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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

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CONTENTS

ORIGINAL PAPERS

Emilia Mikołajewska, Sławomir Wawrzyniak, Piotr Dzięgielewski, Aleksander Goch
Changes of wound dimensions and pain assessment in response to hyperbaric oxygenation therapy. 259

Justyna Ber, Mirosław Malec, Agnieszka Bienert, Małgorzata Nowicka, Łukasz Żurański, Edmund Grześkowiak, Roma Hartmann-Sobczyńska, Paweł Sobczyński
The pharmacodynamics of dexmedetomidine in elderly cardiac patients undergoing analgosedation in the ICU 265

Agnieszka Ulatowska, Katarzyna Plagens-Rotman, Małgorzata Piskorz-Szymendera, Elżbieta Włodarczyk, Natalia Smolarek, Izabela Miechowicz, Andrzej Jóźwiak, Grażyna Bączyk
Clostridium difficile – still a problem among the XXI century of geriatic patients 272

Izabela Warmuz-Stangierska, Monika Gołąb, Adam Stangierski, Katarzyna Piątek, Natalie Musial, Marek Ruchała, Katarzyna Ziemnicka
Personality traits and sex-role schema in adult patients with childhood-onset combined pituitary hormone deficiency not treated with growth hormone 279

Bartosz Sokół, Roman Jankowski, Barbara Więckowska, Łukasz Gąsiorowski, Michael Czekajlo
Development and evaluation of simulation based neurosurgery curriculum. Pilot study at the Poznan University of Medical Sciences 286

Grażyna Bączyk, Dorota Formanowicz, Paweł Kleka, Włodzimierz Samborski
The influence of alendronate therapy on the quality of life in postmenopausal women with reduced bone mineral density 292

Krzysztof Sobczak, Agata Janaszyk, Katarzyna Leoniuk
CONTACT – communication protocol for family practitioners and specialists 300

REVIEW PAPERS

Teresa Matthews-Brzozowska, Monika Łącka, Magdalena Bernacka, Maciej Lichaj
Mesotherapy – a method of facial skin rejuvenation from an interdisciplinary perspective on improving facial aesthetics 308

Łukasz Dobrek
Fatty acid binding proteins (FABPs) – a new laboratory biomarker for kidney diseases . 313

THOUSAND WORDS ABOUT...

Agnieszka Danuta Gaczkowska, Paweł Piotr Jagodziński, Adrianna Mostowska
The molecular basis of non-syndromic orofacial clefts and tooth agenesis 321

Przemysław Wirstlein, Paweł P. Jagodziński, Małgorzata Szczepańska
The importance of epigenome research in the diagnosis and treatment of endometriosis 325

THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

Joanna Bartkowiak-Wieczorek, Ewa Kamińska, Michał Szulc, Joanna Domagała, Przemysław Ł. Mikołajczak, Edmund Grześkowiak, Agnieszka Bienert
Evaluation of efficacy and mechanisms of action of Cannabis sativa extracts with analgesic, anti-inflammatory and antiemetic properties in an *in vivo* model 328

Instructions for Authors 333

ORIGINAL PAPER

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Changes of wound dimensions and pain assessment in response to hyperbaric oxygenation therapy

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ABSTRACT

Introduction. Novel approaches to wound healing can provide decreased risk of complications, wider possibilities of further treatment, rehabilitation and care, and improved patient's quality of life. Most recent studies support the concept that HBOT accelerates the wound healing process.
Aim. This paper aims at presentation and discussion the outcomes of a research on chronic wounds healing using the HBOT. Particular attention was paid to changes of wound dimensions and pain assessment in response to HBOT.
Material and Methods. Inclusion criteria meet the medical records of eighty-nine adult patients with chronic wounds aged 18–85 years treated with HBOT. Wound length, wound width and pain were measured twice: before and after treatment.
Results. There have been observed favourable and statistically significant changes in all measured areas: pain assessment, wound length, and wound width. Improvement of pain assessment occured in 94.38% of patients, improvement of maximal wound length occurred in 94.38% of patients, improvement of maximal wound width occurred in 86.52% of patients.
Conclusions. Application HBOT in adult patients with chronic wound is an effective method of treatment. Age above 62 years, sex (men), lack of obesity, and number of HBOT sessions higher than 29 can be regarded as useful prognostic signs, however there is need for further research.

Keywords: rehabilitation, wounds healing, chronic wounds, hyperbaric oxygenation, hyperbaric oxygen therapy, HBOT.

Introduction

Hyperbaric oxygen therapy (HBOT) becomes more and more popular basic or supplementary method of the severe wound healing. It involves exposing patients to increased gas pressure while inhaling pure oxygen. Hyperbaric oxygenation is the use of 100% oxygen at pressures greater than atmospheric pressure (≥ *1.4 atmospheres absolute pressure* [1–5]). Such method increases the delivery of oxygen to damaged local tissues (wound), stimulates angiogenesis, immune response, collagen synthesis, and stem cell migration, in this way accelerating wound healing [4, 5]. Although pathophysiology underlying improved wound healing as a result of HBOT application is still under

research [1, 6–8] there is common belief that HBOT has two primary mechanisms of action due to:

- hyperoxygenation (increase in dissolved oxygen in plasma due to increased partial pressure of arterial oxygen),
- decrease in bubble size (angiogenesis, vasoconstriction, fibroblast proliferation, leukocyte oxidative killing, toxin inhibition, antibiotic synergy) [5].

This way HBOT helps to maintain optimal wound oxygenation, macrocirculation, microcirculation, and nutrition [1, 6–8].

Main areas of the clinical HBOT application in wound healing were investigated by Bhutani & Vishwanath. They cover i.a. non-healing wounds (diabetic, vascular insufficiency ulcers), infected wounds (clostridial myonecrosis, necrotising soft tissue infections, Fournier's gangrene), traumatic wounds (crush injury, compartment syndrome), skin grafts and flaps, radiation-induced wounds, and thermal burns [5]. Such variety makes difficulties in precise assessment of the real recovery for decision-making process deriving form evidence-based medicine paradigm, usefulness of the prognostic signs, and compartmental studies.

Even if the clinical effectivity of HBOT seems be doubtless there is need for more detailed research on bigger samples researching possible indications, contraindications, and prognostic signs. This paper aims at presentation and discussion of the outcomes of a research on chronic wounds healing using the HBOT. Particular attention was paid to changes of wound dimensions and pain assessment in response to HBOT.

Material and Methods

The research design was a retrospective study. We reviewed the medical records of adult patients with chronic wounds treated with HBOT.

Inclusion criteria covered 18 years of age or older, chronic wounds confirmed by medical records, and lack of contraindications. As absolute contraindications to HBOT were regarded: chemotherapy with certain agents, untreated pneumothorax, history of spontaneous pneumothorax). As relative contraindications to HBOT were regarded: fever, systemic viral infections seizure disorder, retinal surgery, middle ear surgery, cataract exacerbation, spherocytosis, optic neuritis. Eighty-nine patients (63.12%) met the aforementioned inclusion criteria. Their clinical summary is presented in **Table 1**.

Table 1. Clinical summary of the patients

	Patients n = 89 (100%)
Age [years]:	
– Min	18
– Max	85
– Mean	57.87
– SD	14.14
– Median	62
Sex:	
– Females	36 (40,45%)
– Males	53 (59,55%)
Value of Body Mass Index (BMI):	
– Min	20.28
– Max	55.1
– Mean	29.58
– SD	6.65
– Median	28.37
Number of HBOT sessions:	
– Min	5
– Max	70
– Mean	25.8
– SD	9.5
– Median	29

Adult patients with chronic wounds were treated using HBOT receiving ≥ 5 sessions. Patients were treated in Center of Hyperbaric Oxygenation and Wound Healing of the Military Clinical Hospital No. 10 with Polyclinic in Bygoszcz, Poland in 2014. The same twelve-person HBOT chamber HiperTech Zyron 12 (GTC, Sweden) was used in each patient.

Maximal wound length and maximal wound width were measured twice: before and after treatment. Pain assessment (using the numerical rating scale) was done twice: before and after treatment. These values were often impaired in patients with chronic wounds. Selection of aforementioned parameters allow other scientists to replicate the study.

All the data in this study were collected and stored using the MS Access 2013 software. Aforementioned data were analyzed with the software Statistica version 12. Where available, mean, median, minimum value (Min), maximum value (Max) and standard deviation (SD) were calculated to show the results of this study. The Shapiro-Wilk test was used as a powerful normality test. Parametric t-Student's test and non-parametric Wilcoxon's test were used to compare scores. We used $p \leq 0.05$ as the significance level. Correlations (statistical relationships) were assessed between changes of pain assessment, wound length, and wound width observed as the result of the HBOT intervention. Change of results before therapy and after therapy was determined as a result of the substraction. To assess correlations Spearman's rank correlation coeficient (Spearman's rhos) was used.

