <25%, women <35%) and LMI (men <18.9 kg/m^2, women<15.4 kg/m^2) had lower 3-year-survival as opposed to those with higher values. In another meta-analysis [9] studied 15,923 patients chosen in order to the same criteria as in the previously mentioned Romero-Corral. researches. This time aside from BMI, WHR (waist to hip ratio) and WC (waist circumference), which are a measure of central obesity, were used as obesity parameters. High WHR and WC values were related to increased number of deaths. But the connection between BMI and number of deaths was opposite and showed lower mortality for higher BMI. It may indicate the incorrect use of BMI as a parameter of body fat [10]. Paradoxical protective influence of obesity was also noticed in patients who have undergone myocardial infarction. 50,149 patients hospitalized due to STEMI (ST elevation MI) were split according to BMI [11]. The lowest mortality occurred among patients with class I obesity, but it did not diverge significantly from values for patients with normal body weight, overweight and class II obesity. Patients with class III obesity (BMI>40 kg/m^2) had the highest mortality. In MERLIN-TIMI studies patients in one-year period after undergoing an acute coronary syndrome were observed. Dependence between BMI, WC and WHR and recurrence of another myocardial infarction was investigated. It was noticed less frequently among overweight patients in first thirty days of observation but did not vary significantly in the one-year period between groups divided according to BMI. The higher risk of recurrence was detected in a group with lower BMI but high WC [10].

OBESITY PARADOX IN HEART FAILURE

The existence of the obesity paradox, sometimes called as the reverse epidemiology of obesity, has also been demonstrated in heart failure. This is particularly interesting due to several reasons. One of them is the fact that a high BMI index increases the risk of heart failure, which has been demonstrated, among others, in the Framingham Heart Study [12]. Another interesting mechanism is an inverse relationship between adiponectin concentration and BMI. Adiponectin is a cytokine produced by adipocytes that form adipose tissue. The low concentration of this cytokine correlates with a higher risk of cardiovascular disorders and higher mortality. Accordingly, obese patients with heart failure with the more developed adipose tissue should be included in the group of high risk. Meanwhile, some studies show that these people paradoxically have a better prognosis.

Heart failure (HF) is defined as the condition in which occurs impairment of its function and, as a result, the heart cannot cover the body's need for the components delivered with blood: oxygen and nutrients. The paradoxical influence of obesity on longer survival of patients with heart failure has been proven in many studies, among others in French EPICAL study (Épidémiologie de l'Insuffisance Cardiaque Abancée Lorraine), as well as in CHARM (Candidate in Heart Failure-Assessment of Mortality and Morbidity), DIAMOND-CHF (Danish Investigations of Arrhythmia and Mortality on Dofetilide in Congestive Heart Failure), DIG (The effect of Digoxin on Mortality and Morbidity in Heart Failure) or ValHeFT (A Randomized Trial of the Angiotensin-Receptor Blocker Valsartan in Chronic Heart Failure) [13]. The exact pathomechanism, which could clearly explain the obesity paradox in heart failure, remains unknown. There are several theories about the probable causes of this phenomenon. One of them is associated with increased catabolism and limitation of anabolic processes in the people with heart failure. This hypothesis indicates the protective effect of the increased metabolic reserve resulting from the higher content of adipose tissue, which allows better survival of catabolic states, characteristic especially for exacerbations of heart

failure [14, 15, 16]. Another hypothesis concentrates on the production of soluble receptors for tumor necrosis factor alpha (TNF-α) by the adipose tissue which can neutralize the proinflammatory action of this factor [13, 14]. An interesting issue is also the inverse relationship between the concentration of natriuretic peptides and body weight. Natriuretic peptides participate in the regulation of cardiovascular homeostasis, their concentration increases in the case of increased tension on the heart cavities, and they are a clinically useful indicator of left ventricular dysfunction and the advancement of heart failure. Obese patients with HF have been shown to have a lower level of natriuretic peptides, which may be associated with an earlier occurrence and earlier diagnosis of heart failure with lighter symptoms. Therefore, such patients are treated earlier, which can be associated with a better long-term prognosis [13, 17].

OBESITY PARADOX IN HYPERTENSION

In the course of obesity, the concentration of leptin in the blood increases, which results in the activation of the adrenergic system, what may cause an acceleration of the heart rate and, consequently, increased blood pressure. Therefore, obesity is a well-known risk factor for hypertension. However, it has been proven that increased body weight is associated with better treatment results and lower mortality among people with this disease. It was confirmed in a study which involved 22,500 patients with hypertension and coronary artery disease. It showed that patients with a BMI higher than 25 kg/m^2 have a 30% lower risk of death compared to patients with normal body weight. The explanation for these surprising results may be lower peripheral vascular resistance and decreased plasma renin activity in obese patients compared to patients with lower weight, who suffer from hypertension [17, 18].

CONTROVERSIES SURROUNDING THE OBESITY PARADOX

Both the significance and the pathogenesis of the obesity paradox are not fully explained and are controversial among researchers. On the one hand, in many cases research results indicate that a higher BMI index has actually a protective role. It can occur as a result of adipose tissue endocrine function - the secretion of adiponectins with anti-inflammatory and anti-atherogenic effects, although other compounds in this group may have pro-inflammatory activity [19]. In addition, adipose tissue provides a metabolic reserve that facilitates surviving the predominance of catabolic processes associated with chronic disease [4]. The above arguments lead some researchers to the conclusion that patients with chronic diseases should not be recommended to reduce their weight [20].