This study was conducted in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice (GCP). Freely given written informed consent was obtained from every patient prior to the study.

Results

Statistically significant and important changes reflecting recovery in numerical rating scale for pain assessment, maximal wound length, and maximal wound width were observed. The results for whole group of patients are shown in **Table 2** (all changes were statisticaly significant).

Improvement of pain assessment occured in 94.38% of patients, improvement of maximal wound length occurred in 94.38% of patients, improvement of maximal wound width occured in 86.52% of patients. The results of complete recovery are shown in **Table 3**.

Best results of the HBOT administration were achieved in particular groups of patients: men, 62 y.o and older (median of age), with BMI < 30 (i.e. non obese patients), with number of HBOT sessions ≥ 29 (median). Aforementioned outcomes may serve as ground for a clinical prognosis.

Statistically relevant corellations observed in the whole group of patients were as follows: poor (positive) correlations between changes in wound width and wound length, between pain assessment and wound length, and between pain assessment and wound width.

Statistically relevant corellations observed in the group of women were as follows: moderate (positive) correlations between changes in wound width and wound length, and between pain assessment and wound width. Corellations of the study results for men (Spearman's rhos) were not statistically significant.

Statistically relevant corellations observed in the group of patients in the age of 62 (median) and older were as follows: poor (positive) correlations between changes in wound width and wound length, and between pain assessment and wound width.

Corellations of the study results for patients younger than median (Spearman's rhos) were not statistically significant.

Statistically relevant corellations observed in the group of obese patients were as follows: moderate (positive) correlation between changes of wound width and wound length, and moderate (positive) correlation between changes of pain assessment and wound width.

Statistically relevant corellations observed in the group of patients with BMI < 30 (**Table 8**) were as follows: poor (positive) correlation between changes in wound width and wound length, and between pain assessment and wound width.

Table 2. Statistical analysis of the study results for the whole group of patients

	n	Mean	Median	Min	Max	SD
Before therapy						
Numerical rating scale for pain assessment	89	3.7	1	1	10	1,74
Max. wound length	89	6.13	2.1	1	26	2.2
Max. wound width	89	6.69	1.1	1	52	1.78
After therapy						
Numerical rating scale for pain assessment	89	2.95	0	0	10	1.23
Max. wound length	89	5.87	1,3	0	37	1.95
Max. wound width	89	5.94	0.5	0	44	1.59

Table 3. Number and percentage of complete recovery depends on measured parameters

	n	%
Numerical rating scale for pain assessment	66	74.16
Max. wound length	37	41.57
Max. wound width	39	43.82

Table 4. Corellations of the study results for the whole group of patients (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	0.251	0.233
Change of max. wound length		–	0.306
Change of max. wound width			–

n.s. = non significant ($p > 0.05$)

Table 5. Corellations of the study results for women (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	n.s.	0.501
Change of max. wound length		–	0.465
Change of max. wound width			–

n.s. = non significant ($p > 0.05$)

Table 6. Corellations of the study results for patients in the age of 62 years (median) and older (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	n.s.	0.322
Change of max. wound length		–	0.352
Change of max. wound width			–

n.s. = non significant ($p > 0.05$)

Table 7. Corellations of the study results for obese patients i.e. with BMI≥30 (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	n.s.	0.538
Change of max. wound length		–	0.294
Change of max. wound width			–

n.s. = non significant ($p > 0.05$)

Table 8. Corellations of the study results for patients with BMI < 30 (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	0.279	n.s.
Change of max. wound length		–	0.251
Change of max. wound width			–

n.s. = non significant ($p > 0.05$)

Statistically relevant corellations observed in the group of patients with number of HBOT sessions ≥ 29 (**Table 9**) were as follows: moderate (positive) correlation between pain assessment and wound length.

Statistically relevant corellations observed in the group of patients with number of HBOT sessions < 29 (**Table 10**) were as follows: poor (positive) correlation between wound width and wound length.

Table 9. Corellations of the study results for patients with number of HBOT sessions ≥ 29 (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	0.408	0.192
Change of max. wound length		–	0.198
Change of max. wound width			–

Table 10. Corellations of the study results for patients with number of HBOT sessions < 29 (Spearman's rhos)

	Change of numerical rating scale for pain assessment	Change of max. wound length	Change of max. wound width
Change of numerical rating scale for pain assessment	–	0.158	0.199
Change of max. wound length		–	0.39
Change of max. wound width			–

Discussion

Novel approaches to wound healing can provide a decreased risk of complications, wider possibilities of further treatment, rehabilitation and care, and improved patient's quality of life [1–3]. Most recent studies support the concept that HBOT accelerates the wound healing process [1–5], even after limb amputation [9]. The clinical effectivity of hyperbaric oxygenation is doubtless: it increases the percentage of completely healed patients (up to 74–100%), patients with recovery (up to 76–94.7%) and decreases the number of amputations relative to traditional approaches [1, 10, 11]. There is discrepancy among scientists concerning long-term effects of HBOT: improvements may disappear at the two week follow-up [12], even if Boykin and Baylis showed short- and long-term effectivity of HBOT [13]. Clinical trials on HBOT application are difficult to compare due to their heterogeneity in terms of the study design, kind of wounds involved and tools used to assess the outcome [4].

Our results support the hypothesis that HBOT is effective in wounds healing in adult patients. Favorable changes were observed in patients as a result of the therapy. Percentage of recovery and completely healed patients were similar to values observed by other researchers. Correlations may indicate important predictive relationships useful for further studies and in everyday clinical practice (as a part of decision-making process). Values of proposed prognostic signs should not be underestimated – it seems that many factors influencing HBOT efficiency are not identified so far [7, 14–16]. Efficiency of HBOT may depend on many factors (e.g. etiology of wound) – Ueno et al. showed HBOT less effective in wounds caused by diabetes mellitus and in patients who undergone hemodialysis [17], even if Boykin and Baylis showed short- and long-term effectivity of HBOT independently from wound etiology [13]. These findings highlight the increasing value of HBOT in wound healing.

No complications of HBOT (confinement anxiety, ear pain, hypoglycemic event, hyperglycemic event, shortness of breath, etc.) were observed. Despite aforementioned outcomes we should be aware that complications occurrence may vary, depending on e.g. patient clinical conditions, e.g. recent study by Kaur et al. reported incidence of complications and adverse results of HBOT such as ear discomfort/pain (20%), claustrophobia (13%), and generalized seizures (0.5%) [18].

There is need to admit that number of participants was higher than in previous studies concerning HBOT application in wound healing. Tools selection supports replication of the study and usefullnes of our results in clinical practice – they are easy to perform, time-efficient, accurate and inexpensive. Thus such selection of measuriement tools should not be regarded as limitation of our study.

The main limitation of the study is study design (retrospective before-after study) and lack of the reference group. We hope to remove this limitation during further studies. We intent to continue this study on bigger sample of patients based on randomized controlled trail (RCT) design. Current outcomes will be helpful to design better methodology, especially concerning more detailed searching for prognostic signs and correlations. Wound cause, patient history (including secondary changes), age, sex, obesity, number of HBOT sessions, exudation, etc. should be carefully taken into consideration. True may be an assumption that number of factors influencing wound healing may be huge causing neccessity of patient-tailored therapy rather than general method of wound healing.

HBOT is regarded to be useful basic or complementary method in wound healing. Thus directions for further research cover short- and long-term results of the use of HBOT alone and in combination with other therapies (traditional or emerging).

We hope that further studies ensure more independent sources of knowledge and experience necessary to confirm more detailed prognostic signs and correlations needed for clinical guidelines. The ultimate aim is to optimize the wound therapy in clinical setting.

To sum up: application of HBOT in adult patients with chronic wound is an effective method of treatment. Age above 62 years, sex (men), lack of obesity, and number of HBOT session higher than 29 can be regarded useful prognostic signs, however there is need for further research.

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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The pharmacodynamics of dexmedetomidine in elderly cardiac patients undergoing analgosedation in the ICU

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ABSTRACT

Aim. This study aimed to evaluate the pharmacodynamics of dexmedetomidine in elderly cardiac patients.
Material and Methods. Twelve patients of 60 years or older and need for analgesia after surgery or as a result of critical health conditions were included into our study. Dexmedetomidine was administered intravenously as a continuous infusion without the initial dose. At the beginning the infusion was started at the rate of 0.7 μg/kg/h and then it was continued in the range of 0.17–1.39 μg/kg/h according to desired level of sedation. Information about heart rate, systolic, diastolic and mean arterial blood pressure, bispectral index and cardiac index were collected a few minutes before, during and in 12 hours after infusion of dexmedetomidine.
Results. The hemodynamic data as well as BIS level were collected from 12 patients. The duration of dexmedetomidine infusion was less than 9 hours. For each patient the reduction in blood pressure and heart rate compared to the value before dexmedetomidine infusion was observed. We did not observe bradycardia in any patient. Appropriate sedation level was achieved using only dexmedetomidine and ranged from 60 to 80. In only 2 cases it was necessary to give a single dose of another sedative.
Conclusions. To conclude, in the patients' population involved in the study, which included older cardiac patients dexmedetomidne has been shown as a sedative agent which enabled to achieve desire level of sedation in the recommended ranges without episodes of bradycardia, however hypotension events were noted.

Keywords: dexmedetomidine, pharmacodynamics, bradycardia, hypotension, sedation.

Introduction

Dexmedetomidine (dex) is a newly discovered drug that has gained great popularity in neuroanesthesia, intensive care unit (ICU) and cardiac anesthesia in recent years. It was approved in 1999 by the FDA as a short-acting sedative. In Europe, it was introduced to health care in 2011 [1, 2]. Dexmedetomidine is a highly selective α2-adrenergic receptors agonist with high affinity for the α2-receptor (α2/α1 1600:1) compared with clonidine (α2/α1 200:1), which makes it a complete α2 agonist [3, 4]. Dexmedetomidine by α2-adrenergic receptor activation causes sedation similar to natural sleep, which helps in the early postoperative period [5, 6]. It also exhibits analgesic, anxiolytic and sedative effects without causing severe respiratory depression. Sedative action is responsible for the stimulation of receptors located at the sinus of the upper part of the brain stem [7]. Analgesic activity consists of a central component – stimulation of receptors in the brain stem and hind corners of the spinal cord, and peripheral – stimulation of the receptors in the nerve roots of the posterior nerve roots [8].

Dexmedetomidine is a highly lipophilic drug. After *i.v.* administration it shows rapid distribution with biological half-life ($t_{0,5}$) in the distribution phase being of 6 min. The drug is 94% bound to plasma proteins. Metabolism occurs as a result of direct conjugation to glucuronic acid and cytochrome P-450 isozymes. $T_{0,5}$ in the elimination phase equals 2 h. The drug is in 95% excreted in the urine, 4% in the feces in the form of metabolites [3, 9, 10].

Unique action allows for sedation without causing excessive sedation (cooperative sedation), difficult to achieve by other drugs. Drug administration is particularly useful in situations where there is a need for awareness during sedation [11]. The sedation combined with the analgesic effect without respiratory depression is used at the time of weaning from ventilation therapy, after long-term use of other sedative medicinal products, and in the elderly or other severe illness. Sedation and diminution in muscle tone is well tolerated in patients with withdrawal syndrome and delusional syndromes after major and long-term surgical procedures such as extracorporeal cardiac surgery [9]. This action reduces psychomotor excitability and has cardioprotective activity. Reduction of muscle tremor is used to control chills while cooling the body as well as for hypothermia. Because of its different mechanism of action, it can also be given in terminal illness as a supplement to other painkillers and sedatives. Dexmedetomidine has also promising results in patients with pulmonary hypertension undergoing mitral valve replacement [11].

Side effects of dexmedetomidine are mainly limited to hemodynamic changes. These include hypertension, bradycardia and hypotension due to pre-and post-synaptic activation of the α2 receptor, which causes vasospasm. Furthermore, it has been shown that dexmedetomidine alleviates stress responses, thus creating a more stable hemodynamic profile during surgery or induction of anesthesia. In case of overdosage atrioventricular block I degree, bradycardia and hypotension had been seen. Co-administration of dexmedetomidine with anesthetics, sedatives, hypnotics and opioids may increase observed effects [9, 11].

Aim

The aim of the study was to evaluate the pharmacodynamics of dexmedetomidine in elderly cardiac patients. Cardiac patients require comprehensive interdisciplinary teamwork to ensure the best possible outcome. Perioperative care is to carefully select sedatives to provide comfort to the patient, while avoiding physiological stress and heart instability [1].

Material and Methods

The study was conducted among intensive care patients in clinical hospital after approval of protocol by institutional Bioethics Committee. The approval number was 213/13 and 572/16. The inclusion criteria were: age – 60 years or older and need for analgosedation after surgery or as a result of critical health conditions. We excluded patients that were younger than 60 years and/or have hemodynamic instability. Dexmedetomidine (Dexdor, Orion Pharma Poland Sp. z.o.o.) was administered in continuous infusion without a loading dose. The infusion was started at the rate of 0.7 μg/kg/h and was continued in the range 0.17–1.39 μg/kg/h according to desired level of sedation. Information was recorded about heart rate (HR), systolic, diastolic and mean arterial blood pressure (SBP, DBP and MAP), bispectral index (BIS), cardiac index (CI) a few minutes before, during and in 12 hours after infusion of dexmedetomidine. BIS was monitored by Philips Medical Systems B.V (Netherlands) and CI by FloTrac System (Edwards Lifescences, USA). All the parameters were recorded every hour during the infusion as well as in any case when other drug which might have influenced collected parameters was administrated, e.g. noradrenalin, midazolam, ephedrine, propofol.

Bradycardia and hypotension were monitored among the patients as the pharmacodynamics of dex and potential side effects of the drug.

Heart rate was measured in bites per minutes (bpm) and blood pressure in millimetres of mercury (mmHg). Bradycardia was defined for the heart rate less than 40 bites per minutes. Hypotension was defined for systolic blood pressure less than 80 mmHg and diastolic blood pressure less than 50 mmHg. Bradycardia and/or hypotension was defined also when the fall by at least 30% compared to baseline value was noticed (heart rate and/or blood pressure, respectively) [12].

Bispectral index was used to assess the level of consciousness. It is a parameter to measure brain activity and is based on electroencephalogram. BIS is used to estimate the level of sedation and anaesthesia. BIS values ranged between 0 (no cortical activity) and 100 (completely awake). The values between 40 and 60 signified general anaesthesia, whereas for adequate sedation in ICU BIS should remain in the range 60–80 [13, 14].

Cardiac index is a cardiac output (CO) indexed to body surface area (BSA). Cardiac output is the sum of the systemic flow per minute and calculated by the product of heart rate and stroke volume. The cardiac index reference range for elderly patients is 2.2–3.8 L/min/m^2 [15]. According to study performed by Cattermole at al [16], reference range of CI is 1.88–4.71 L/min/m^2. They offered this range for healthy patients over 60. In our study we used the norm given by Cattermole et al.

Results

12 patients were enrolled to the study, 10 of which were postsurgical patients. **Table 1** lists patients' demographics and characteristics of dexmedetomidine infusion. The monitored hemodynamic parameters e.g. DBP, SBP, MAP, HR, CI as well as BIS were also presented (**Table 2**). The duration of dexmedetomidine infusion was less than 9 hours. In each patient a decrease in heart rate and blood pressure was calculated by comparing the values of these parameters before and during the infusion (**Table 2**). During the study period any episodes of bradycardia were not observed among the patients. However, in 8 patients incidents of hypotension were observed. In one case the administration of noradrenalin was needed whereas in the other dosage changes ensured the adequate hemodynamic stability.

The infusion of dex started after the surgery, it was 39 (± 20) minutes after stop of sevoflurane administration and 226,5 (± 51,8) minutes after premedication with propofol. In one patient values were above 60, in 3 patients they were less than 60. In two cases 5 mg midazolam was given less than one hour (45 and 55 minutes) before beginning of dexmedetomidine administration and as the result the baseline values of BIS were affected. Adequate sedation dex infusion was started 15 minutes after end of sevoflurane administration so that the baseline BIS could have been affected by the anesthesia period. At the start of infusion BIS level was maintained by using only dexmedetomidine in the range between 60 and 80. In only 2 cases it was necessary to give a single dose of another sedative (midazolam or propofol). However, it caused decreasing BIS values below 60. Bispectrac index values during dex infusion were presented on **Figure 1**.

Cardiac index was registered in 11 patients. In general, there was a reduction in cardiac index following commencement of dex infusion when compared to the baseline. In two patients there were also such episodes during continued administration of dex, necessitating adjustment of the rate of infusion (**Table 2**, **Figure 2**). In two patients before start of dex infusion CI was above references values (7, 6 and 5.6) and during infusion CI was reduced to normal range (average values: 2.7, 3.8 and 3.7, respectively).