Opponents of this theory point the wrong selection of factors whose occurrence results in the appearance of an apparent direct relationship between the increase in body weight and reduced mortality. One of them may be the fact that obese patients, after the diagnosis of the disease, are treated with a more aggressive therapy that provides better results [21]. In addition, some patients lose their weight during the course of the disease and if the studies take into consideration only patients with constant body weight, the results for both groups are very similar. Narrowing the study group to non-smokers, the mortality rate is significantly higher among obese and overweight people. This is due to the fact that smoking leads to a reduction in body weight and, at the same time, a poorer patient condition [22]. Additionally, the use of BMI in these studies is criticized because it does not characterize the distribution of body fat and the level of visceral obesity. In studies in which waist circumference (WC) or waist-hip ratio (WHR) was used, it was shown that the higher values of these two factors of overweight and obesity were associated with higher mortality [23].

CONCLUSIONS

Obesity is one of the basic modifiable risk factors for cardiovascular system diseases among the population. Therefore, it seems surprising that the BMI value above 25 is connected with a better prognosis in patients with heart failure, coronary artery disease or hypertension. This indicates potentially a protective effect of overweight and obesity on the condition of these patients. However, a broader approach and taking into consideration not only BMI but also other metabolic indicators such as WHR and WC are needed to fully evaluate the phenomenon of the obesity paradox. The association of the last two with prognosis in patients with the cardiovascular disease does not show any protective effect or it is observed solely in the short period after exacerbation of the disease. The possible differences in the treatment methods and intensity in different groups of patients also should be taken into account.

The obesity paradox is undoubtedly an interesting phenomenon that can affect the prognosis of people with cardiovascular disease. Determining its exact meaning will require further research on its pathomechanism and linking this phenomenon not only with the BMI index which is used to recognize overweight or obesity, but also other indicators used to the measurement of the distribution of body fat content such as WHR or WC.

CITE THIS AS

MEDtube Science Jan, 2018, Vol. V (2), 51 – 54

ABBREVIATIONS

BF – body fat
BMI – Body Mass Index
CAD – coronary artery disease
DALYs – disability-adjusted life years
HF – heart failure
ICD-10 – International Statistical Classification of Diseases and Related Health Problems
LMI – lean mass index
STEMI – ST elevation myocardial infarction
TNF-α – tumor necrosis factor alpha
WC – waist circumference
WHO – World Health Organization
WHR – waist to hip ratio

REFERENCES

1. Bhurosy T, Jeewon R. Overweight and Obesity Epidemic in Developing Countries: A Problem with Diet, Physical Activity, or Socioeconomic Status? Scientific World Journal 2014; 2014: 964236.
2. Hruby A, Hu F. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics 2015; 33 (7): 673–689.
3. Forouzanfar MH, Afshin A, Alexander LT et al. Global, regional, and national comparative risk assessment of 79 behavioral, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis of the Global Burden of Disease Study 2015. The Lancet Volume 2016; 388: 1659-1724.
4. Doehner W, Clark A, Anker S. The obesity paradox: weighing the benefit. European Heart Journal 2010; 31: 146–148.
5. Bogolowska-Stieblich A, Talalaj M. Otyłość a choroby układu sercowo-naczyniowego. Postępy Nauk Medycznych 2013; 5b: 19-25.
6. Sayols-Baixeras S, Lluís-Ganella C, Lucas G, Elosua R. Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. Appl Clin Genet 2014; 7: 15–32.
7. Romero-Corral A, Montori VM, Somers VK et al. Association of body weight with total mortality and with coronary events in coronary artery disease: a systematic review of cohort studies: a systematic review of cohort studies. Lancet 2006; 368: 666–678.
8. Lavie CJ, De Schutter A, Patel DA et al. Body composition and survival in stable coronary heart disease: Impact of lean mass index and body fat in the “obesity paradox”. J Am Coll Cardiol 2012; 60: 1374–1380.
9. Coutinho T, Goel K, Correa de Sa D et al. Central obesity and survival in subjects with coronary artery disease. A systematic review of the literature and collaborative analysis with individual subject data. J Am Coll Cardiol 2011; 57: 1877–1886.
10. Cybulska B, Klosiewicz-Latoszek L. Co znaczy paradoks otyłości w chorobie wieńcowej? Kardiologia Polska 2013; 71 (9): 963–968.
11. Das SR, Alexander KP, Chen AY et al. Impact of body weight and extreme obesity on the presentation, treatment and in-hospital outcomes of 50 149 patients with ST-segment elevation myocardial infarction. J Am Coll Cardiol 2011; 58: 2642–2650.
12. Kenchaiah S, Evans JC, Levy D et al. Obesity and the risk of heart failure. N Engl J Med 2002; 347 (5): 305-13.
13. Rozentryt P, Myrda K, Nowak J, Niedziela J, Kawecka E, Iwinski J, Polonski L. Nadwaga i otyłość a śmiertelność wśród chorych z niewydolnością serca. Paradoks czy biologiczna prawidłowość? KOF 2010; 3: 273–279.
14. Kleinrok A, Glowa B. Otyłość i jej znaczenie w chorobach układu krążenia Cz. 2. Paradoks otyłości. Medical Review 2015; 2: 173-179.
15. Myrda K, Rozentryt P, Nowak J, Niedziela J, Kawecka E, Polonski L. Tkanka tłuszczowa w niewydolności serca — wróg czy przyjaciel? Folia Cardiologica Excerpta 2010; 5 (4): 232–241.
16. Hamzeh N, Ghadimi F, Farzaneh R, Hosseini S. Obesity, Heart Failure, and Obesity Paradox. J Tehran Heart Cent. 2017; 12 (1): 1–5.
17. Artham SM, Lavie CJ, Milani RV, Ventura HO. Obesity and Hypertension, Heart Failure, and Coronary Heart Disease—Risk Factor, Paradox, and Recommendations for Weight Loss. Ochsner J 2009; 9 (3): 124–132.
18. Drzewoski J. Paradoks otyłości. Diabetologia po Dyplomie 2013; 10 (3): 15-17.
19. Jasinska A, Pietruczuk M. Adipocytokines – proteins of multidirectional function. Journal of Laboratory Diagnostics 2010; 46 (3): 331-338.
20. Lainscak M, Haehling S, Doehner W, Anker S. The obesity paradox in chronic disease: facts and numbers. Journal of Cachexia, Sarcopenia and Muscle 2012; 3 (1): 1–4.
21. Diercks DB, Roe MT, Mulgund J, Pollack CV Jr, Kirk JD, Gibler WB, Ohman EM et al. The obesity paradox in non-ST-segment elevation acute coronary syndromes: results from the Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the American College of Cardiology/American Heart Association Guidelines Quality Improvement Initiative. American Heart Journal 2006; 152 (1): 140-8.
22. Stokes A, Preston SH. Smoking and reverse causation create an obesity paradox in cardiovascular disease. Obesity (Silver Spring) 2015; 23 (12): 2485-90.
23. Chrysant SG, Chrysant GS. New insights into the true nature of the obesity paradox and the lower cardiovascular risk. Journal of the American Society of Hypertension 2013; 7 (1): 85-94.