Table 1. Demographic characterization of patients. Results are expressed as number or median with range

Parameter [Unit]	Number or Median [Range]
Male/Female	11/1
Age [years]	64.5 [61–79]
Weight [kg]	74.5 [55–85]
Height [cm]	169 [160–177]
Infusion time [minutes]	287.5 [220–505]
Total dose of dexmedetomidine [μg]	276.35 [142.40–602.40]
Mean ratio of infusion [μg/kg/h]	0.83 [0.46–1.15]
Use of inotropes during infusion [yes/no]	1/11

Table 2. Hemodynamic and pharmacodynamic (BIS) parameters of dexmedetomidine infusion (mean values with standard variation for all subjects)

Parameter [unit]	Baseline[1]	During infusion	After infusion	% changes[2]
Bispectral index	68,0 ± 11,0	67,7 ± 10,2	82,8 ± 6,9	1,0
Systolic blood plessure [mmHg]	146,0 ± 36,0	117,3 ± 23,9	115,2 ± 10,9	19,7
Diastolic blood plessure [mmHg]	72,0 ± 17,0	59,1 ± 10,6	58,0 ± 8,8	17,9
Heart rate [bpm]	90,0 ± 18,0	71,3 ± 9,6	74,0 ± 11,6	20,8
Cardiac index [L/min/m^2]	3,9 ± 1,7	2,9 ± 0,7	2,9 ± 0,8	25,6

[1] just before the start of the infusion
[2] percentage change of the mean value of the infusion period compared to the baseline

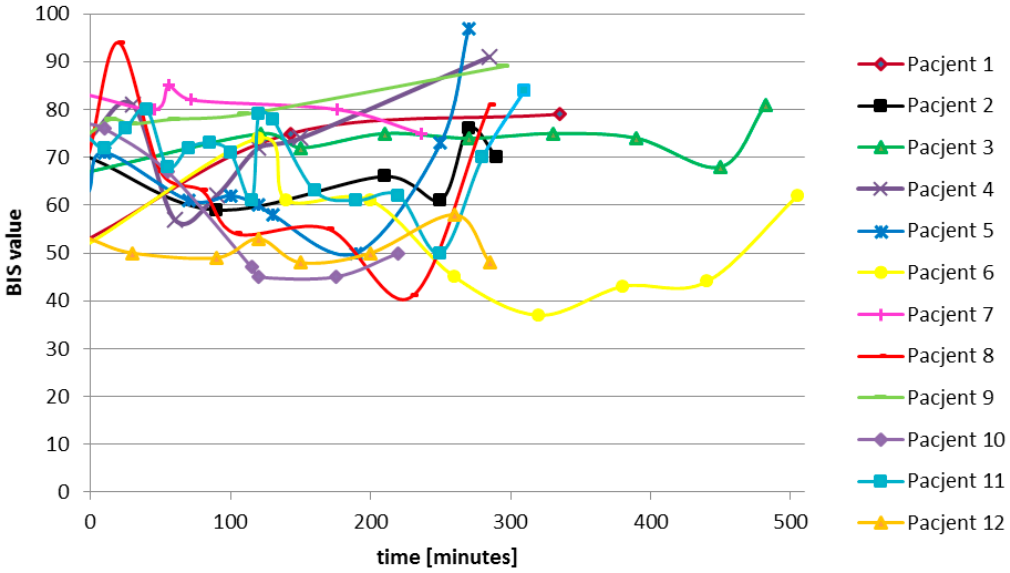

Figure 1. Bispectral index values for each patient before and during dex infusion

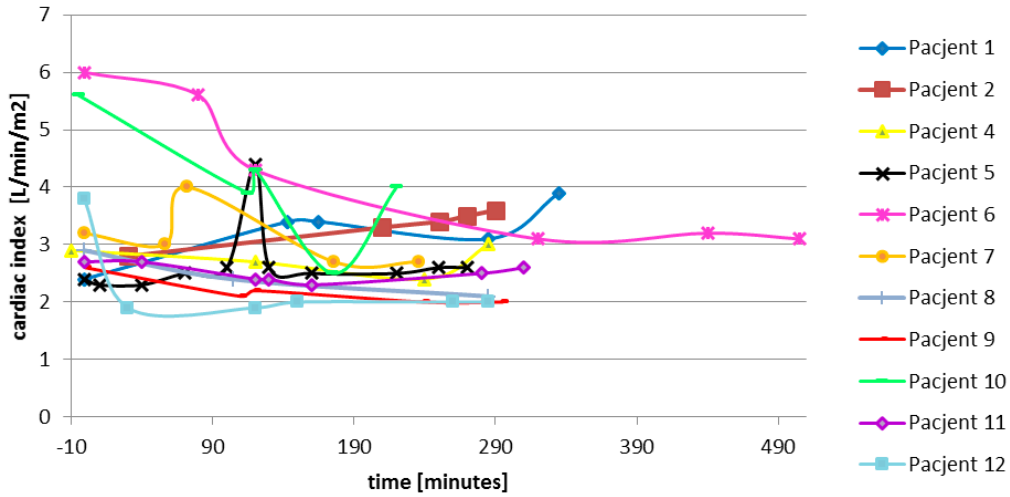

Figure 2. Cardiac index values for each patient before and during dex infusion

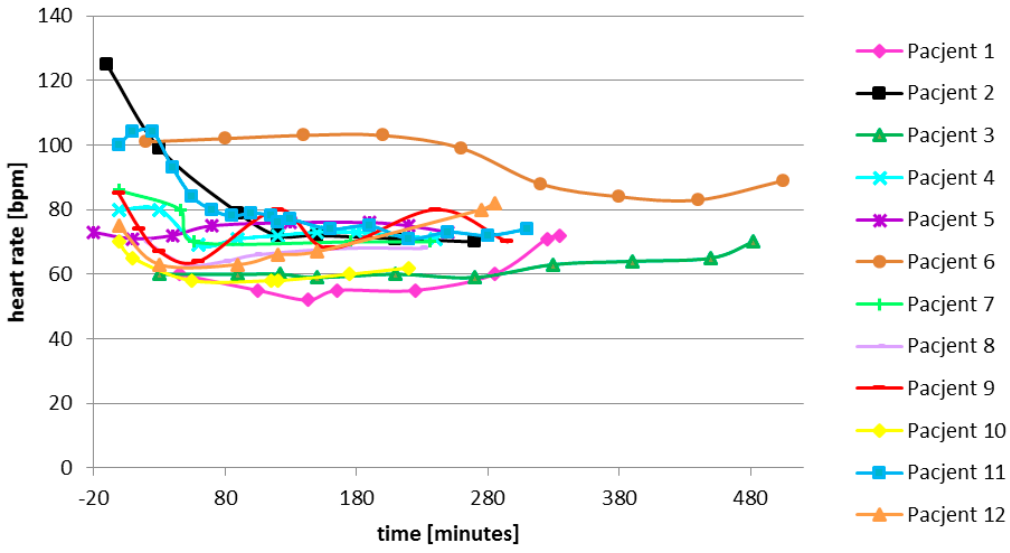

Figure 3. Heart rate values for each patient before and during dex infusion

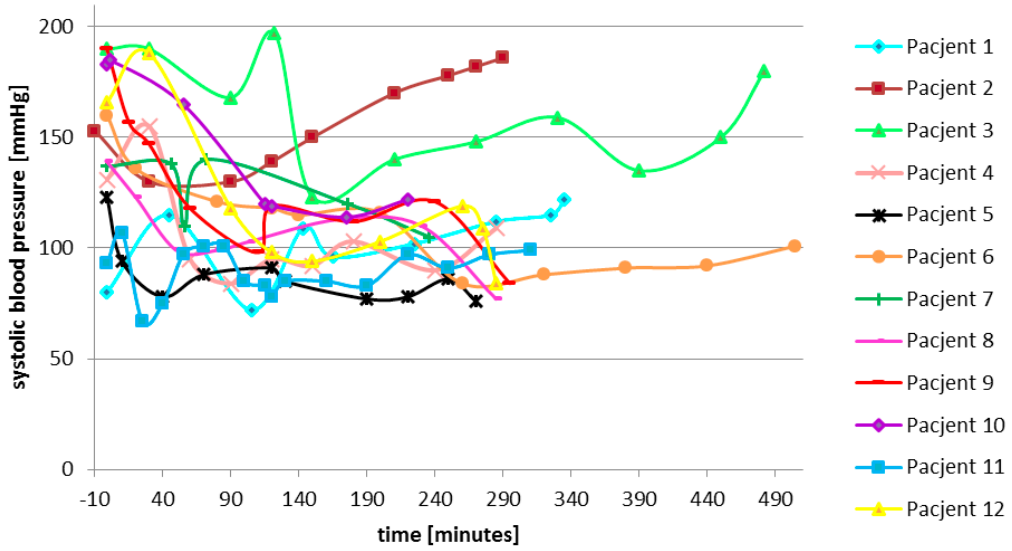

Figure 4. Systolic blood pressure values for each patient before and during dex infusion

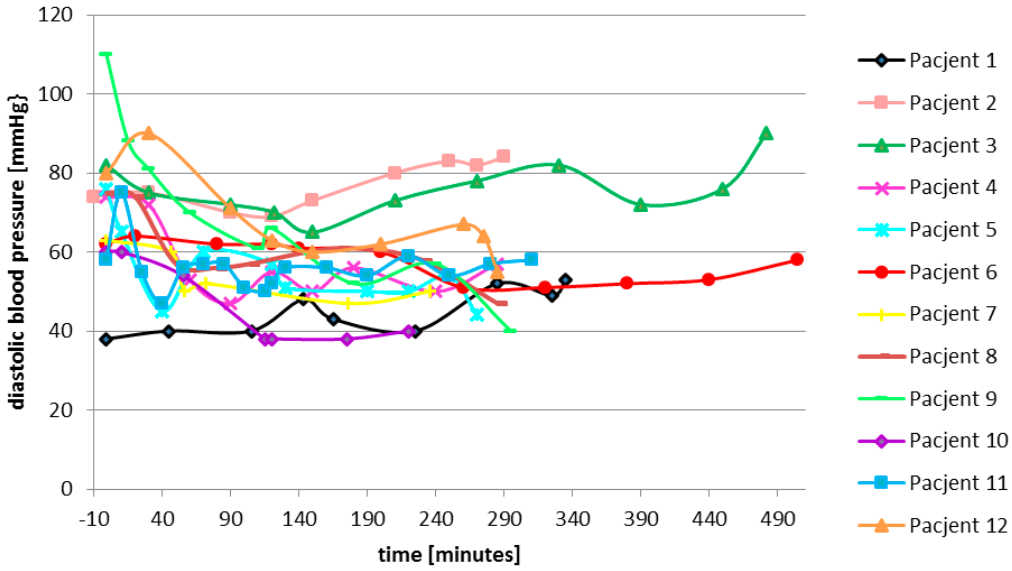

Figure 5. Diastolic blood pressure values for each patient before and during dex infusion

Discussion

Our results showed that dexmedetomidine may have stabilizing effect on blood pressure and heart rate in postoperative period in ICU. Dexmedetomidine has a binary effect: on one side it decreases blood pressure response to surgical stress and on the other hand minimizes surge in blood pressure and heart rate during operation and postoperative ICU. The use of α2-agonists aims at blunting the hemodynamic stress response. Dexmedetomidine is a good sedative agent in cardiac patients as it is a sympatholytic and reduces heart rate [17].

In the study group, the recommended dosage of dexmedetomidine was sufficient to maintain postoperative sedation. During the entire period of dexmedetomidine infusion, deep sedation was maintained. Difference between the baseline value of BIS and the average value of dex infusion was small – 1%. It was due to short time from end of sevoflurane administration and beginning of infusion. Baseline value for each patients was in the range 52–83 denoting deep sedation. Dex administration caused maintaining the appropriate level of sedation for ICU. It is very important that it is possible to keep deep sedation (BIS in the range

60–80) using only dexmedetomidine. In the present study in three cases baseline BIS value was below 60. Two of them received additionally 5 mg midazolam 45 and 55 minutes before beginning of dexmedetomidine infusion and one patient had started infusion only 15 minutes after end of sevoflurane administration.

Dexmedetomidine has been shown to affect the patients' hemodynamic parameters. On the Figures 3–5 a decrease is visible of blood pressure and heart rate values after start of infusion. However, we have not reported any bradycardia episodes in patients. Hui et al [18] reported clinically significant bradycardia during simultaneous administration of dexmedetomidine and fentanyl. We didn't find this relationship using dex in combination with oxycodone or morphine, heart rate did not differ between patients who were administered opioids and patients that were administered another analgesics. Nevertheless, we reported in 8 patients episodes of hypotension and in 2 cases the hypotension was followed by too deep sedation (BIS below 60). In all these three cases dex infusion was stopped and patients were recovered from sedation. No other sedative agent was given instead because there weren't any further indications to continue sedation in these patients.

In all patients the infusions were during the day, and the parameters after the infusion were measured in the afternoon, in the evening and at night. In these periods the pressure is normally more than 10 mmHg at night lower than in the day [19]. In our opinion, this might had an effect on the median of blood pressure and heart rate after stopping the infusion however to make a conclusion on this field circadian rhythmicity of the physiological parameters should be included in the protocol of further studies.

During the infusion, a significant decrease in CI (25,6%) was observed when compared the baseline value to the average value of the infusion period. In two patients, CI values were above the reference range before the infusion of dexmedetomidine, and then during the infusion period they fell to the references values whereas at the end of the infusion increased again to the above the references values. Further studies are needed to assess the relation between dexmedetomidine and cardiac output in which the influence of this hemodynamic parameter on the drug elimination clearance should be also taken into account. Lower cardiac output may potentially decrease the elimination rate of the drug and as a result increase its pharmacological effect [20].

In conclusion, in our patients' population including elderly cardiac patients, dexmedetomidine has been shown as a drug which given as the only sedative agent enabled to achieve desire level of sedation in the recommended ranges (60–80 of BIS) without any episodes of bradycardia. However hemodynamic parameters should be closely monitored during the infusion, because hypotension events were reported.

Acknowledgements

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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Clostridium difficile – still a problem among the XXI century of geriatic patients

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ABSTRACT

Introduction. The disease caused by *Clostridium difficile* (CzcD – *Clostridium difficile* -associated disease) – was defined by the Centres for Disease Control and Prevention in Atlanta in 2007 in order to standardize monitoring conditions of diarrhea caused by the bacterium *Clostridium difficile* [1]. It is a gram-positive bacterium forming part of intestinal flora that causes, among other, pseudomembranous colitis in elderly patients. It occures due to the destruction of anaerobic flora through the application of antibiotics and mass colonization of the bacterium *Clostridium difficile* in the large intestine. The diarrhea may resolve spontaneously but in older people often causes a severe form of life-threatening condition. [2–3]. The determinants which are the criteria for diagnosis of *Clostridium difficile* is a toxin A and/or B in the stool or demonstration of the presence of *Clostridium difficile* strain.

Aim. The aim of the study was evaluation of the bacterium *Clostridium difficile* infection in geriatric patients among hospitalized in Department of Geriatrics at Regional Hospital for Mental Diseases "Dziekanka" in Gniezno in the years 2015–2016 and comparison with the information of infections in the years 2012–2014 in the same department and the same hospital.

Material and Methods. The studied material consisted of data from the medical records based on 1342 patients from Regional Hospital for Nervous and Mental Patients "Dziekanka" in Gniezno. The following parameters were analysed: gender, age of the patient, duration of hospitalization, antibiotics before diarrhea, basic diseases and coexisting diseases.

Results. The study included in total 1342 patients. *Clostridium difficile* was diagnosed in 4 people which was 0.3% of all diagnosed patients. Among the coexisting diseases was diagnosed heart failure (50%), anemia (75%) and renal failure (50%). First-line treatment was vancomycin and metronidazole.

Conclusions. Prevention against infection with *Clostridium difficile* must be taken through early detection and implementation of medical procedures, medicines and sanitary-epidemiological procedures.

Keywords: infection, *Clostridium difficile*, elderly person.

Introduction

During the aging process a lot of irreversible changes in the systems and organs occur. This period is related to the presence of specific health problems, which result in a limitation of independence and self-reliance elderly patients. Moreover it is decreases the quality of life [4–5]. A major problem is a disruption the immune system lead to the reduction of vaccination response

and increased susceptibility of infection. Additionally the susceptibility of various types infection is also increased because of many chronic and devastating diseases which are presence. Clostridium difficile is dangerous pathogenic microorganism for elderly patients. It is caused by antibiotic opportunistic bacterium that causes gastrointestinal illness in humans. It produces toxins (A and B) and disputes resistant to high temperatures and any cleaner substance [6–7]. *Clostridium difficile* causes diarrhea that can be life-threatening to elderly patients which leads to hospitalization. Almost always the causes is prior antibiotic use (over 95%) especially fluoroquinolones, clindamycin and cephalosporins. Factors additional cause infection can be: bad hygiene staff (especially hands), age over 65 years, contact with infected persons, incorrect and inaccurate washing, cleaning. Because of bacteria items are (sinks, showers, bathtubs, toilet bowls, beds, tables and other equipment used for the care and rehabilitation of patients), lack of cleaning agents and appropriate disinfectants in this type of infection. The infection caused by the bacterium *Clostridium difficile* are; 1. not heavy, 2. heavy, 3. severe, fulminant, complicated [8]. In the case of diagnosis a particular form is taking a standard treatment procedure (**Figure 1**).

The diagnosis of *Clostridium difficile* included medical history, antibiotic testing of stool for the presence of toxins and bacteria, additional lab results and imaging studies [8]. The results confirm the presence of *Clostridium difficile* and they are the basis for implementation of medical procedures and also they are prevent spread of infection.