ELICITATION AND ELICITATION SUPPORTED WITH THE PHENYLPROPANOIDS PATHWAY FEEDING FOR THE ELEVATION OF PHENOLICS CONTENT IN QUINOA SPROUTS

Justyna Bochnak, Małgorzata Sikora, Michał Swieca

Department of Biochemistry and Food Chemistry, University of Life Sciences, Lublin, Poland

#Corresponding author: Justyna Bochnak, e-mail: just.bochnak@gmail.com, Department of Biochemistry and Food Chemistry, University of Life Sciences, Skromna 8 St, p. o. box 20-704 Lublin, Poland, phone number: +48 530 554 252

RUNNING TITLE	Elevation of phenolics content in quinoa sprouts
KEYWORDS	elicitation; precursor feeding; antioxidant activity; quinoa; phenolics
WORD COUNT	2104
CONFLICT OF INTERESTS	no conflicts of interest

ABSTRACT

The aim of this study was to evaluate the combined action of phenylpropanoids feeding (shikimic acid, phenylalanine, tyrosine) and elicitation as a strategy for the elevation of phenolic content in quinoa sprouts. The highest increase of flavonoids content was found for the sprouts treated with shikimic acid. All the studied modifications increased the antioxidant potential of sprouts. The highest reducing power was found for the sprouts treated with 200 mM H_2O_2 obtained by phenylalanine feeding (2.65 mg TE/g d.m.), and those treated with 50 mM H_2O_2 and fed with phenylalanine (2.54 mg TE/g d.m.). Therefore, elicitation and elicitation supported can be considered as a promising approach to improve the content of phenolics and allows to increase the nutraceutical potential of quinoa sprouts.

BACKGROUND

In recent years, quinoa sprouts have emerged as one of the most nutrient-packed, beneficial, and easy-to-make health food. Sprouts have a lot of nutrients, minerals, vitamins and antioxidants. The production of sprouts is easy and fast. Sprouting begins with soaking, where the endogenous enzymatic systems are activated in seeds. As a result, amylolytic, proteolytic and lipolytic enzymes are produced. During germination, reserve substances (proteins, fats and carbohydrates) are broken down to the simple compounds. The content of proteins in grain decreases, while their availability in sprouts increases. Proteins present in the sprouts have greater digestibility and nutritional value than the protein found in plant seeds. Similarly, carbohydrates are broken down from complex forms (polysaccharides) to simple compounds (disaccharides and simple sugars) [1].

Quinoa is one of the most nutritive grains. Quinoa is gluten-free, high in protein and contains all nine essential amino acids. The content of lysine, methionine and cysteine in quinoa is higher than in common cereals and legumes, making it complementary to these crops. It has a glycemic index of 53, which is considered low. Quinoa is rich in oil, containing beneficial fatty acids and a high content of tocopherols. In addition, it contains a large range of vitamins (ascorbic acid and tocopherols) and microelements (i.e., phosphorus, copper, manganese, iron, zinc, calcium, magnesium, sodium, and potassium) [2, 3].

The human body is unable to synthesize aromatic compounds, such as polyphenols, so they should be supplied with food. Rich in polyphenol compounds are fruits and vegetables in raw as well as processed form. Phenolics are also found in coffee, tea, wine and chocolate. Polyphenols have a significant impact on the health of our body. They have the ability to reduce the risk of diet-related diseases. They can minimize the effects of stress, inhibit the oxidation of lipids and nucleic acids, neutralize free radicals and the chelate metal ions. Flavonoids have also the ability to strengthen capillary walls and more over inhibits free radical oxidation reactions [4]. Previous studies have shown that phytochemicals can be increased after germination. The enhancement of the antioxidant activity during germination due to an increased content of polyphenols has been reported by several authors [5, 6].

Polyphenols in plants play diverse functions and are immensely important (act as agents in plant defense, hormones). Phenolics are mainly produced through the pentose phosphate, the shikimate, and the phenylpropanoid pathways. Phenylalanine and tyrosine ammonia-lyases, playing a fundamental role in the phenylpropanoids metabolism, transform aromatic amino acids into trans-cinnamic and p-coumaric acids, respectively. The function of these enzymes is increased under stress conditions, which results in an accumulation of “pathogen-related compounds,” including phenolics. This occurrence may be used for improving phenolics overproduction in plant systems [7]. Several authors have applied exogenous elicitors during germination of seeds to stimulate induction of phenylpropanoids metabolism for the production of buckwheat [8] broccoli [9], lentil [10], wheat [11] or mung bean sprouts [12].

In this study, the effects of hydrogen peroxide treatment and the phenylpropanoid pathway feeding on changes in the phenolics and antioxidant capacity of quinoa sprouts were studied.

MATERIAL AND METHODS

Chemicals

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), ammonium thiocyanate, and polyvinylpyrrolidone were purchased from Sigma-Aldrich Company (Poznan, Poland). All other chemicals were of analytical grade.

Sprouting conditions

Quinoa (*Chenopodium quinoa*) seeds were bought in local organic shop. Seeds were sterilized in 1% (v/v) sodium hypochloride for 3 min. and washed with distilled water. Before sprouting, quinoa seeds were soaked for 4 h in distilled water (control; C, C1, and C2) or phenolic precursors (0.1 mM shikimic acid, S, S1, and S2; 0.1 mM L-phenylalanine, F, F1, and F2; 0.1 mM L-tyrosine, Y, Y1, and Y2). Seeds were sprouted on petri dishes (Ø 125 mm) in darkness at 25 °C and 75% of humidity. Seeds were watered daily with water (control experiment) or with elicitor – hydrogen peroxide. For the treatment 1-day-old sprouts were sprayed with 5 mL of 50 mM (C1, F1, and Y1) or 200 mM (C2, F2, and Y2) hydrogen peroxide. After 3 days, sprouts were collected, freeze-dried, milled and stored in vacuum bags at −20 °C until further analysis.

Analysis of phenolics and antioxidant capacity

Extraction Procedure

500 mg of quinoa flour was extracted three times with 5 ml of 80% ethanol. The samples were shacked for 30 minutes at 40ºC at 50 rpm/min. After this time, the samples were centrifuged for 5 min, 6900 ×g. All fractions were collected, combined, and used for further analysis.

Total phenolics content

The amount of total phenolics was determined using Folin-Ciocalteau reagent [13]. To 100 µl of the extract 100 µl of H_2O and 400 µl of Folin–Ciocalteau reagent (1:5 H_2O) were added. After 3 min 2.5 mL of 10% Na_2CO_3 was added and samples were allowed to stand for 30 min at room temperature. After that time absorbance was measured at 725 nm. The amount of total phenolics was expressed as gallic acid equivalents (GAE) in mg per g of sprouts dry mass (d.m.).

Total flavonoid content

Total flavonoid content was determined according to the method described by Lamaison and Carnat [14]. 1 mL of the extract was mixed with 1 mL of 2% $AlCl_3$ x $6H_2O$ solution. Mixture was incubated at room temperature for 20 min. Following, absorbance was measured at 430 nm. Total flavonoids content was expressed as quercetin equivalents (QE) in mg per g of sprouts dry mass (d.m.).

Reducing power

Reducing power was determined by the method of Pulio et al. (2000). The extract (0.5 mL) was mixed with

phosphate buffer (0.5 mL, 200 mM, pH 6.6) and potassium ferricyanide $K_3[Fe(CN_6)]$ (0.5 mL, 1%). The mixture was incubated at 50 ºC for 20 min. Reactions were stopped with 0.5 mL 10% TCA and centrifuging for 10 min at 6500 × g. The upper layer of solution (2.5 mL) was mixed with distilled water (1.5 mL) and 300 µl of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Reducing power was expressed as Trolox equivalents in mg per g of sprouts dry mass (d.m.).