Aim

The aim of the study was evaluation of the bacterium *Clostridium difficile* infection in geriatric patients among hospitalized Department of Geriatrics at the Regional Hospital for Mental Diseases "Dziekanka" in Gniezno in the years 2015–2016 and compare the information of infections in the years 2012–2014 in the same department and the same hospital.

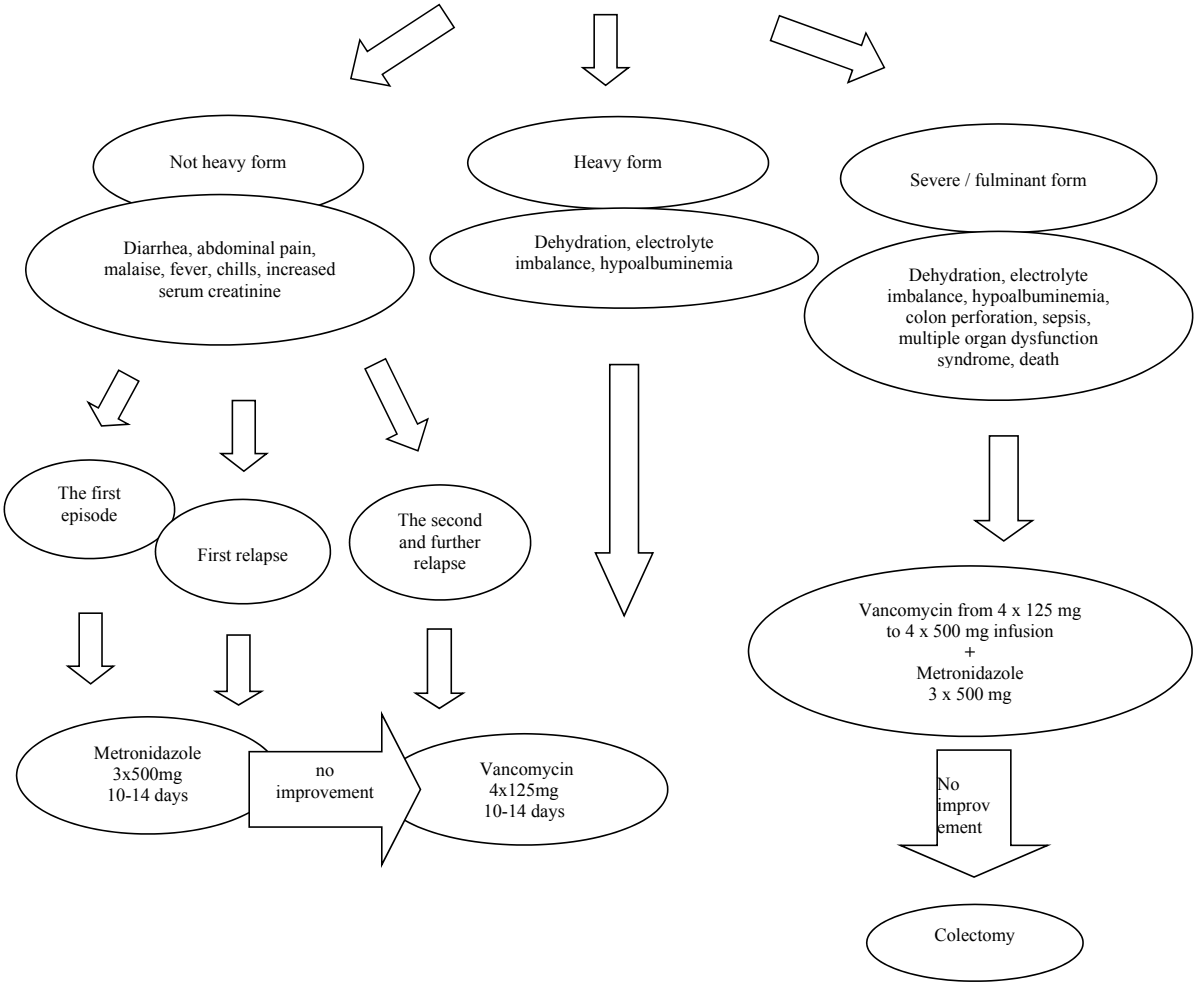

Figure 1. Forms of Clostridium difficile, clinical and standard treatment [8–9]

Material and Methods

Studied material consisted of data from the medical history of 1342 patients between 81 and 84 years old from Regional Hospital for Nervous and Mental Patients "Dziekanka" in Gniezno treated in the period from 01.01.2015 to 31.05.2016.

Retrospectively analyzed medical histories of patients with laboratory-confirmed infection of *Clostridium difficile*.

Similarly to that in the years 2012–2014 were taken into consideration the following parameters: gender, age of the patient, duration of hospitalization, antibiotics before the diarrhea, primary disease and coexisting disease.

The research results are based on Student's t-test for unrelated samples and the coefficient of Spearman's rank correlation. The level of statistical significance adopted level of $p < 0.05$.

Results

In the years 2015–2016 in the geriatric department of the Regional Hospital for Nervous and Mental Patients "Dziekanka" in Gniezno hospitalized in total 1342 patients. In the laboratory-confirmed infection of *Clostridium difficile* were 4 patients (2 females and 2 males, respectively 50% and 50%) between 81 and 84 years old (average age 84.5 years), representing 0.3% of the treated patients. In the years 2012–2014 *Clostridium difficile* was found in 16 patients (11 females and5 males, respectively 68.75% and 31.25%) between 71 and 96 years old (average age 83.3 years) acting 0.92% of all patients.

Analysis of aged patients with *Clostridium difficile* are presented in **Table 1**.

The average time of hospitalization in the department was 12.5 days (from 1 to 31 days). The data are presented in **Table 2**.

Nosocomial infection was diagnosed in 2 men (50%). The average age for this group of patients was 86.5 years. While community-acquired infections were observed in 2 women (50% average age 82.5 years). Indication for antibiotic treatment against diarrhea was bronchitis (1 male, 25%). In other cases they was no information about previous diseases.

Table 3 shows used antibiotics for patients with *Clostridium difficile* before diarrhea.

Clostridium difficile is the most frequently diagnosed with coexistence of heart failure (50%), anemia (75%), renal failure (50%). Other figures and percentages presented in **Table 4**. Moreover if patient has more coexisting diseases, the duration of hospitalization become to be longer ($p = 0.012719$). The data are shown in **Figure 2**.

Treatment of *Clostridium difficile* are presented in **Table 5**.

Among the analyzed group was found 1 death (50% in the group of females) – 84 years old woman with dementia, renal failure, malnutrition and dehydration. The differences were statistical significant ($p = 0.00452$).

For decrease of the *Clostridium difficile* incidence in the analyzed material is probably the impact of the

Table 1. The age distribution of patients with *Clostridium difficile* in the analyzed groups in the years 2012–2014 and 2015–2016

Age distribution	1.X.2012–31.XII.2014				1.I.2015–31.V.2016				p
	Females		Males		Females		Males		
	N	%	N	%	N	%	N	%	
65–75 years	1	9,09	2	40	0	0,00	0	0,00	0.468485
76–85 years	5	45,46	2	40	2	100,00	1	50,00	
86–95 years	4	36,36	1	20	0	0,00	1	50,00	
Over 95 years	1	9,09	0	0,00	0	0,00	0	0,00	
Total number	11	100,00	5	100,00	2	100,00	2	100,00	

Table 2. The average time of hospitalization patients with Clostridium difficile in 2012–2014 and 2015–2016 years

Hospitalization time	1.X.2012–31.XII.2014		1.I.2015–31.V.2016		p
	N	%	N	%	
1–10 days	3	18,75	2	50,00	0.012719
11–21 days	8	50,00	1	25,00	
22–35 days	4	25,00	1	25,00	
Over 36 days	1	6,25	0	0,00	

Table 3. Antibiotics used before diarrhea

Antibiotics used	1.X.2012–31.XII.2014		1.I.2015–31.V.2016	
	N	%	N	%
Amoxycillin with clavulanic acid	4	25,00	0	0,00
Amoxycillin with clavulanic acid + Clarithromycin	1	6,25	0	0,00
Amoxycillin with clavulanic acid + Ceftriaxone	1	6,25	0	0,00
Amoxycillin with clavulanic acid + Ciprofloxacin	1	6,25	0	0,00
Cefuroxime	4	25,00	1	25,00
Cefuroxime + Ciprofloxacin + Gentamicin	1	6,25	0	0,00
Ciprofloxacin	1	6,25	1	25,00
Unknown	3	18,75	2	50,00