Antiradical activity (ABTS)

The experiments were carried out using the ABTS decolourization assay (Re et al. 1999). The ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature prior to use. The $ABTS^{+\bullet}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Lambda 40 UV-Vis spectrophotometer, Perkin Elmer Inc. Waltham, USA). Then, 40 mL of the extract obtained after digestion *in vitro* were added to 1.8 mL of $ABTS^{+\bullet}$ solution and the absorbance was measured at the end time of 5 min. The affinity of test material to quench ABTS free radical was evaluated according to the following equation:

$$\text{scavenging \%} = [(A_C - A_A) / A_C)] \times 100, \text{ where:}$$

A_C – absorbance of control,
A_A – absorbance of the extract obtained after digestion *in vitro*

Free radical scavenging ability was expresses as Trolox equivalents in mg per g of sprouts dry mass (d.m.).

Statistical Analyses

All experimental results were mean ± SD of three parallel experiments (n= 9). The obtained data was subjected to a statistical analysis and the consequent evaluations were analysed for a variance analysis. The statistical differences ($p < 0.05$.) were estimated through Tukey's test using the Statistica 6.0 software (StatSoft, Inc., Tulsa, USA).

RESULTS

The phenylpropanoids pathway was stimulated by elicitation with hydrogen peroxide as well as feeding with shikimic acid, phenylalanine, tyrosine. Research results showed that elicitation enhanced significantly ($p \leq 0.05$) the phenolic content of quinoa sprouts (Tab. 1). Compared to the control, total phenolics content was significantly increased by treatment of control sprouts with 50 mM (2.43 mg/g d.m.) and 200 mM H_2O_2 (2.52 mg/g d.m.). A significant increase was also determined in the sprouts fed with shimic acid (2.34 mg/g d.m.) and those fed with shikimic acid treated with 50 mM H_2O_2 (2.06 mg/g d.m.). However, the lowest content of polyphenols was determined in the sprouts fed with tyrosine and treated with 50 mM H_2O_2. It should be emphasized that the enrichment of seeds with phenylalanine and tyrosine and their subsequent elicitation with 200 mM H_2O_2 allowed to increase the polyphenols content by 16% and 23%, respectively.

The highest increase of flavonoids content was found in the sprouts fed with shikimic acid (2.08 mg/g d.m.). Among the analysed sprouts, the lowest content of flavonoids was determined in the sprouts from seeds supplemented with shikimic acid and treated with 200 mM H_2O_2.

Fig. 1 presents the effect of elicitation with hydrogen peroxide and elicitation supported by precursors of the phenylpropanoid pathway on the antiradical potential of the received sprouts. The germination process increased the ability to neutralize free radicals in relation to seeds. It should be noted that in all the sprouts, the elicitation caused an increase in the ability to neutralize free radicals. The highest ability to neutralize free radicals was demonstrated by the sprouts fed with tyrosine and treated with 200 mM H_2O_2.

The highest reducing power was found for the sprouts treated with 200 mM H_2O_2 obtained by phenylalanine feeding (2.65 mg TE/g d.m.) and those obtained from the seeds fed with shikimic acid (2.45 mg TE/g DW) (Fig. 2). Germination caused more than twice increase in the reduction power as compared to seeds. Elicitation of the sprouts with hydrogen peroxide slightly changed the value of the reduction power. The lowest value was recorded for sprouts fed tyrosine and treated with 200 mM H_2O_2.

DISCUSSION

Recently many authors paid a huge attention on phenolic compounds because of their antioxidant properties and different protective roles against diseases related with oxidative stress, such as cancer or cardiovascular either neurodegenerative diseases [17]. The application of elicitation for improving the nutraceutical potential of sprouts is described previously in many publications [18-20]. It is well known that sprouts are an excellent source of substances that enhance food and improve its functionality [21]. Through germination, the capacity and bioactivity of compounds with nutraceutical potential change dynamically, and most importantly, may be strongly affected by the elicitation [22, 23]. Sprouting is a very complex process; however in quinoa sprouts phenolics level is significantly lower than in seeds.

In our study, all the studied modifications caused the overproduction of polyphenolics. An elevation of plant polyphenolics in response to elicitors was also observed by Świeca et al. [24]. Quinoa sprouts have the highest content of polyphenols (Y200: 2.43±0.29 mg/g d.m.) The results showed that the content of polyphenols was most effectively increased by the elicitation of sprouts obtained from seeds soaked with phenylpropanoids pathway precursors with hydrogen peroxide. This result is was lower than that presented in the work Paśko et al. [25] where the content of polyphenols was 3.75±0.05 mg GAE g^{-1}. The difference may be due to the origin of grain and the use of other methods of grain preparation, germination conditions or research methods. Moreover, different research studies reported that germination is an efficient process to increase the total phenolics in soybean, black bean [26], broccoli radish and sunflower [27, 28]. After analysing the content of flavonoids in sprouts, it was unexpectedly noticed a decrease in their amount compared to the quinoa grains. However, when the elicitor (hydrogen peroxide) was used the amount of

flavonoids increased. The highest increase of flavonoids content was found for the sprouts treated with shikimic acid.

Phnolics were proved to have a significant correlation with antioxidant activity [29, 30]. Changes in phenolic content were generally associated with the changes in antioxidant capacity of the studied sprouts. Based on the data in the present work, antioxidant activity of quinoa sprouts is much higher than the dry grains. Both hydrogen peroxide treatment and precursors feeding caused about a double increase of reducing ability. The highest activity was found for the sprouts obtained from seeds fed with tyrosine and treated with 200 mM H_2O_2 (2,17 mg TE/g DW). The reason for this are differences in the content of polyphenols. Elicitation with the precursors of the phenylpropanoids pathway additionally allows to increase the antioxidant potential [18, 22, 25]. Researchers in the cited study observed the effect the modification of germination on the antioxidant activity of sprout.

CONCLUSIONS

In conclusion, improvement of quinoa sprout quality by elicitation with H_2O_2 and elicitation supported by the addition the phenylpropanoids pathway precursors is a useful tool for designing some new product with an increased nutraceutical potential. It can also be used as an alternative to conventional techniques applied to improve the levels of health-promoting phytochemicals and bioactivity in the low-processed food. Elicitation may improve health-promoting potential of sprouts, although selection of elicitor is crucial to deliver marketplace ready-to-eat sprouts enriched in specific bioactive phytochemicals.

CITE THIS AS

MEDtube Science Jun, 2018, Vol. VI (2), 55 – 60

ABBREVIATIONS

d.m – dry mass
GAE – Gallic Acid Equivalents
SD – Standard Deviation
TE – Trolox Equivalents
QE – Quercetin Equivalents

REFERENCES

1. Czerwińska D. Wartość odżywcza i walory zdrowotne kiełków zbożowych. Przegląd Zbożowo-Młynarski 2012; 8: 9-10.
2. Jacobsen SE. The worldwide potential for quinoa (Chenopodium quinoa Willd.). Food Reviews International 2003; 19: 167-177.
3. Gawlik-Dziki U, Świeca M, Sułkowski M, et al. Antioxidant and anticancer activities of Chenopodium quinoa leaves extracts—in vitro study. Food and Chemical Toxicology 2013; 57: 154–160.
4. Koehler P, Hartmann G, Wieser H, Rychlik M, Changes of folates, dietary fiber, and proteins in wheat as affected by germination. Journal of Agricultural and Food Chemistry 2007; 55 (12): 4678–4683.
5. Dikshit M, Ghadle M. Effect of sprouting on nutrients, antinutrients and in vitro digestibility of the MACS-13 soybean variety. Plant Foods Hum Nutrition 2003; 58: 1–11.
6. Miranda M, Vega-Gálvez A, López J, et al. Impact of air-drying temperature on nutritional properties, total phenolic content and antioxidant capacity of quinoa seeds (Chenopodium quinoa Willd.). Industrial Crops and Products 2010; 32 (3): 258–263.
7. Zhao J, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnology Advances 2005; 23 (4): 283–333.
8. Świeca M. Potentially bioaccessible phenolics, antioxidant activity and nutritional quality of young buckwheat sprouts affected by elicitation and elicitation supported by phenylpropanoid pathway precursor feeding. Food Chemistry 2016; 192: 625–632.
9. Yang R, Guo L, Jin X, et al. Enhancement of glucosinolate and sulforaphane formation of broccoli sprouts by zinc sulphate via its stress effect. Journal of Functional Foods 2015; 13: 345–349.
10. Świeca M. Production of ready-to-eat lentil sprouts with improved antioxidant capacity: optimization of elicitation conditions with hydrogen peroxide. Food Chemistry 2015; 180: 219–226.
11. Dziki D, Gawlik-Dziki U, Kordowska-Wiater M, Domań-Pytka M. Influence of elicitation and germination conditions on biological activity of wheat sprouts. Journal of Chemistry 2015; 2015: 1-8.
12. Randhir, Reena & Lin, Yuan-Tong & Shetty, Kalidas. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochemistry 2004.
13. Singleton VL, Rossi A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstics acid reagents. American Journal of Enology and Viticulture 1965; 16 (3): 144–158.
14. Lamaison J, Carnet A. Teneurs en principaux flavonoids des fleurs de Crataegeus monogyna Jacq et de Crataegeus laevigata (Poiret D. C) en fonction de la vegetation. Pharmaceutica Acta Helvetiae 1990; 65: 315–320.
15. Pulido R, Bravo L, Saura-Calixto F, Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing antioxidant power assay. Journal of Agricultural and Food Chemistry 2000; 48 (8): 3396-3402.
16. Re R, Pellegrini N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 1999; 26 (9-10): 1231–1237.
17. Riedel H, Akumo DN, Saw NMMT, et al. Elicitation and precursor feeding influence phenolic acids composition in Vitis vinifera suspension culture. African Journal of Biotechnology 2012; 11 (12): 3000-3008.
18. Świeca M, Sęczyk Ł, Gawlik-Dziki U. Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts. Food Chemistry 2014; 161: 288-295.