Table 4. Coexisting diseases in hospitalized patients

Coexisting diseases	1.X.2012–31.XII.2014		1.I.2015–31.V.2016	
	N	%	N	%
Diabetes	7	43,75	0	0,00
Heart failure	9	56,25	2	50,00
COPD	4	25,00	0	0,00
Malnutrition, dehydration	9	56,25	1	25,00
Delirium	7	43,75	0	0,00
Stupor	7	43,75	1	25,00
Cancers	1	6,25	1	25,00
Strokes	5	31,25	0	0,00
Renal failure	6	37,5	2	50,00
Anemia	9	56,25	3	75,00

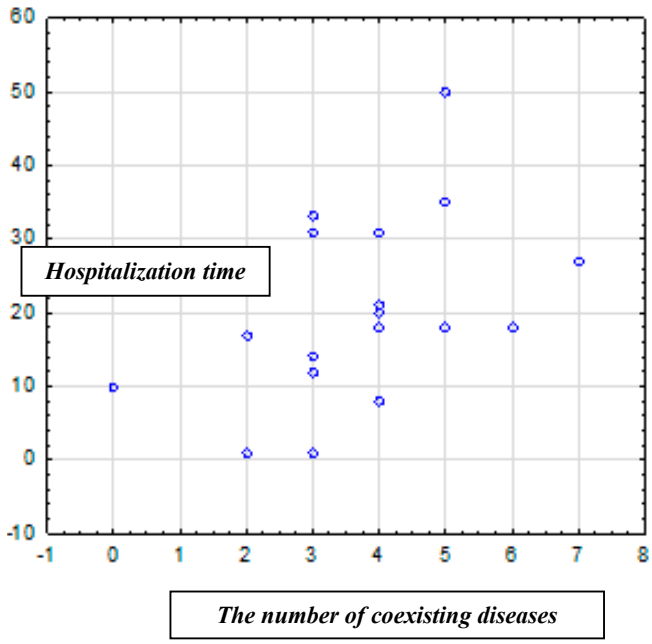

Figure 2. Dependence between the number of coexisting diseases and hospitalization time

effective control of infections. Aamong others; inclusion of sporicidal agents, disposable equipment, hand washing, isolation of the infected patient and microbiological diagnostics.

Screening test for *Clostridium difficile* infections is a test to detect the antigen GDH, which is characterized by high sensitivity. Furthermore it is recommended that in the case of a positive result GDH test. Should be use other available methods to confirm result – PCR (NAAT – amplification of nucleic acids), positive test A // B EIA test and Gene – Xpert *Clostridium difficile* PCR.

Table 5. Treatment of Clostridium difficile

Used antibiotic	1.X.2012–31.XII.2014		1.I.2015–31.V.2016	
	N	%	N	%
Vancomycin	10	62,5	2	50,00
Metronidazole	0	0,00	1	25,00
Vancomycin+ Metronidazole	5	31,25	0	00,00
No treatment	1	6,25	1	25,00

Table 6. Mortality in patients hospitalized in the 2012–2014 and 2015–2016 years

Deaths	1.X.2012–31.XII.2014				1.I.2015–31.V.2016			
	Females		Males		Females		Males	
	N	%	N	%	N	%	N	%
	5	45,45	3	60,00	1	25,00	0	0,00
Total number	5	100	3	100	1	100	0	100

The use of a reasonable antibiotic therapy – empiric therapy – should be used only until a positive result of microbiological examination.

Then applied antibiotic should be strict according to antibiogram. Antibiotics are not given without observed clinical symptoms of infection and colonization occurs.

The administration of probiotics is one way of preventing diarrhea. Probiotics reduce the risk of diarrhea associated with antibiotic therapy. The oral administration of Lactic acid bacteria, Saccharomyces boulardii in an amount of 250 mg twice a day for 4 to 6 weeks. Furthermore probiotics Lactobacillus rhamnosus, Saccharomyces boulardii takes in conjunction with vancomycin to reduce the incidence of recurrence of *Clostridium difficile* from 50 to 16%.

Discussion

Clostridium difficile at the beginning of the twenty-first century remains a serious medico – social problem. Some of the most common risk factors of *Clostridium difficile* include hospitalization constituting 20–30% compared to 3% of the general population [10]. The incidence of *Clostridium difficile* among the patients hospitalized depends on the frequency of used antibiotics and it is 1–10/1000 patients [11–12]. This is confirmed by our research. During the years 2012–2014 *Clostridium difficile* constituted 6.97 / 1000 hospitalization, while currently 2.98 / 1000 hospitalization.

Decrease of immunity, age and gender are the risk factors for nosocomial of *Clostridium difficile*. Furthermore medical procedures, ie. mechanical ventilation, antibiotics, parenteral nutrition, steroid treatment, diabetes take a significant role in the development of *Clostridium difficile* [13]. In the case of recurrence risk factors are; age over 65 years, continue antibiotic treatment and severe disease. Studies of Brown et al. [14] showed increased risk for *Clostridium difficile* in patients treated with cephalosporins, carbapenems, monobactams and clindamycin and the reduction after use of macrolides, penicillins and sulphonamide.

Currently there are unknown accurate statistics associated with mortality of *Clostridium difficile* in Poland. In the United States the overall mortality rate is estimated at 23.7 / 1,000,000 [15–16] while the mortality rate among patients treated in the Intensive Care Unit is estimated at 6.1%. In our studies in the years 2012–2014 mortality was high – 50%, while in the years 2015 to 2016 – 25%.

Currently all over the world as well as in Poland it's tends to seek the best solutions in the case of a patient diagnosed with the pathogen- *Clostridium difficile*:

- inform the State Sanitary Inspectorate – in the first 24 hours,
- patient isolation,
- observance the rules of contact isolation,
- daily disinfection of surface with Chlorine-Clean liquid,
- use disposable bed sheets,
- worn bedding disposable to the medical waste, and then burn it,
- for washing hands, only the chlorhexidine – (Hydrex); disinfection alcohol only after washing your hands above,
- in the case of discharge or relocation the patient: thorough disinfection of surfaces and equipment with Chlorine-Clean 10,000 ppm for 15 minutes and then ventilate the room,
- newspapers, books should be packed inside a red bag and pass to burn,

– personal clothes should be utilization or disinfected after consultation with the patient or patient family.

Effective method of limiting the spread of *Clostridium difficile* infection is the isolation of a patient suspected of being infected and isolation of a patient diagnosed with infection by providing:

– separate room with a sink with a battery that runs without any contact with the hands,
– dispenser with disinfectant that run without contact with the hands,
– container with disposable towels and container for used towels,
– isolation room equipped with negative pressure ventilation,
– lock room, sink – aprons equipped with: a sink with a battery that runs without any contact with the hands; dispenser of liquid soap; dispenser with disinfectant that run without contact with the hands; container with disposable towels and a container for used towels; closed container for dirty clothes; space for clothes by separating clean and dirty clothes [17].

Furthermore;

– before entering the room use disposable aprons,
– washing your hands after contact with a patient,
– isolation of the patient for period of 48 hours after the resolution of diarrhea and stool formation [18],
– use sterile disposable gloves for treatment under aseptic conditions, sterile handling equipment,
– use non-sterile disposable gloves for all procedures that may lead to contact with blood, body fluids, excretions, secretions; contact with mucous membranes or broken skin,
– use masks during procedures with the existing risk of aerosols formation, splashes of blood or body fluids; preventing the spread of microorganisms from the nose and mouth during coughing and sneezing.

Rules of preventive procedure in patients with geriatric age:

– use antibiotics only in justified cases,
– use antibiotics after microbiological diagnostics,
– prevention of infections,
– compliance with sanitary regime.

Conclusions

– Take preventive actions of infection with *Clostridium difficile* through early detection and implementation of medical procedures, medicines and sanitary-epidemiological procedures.
– Use and observance the principles of prevention reduced the incidence of *Clostridium difficile* infections in the geriatric ward.

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Personality traits and sex-role schema in adult patients with childhood-onset combined pituitary hormone deficiency not treated with growth hormone

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ABSTRACT

Background. Patients with combined pituitary hormone deficiency have quantitative and qualitative abnormalities of pituitary hormone production that may trigger psychological consequences. Several studies have evidenced symptoms of social disturbances in these patients.

Aim. The aim of this study was to evaluate personality traits and psychological sex-role schema influencing social adaptation in patients with childhood-onset combined pituitary hormone deficiency.

Material and Methods. Study involved a unique group of 28 adult patients with childhood-onset combined pituitary hormone deficiencies that were never treated with growth hormone. To psychological assessment the short Polish version of Bem's Sex Role Inventory and the Polish version of Minnesota Multiphasic Personality Inventory were used in the study.