19. Pérez-Balibrea S, Moreno DA, García-Viguera C. Improving the phytochemical composition of broccoli sprouts by elicitation. Food Chemistry 2011; 129 (1): 35-44
20. Oh MM, Rajeshekar CB. Antioxidant content of edible sprouts: effect of environmental shocks. Journal of the Science of Food and Agriculture 2009; 89: 2221-2227.
21. Świeca M, Baraniak B. Influence of elicitation with hydrogen peroxide on health promoting phytochemicals and nutritional quality of dark germinated Lens culinaris sprouts Journal of the Science of Food and Agriculture 2013.
22. Świeca M, Gawlik-Dziki U, Kowalczyk D, Złotek U. Impact of germination time and type of illumination on the antioxidant compounds and antioxidant capacity of Lens culinaris sprouts. Scientia Horticulturae 2012; 140: 87-95.
23. Zhao J. Nutraceuticals, nutritional therapy, phytonutrients, and phytotherapy for improvement of human health: A perspective on plant biotechnology application. Recent Patents on Biotechnology 2007; 1: 75-97.
24. Świeca M, Baraniak B, Gawlik-Dziki U. Effect of selected divalent cations on protein mobilization in lentil (Lens culinaris) sprouts. Journal of Elementology 2014; 19 (2): 577-585.
25. Paśko P, Bartoń H, Zagrodzki P, et al. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chemistry 2009; 115 (3): 994-998.
26. Xue Z, Wang C, Zhai L, et al. Bioactive compounds and antioxidant activity of mung bean (Vigna radiata L.), soybean (Glycine max L.) and black bean (Phaseolus vulgaris L.) during the germination process. Czech J. Food Sci. 2016; 34: 68–78.
27. Drużyńska B, Kostrzewski K, Majewska E, et al. Content of components and their bioactive antioxidant activity sprouts seeds. Zeszyty Problemowe Postępów Nauk Rolniczych 2015; 583: 45–54.
28. Díaz-Batalla L, Widholm JM, Fahey GC, et al. Chemical components with health implications in wild and cultivated Mexican common bean seeds (Phaseolus vulgaris L.). Journal of Agricultural and Food Chemistry 2006; 54: 2045–2052.
29. Hossain MA, Rahman SMM. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. Food Research International 2011; 44: 672–676.
30. Kou X, Chen Q, Li X, et al. Quantitative assessment of bioactive compounds and the antioxidant activity of 15 jujube cultivars. Food Chemistry 2015; 173: 1037–1044.

LIST OF TABLES

Tab. 1. The effect of elicitation and precursor feeding on the flavonoids and phenolics content.

LIST OF FIGURES

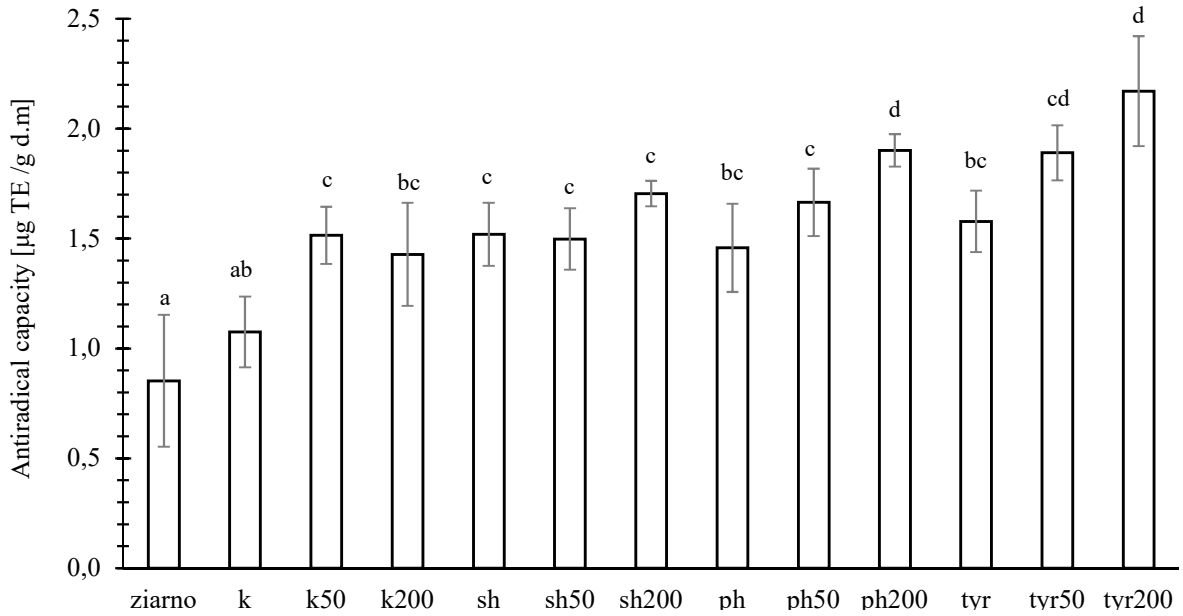
Fig. 1. The effect of elicitation and precursor feeding on the antiradical capacity of quinoa sprouts.
Fig. 2. The effect of elicitation and precursor feeding on the reducing power of quinoa sprouts.

TAB. 1. THE EFFECT OF ELICITATION AND PRECURSOR FEEDING ON THE FLAVONOIDS AND PHENOLICS CONTENT.

		Total flavonoids [mg/g d.m.]	Total phenolics [mg/g d.m]
Sample	Seeds	1.60±0.17^{a}	1.10±0.08^{a}
	C	1.78±0.08^{ab}	2.31±0.25^{b}
	C50	1.78±0,07^{ab}	2.43±0,05^{b}
	C200	1.90±0.05^{b}	2.52±0,45^{b}
	S	2.08±0.09^{c}	2.34±0,23^{b}
	S50	1.92±0.08b^{c}	2.06±0,11^{bc}
	S200	1.62±0.03^{a}	2.19±0,32^{bc}
	F	1.92±0.03^{bc}	1.98±0,17^{c}
	F50	1.91±0.06^{b}	1.86±0,18^{c}
	F200	1.75±0.03^{a}	2.29±0,43^{bc}
	Y	1.84±0.03^{b}	1.85±0.08^{c}
	Y50	1.81±0.18^{abc}	1.89±0.07^{c}
	Y200	1.84±0.13^{ab}	2.27±0.29^{b}

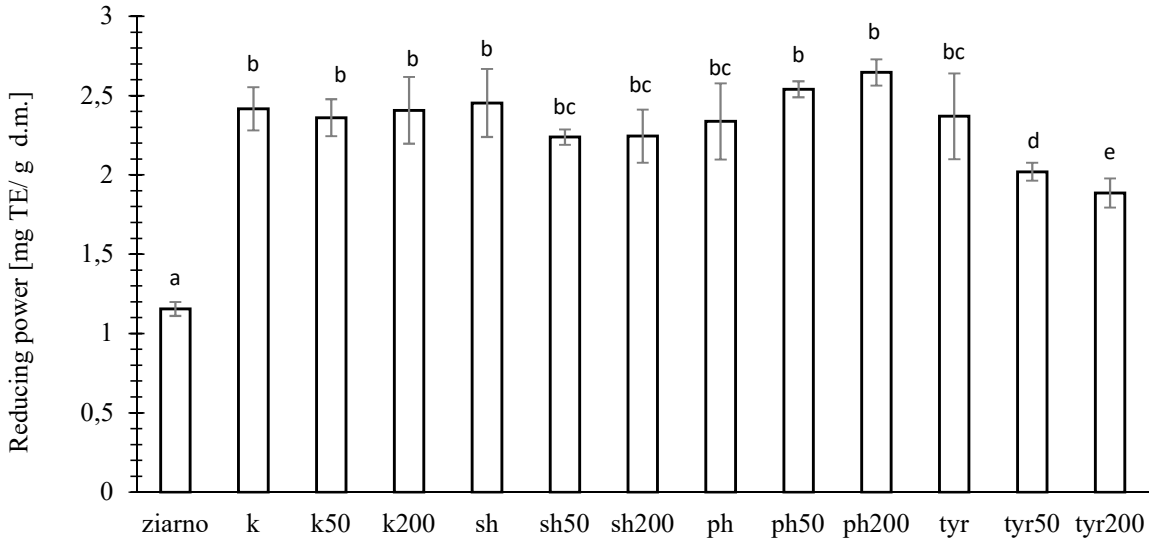
Value represents the mean of three independent experiments (±SD).
Means in columns followed by different letters are significantly different at $p \leq 0.05$.
C - control sprouts; C50- sprouts treated with 50 mM H_2O_2; S50- sprouts fed with shimic acid and treated with 50 mM H_2O_2; F50- sprouts fed with phenylalanine and treated with 50 mM H_2O_2; Y50- sprouts fed with tyrosine and treated with 50 mM H_2O_2; C200- sprouts treated with 200 mM H_2O_2; S200- sprouts fed with shimic acid and treated with 200 mM H_2O_2; F200- sprouts fed with phenylalanine and treated with 200 mM H_2O_2; Y200- sprouts fed tyrosine and treated with 200 mM H_2O_2.

FIG. 1. THE EFFECT OF ELICITATION AND PRECURSOR FEEDING ON THE ANTIRADICAL CAPACITY OF QUINOA SPROUTS.



Means followed by different small letters are significantly different ($p \leq 0.05$).
Each value represents the mean of three independent experiments (±SD).
C - control sprouts; C50- sprouts treated with 50mM H_2O_2; S50- sprouts fed with shimic acid and treated with 50mM H_2O_2; F50- sprouts fed with phenylalanine and treated with 50mM H_2O_2; Y50- sprouts fed with tyrosine and treated with 50mM H_2O_2; C200- sprouts terated with 200mM H_2O_2; S200- sprouts fed with shimic acid and treated with 200mM H_2O_2; F200- sprouts fed with phenylalanine and treated with 200mM H_2O_2; Y200- sprouts fed tyrosine and treated with 200mM H_2O_2.

FIG. 2. THE EFFECT OF ELICITATION AND PRECURSOR FEEDING ON THE REDUCING POWER OF QUINOA SPROUTS.



Note: means with different small letters are significantly different ($p \leq 0.05$). Each value represents the mean of three independent experiments (±SD). C - control sprouts; C50- sprouts treated with 50mM H_2O_2; S50- sprouts fed with shimic acid and treated with 50mM H_2O_2; F50- sprouts fed with phenylalanine and treated with 50mM H_2O_2; Y50- sprouts fed with tyrosine and treated with 50mM H_2O_2; C200- sprouts terated with 200mM H_2O_2; S200- sprouts fed with shimic acid and treated with 200mM H_2O_2; F200- sprouts fed with phenylalanine and treated with 200mM H_2O_2; Y200- sprouts fed tyrosine and treated with 200mM H_2O_2


MEDtube
sharing
medical
knowledge™