Results. The analysis of scores on the Polish version of Minnesota Multiphasic Personality Inventory showed significantly elevated results in the scales for lying, hysteria, psychopathic deviation, hypochondria, and schizophrenia as well as decreased scores in hypomania indicating a number of symptoms of maladjustment in many different areas of life. The short Polish version of Bem's Sex Role Inventory scores indicated that most of combined pituitary hormone deficiency patients were sex-undifferentiated and no one was androgynous.

Conclusions. The sex-role schema and certain personality traits seem to predispose childhood-onset combined pituitary hormone deficiency patients not treated with growth hormone to problems with social adaptation and greater susceptibility to situational stressors. Neurotic reactions, tendency for social alienation, and lack of flexibility have all been observed in these patients. Therefore, combined pituitary hormone deficiency patients may more often need special support when it comes to coping with disease.

Keywords: personality; sex role schema; gender identity; hypopituitarism; growth hormone.

Introduction

The term Combined Pituitary Hormone Deficiency (CPHD) is used to describe the condition where the pituitary gland ceases to produce and release two or more hormones, one of which includes growth hormone (GH). Symptoms of GH deficiency (GHD) in adults commonly include fatigability, poor exercise performance, and symptoms of social isolation and cognitive impairment [1, 2]. Deficiency of pituitary hormones cannot only affect the development of the body, but also has been proven to result in psychological consequences [3]. Hormones play a crucial aspect in almost all areas of development so it is no surprise it can present effects on a psychological scale. It has been found

that many patients exhibit a lack of concentration along with impairments of memory performance when pituitary hormones are deficient [4, 5]. However, little is known about the quality of social existence, dimensions of self-esteem, and psychical health in patients with CPHD. Most past studies dealing with this topic were performed on a group of short stature children [6–8]. Relationships with older patients who suffer from CPHD and the psychological and social aspects that are implicated are not currently known.

Furthermore, along with physical and psychological well-being, an important attribute of human existence is personality [9]. Personality can impact individuals in how they interact with the world around them. The influence of personality along with psychological sex roles can vary greatly in their effect on behavior and cognition in individuals [10]. It was suggested that psychological gender affects social competence and psychical health with an emphasis on stress resistance. Sandra Bem claimed that there are four sex types that differentiate people and further condition their behavior, intelligence, and emotional reactions, which can be measured with the Bem Sex-Role Inventory (BSRI). Bem called these types: masculine, feminine, androgynous, and undifferentiated. Of course, the masculine and feminine categories can be cross-typed leading to a different psychological sex than an individual's biological sex. Sex type can have a tremendous impact on how individuals view themselves along with their interactions with others and the environment surrounding them. A common understanding of masculinity or femininity within oneself results in visible proprieties and behaviors associated with sexuality. These associations contribute to cultural attitudes and beliefs [11, 12].

All of these factors play a role in the social well-being of individuals. The aim of this study was to further investigate factors influencing social adaptation such as personality traits and sex role schema as determinants of style of reaction, perception, and stress management in childhood-onset CPHD patients not treated with growth hormone.

Material and Methods

Subjects

The study was carried out on 28 adult patients (16 males and 12 females) and referred to the Department of Endocrinology due to childhood-onset combined pituitary hormone deficiency.

The mean age when the first hormonal deficiency diagnosis in CPHD patients has been made was 8.7 ± 7.0 (range 2–26 years old). In all patients, GH, thyrotropin (TSH), and gonadotropin (LH/FSH) deficiencies were diagnosed and hypoplasia of the anterior pituitary lobe was found on MRI study. Seventeen patients (60.7%) were receiving hydrocortisone because of the early or late onset of adrenocorticotropin (ACTH) deficiency and 12 patients (42.9%) also exhibited prolactin (PRL) deficiency. All patients were receiving hormonal replacement therapy including levothyroxine and sex hormones but no one was treated with recombinant human GH before this study. This criterion emphasized the importance and unique character of the studied group but simultaneously limited the number of examined subjects. The control group consisted of 28 healthy persons matched regarding age, sex, and level of education. Demographic data of CPHD patients and controls were shown in the **Table 1**. The psychiatric diseases in both groups were excluded in preliminary psychological consultation.

Table 1 Characteristics of patients with CPHD.

Variable	CPHD patients (n = 28)
Sex – n (%): – males – females	 16 (57.1) 12 (42.9)
Age (years) – mean ± SD (range) at the time of psychological study	41.7 ± 11.1 (18–59)
Age, when the testosterone or estradiol/progesterone therapy was initiated – mean ± SD (range)	19.8 ± 4.6 (9–30)
Age, when the thyroid hormone therapy was initiated – mean ± SD (range)	15.6 ± 6.8 (6–29)
Education – n (%): – elementary level – high school – university level	 15 (53.6) 10 (35.7) 3 (10.7)

Methods

Personality traits

Minnesota Multiphasic Personality Inventory (MMPI) developed by Hathaway and McKinley, adapted to the Polish version, was used to assess personality [13, 14]. This questionnaire consists of 10 clinical scales, which are used to identify different psychological conditions. Scales include: hypochondriasis (Hd), depression (D), conversion hysteria (Hy), psychopathic deviation (Pp), masculinity and femininity (Mf), paranoia (Pa), psychasthenia (Pt), schizophrenia (Sc), hypomania (Ma)

and social introversion (Si). There are also 3 validating scales including: lie (L), infrequency (F), and defensiveness (K). The psychometric investigation was conducted both in studied and control groups.

Sex Role Inventory

The Polish version of Bem's Sex Role Inventory (IPP) adapted by A. Kuczynska [15, 16] was used to assess psychological sex role. The short form has 35 items, which represents half of the items that compose the original form. The 35 items include 15 masculine adjectives, 15 feminine adjectives, and 5 neutral adjectives (**Table 2**). This scale assesses masculinity, femininity, androgyny, and undifferentiated roles. One can be defined as sex-typed (men score high on the masculinity scale and low on the femininity or vice-versa for women) or cross-sex typed (men score low on the masculinity scales and high on the femininity scales or vice-versa for women). Those individuals classified as masculine have characteristics like the "typical male" while those who identify as feminine have characteristics like the "typical female" according to traditional views of society. Those named undifferentiated represent individuals for whom the dimensions of masculinity and femininity are not essential and do not identify fully with either. People who are defined as androgynous have high levels of both masculine and feminine characteristics [11]. The IPP is a standardized and normalized psychometric tool that allows conducting the study without the requirement for a comparison of the control group.

Statistical analysis

The grouped data were expressed as the mean ± standard deviation (SD). The D'Agostino & Pearson test was used to check the normality of the data distribution. All data was compared using the Student t-test or Mann-Whitney's test.

Table 2. Short sex-role schema inventory used in the study [15, 16]

Feminine	Masculine	Neutral
– sensitive – affectionate – eager to soothe hurts feeling – yielding – having a sense of aesthetics – grumpy – able to make sacrifices – tactful – image-conscious – sensitive to other's needs – tender – warm – gentle – coquettish – gullible	– dominant – athletic – cheerful – acts as a leader – self-confident – self-sufficient – independent – competitive – set for success – willing to take a stand – forceful – clever – makes decisions easily – open-minded – experimenting in sexual life	– reliable – likable – truthful – tolerant – sympathetic

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments on comparable ethical standards.

Results

The analysis of the MMPI results (clinical scales results are showed in **Table 3**) in CPHD patients in comparison with healthy controls showed significantly different personal characteristics. CPHD patients exhibited evidence of numerous somatic symptoms, tendency to complain, a sense of dissatisfaction, and deductions from life (hypochondriasis; $P = 0.003$); egocentrism, excessive expectations, and care and support of the environment (conversion hysteria; $P < 0.0001$); social alienation with irresponsibility, failure to conform to social norms, deceit, and impulsivity (psychopathic

Table 3 The results of clinical scales of MMPI in CPHD patients and controls.

Groups	Hypochondriasis (Hd)	Depression (D)	Conversion hysteria (Hy)	Psychopatic deviation (Pp)	Masculinity/Feminity (Mf)
CPHD patients	72.0 ± 8.1	78.0 ± 13.0	56.0 ± 5.9	69.0 ± 14.0	50.0 ± 4.8
Controls	64.0 ± 9.0	75.0 ± 6.2	48.0 ± 5.7	63.0 ± 6.0	58.0 ± 5.5
P value	0.003	0.123	< 0.0001	0.047	< 0.0001
	Paranoia (Pa)	Psychastenia (Pt)	Schizophrenia (Sc)	Hypomania (Ma)	Social introversion (Si)
CPHD patients	62.0 ± 9.0	72.0 ± 13.0	78.0 ± 18.0	58.0 ± 15.0	62.0 ± 10.0
Controls	59.0 ± 11.0	68.0 ± 12.0	66.0 ± 10.0	64.0 ± 11.0	63.0 ± 6.8
P value	0.323	0.278	0.003	0.006	0.662

deviation; P = 0.047). They also presented with feelings of being pushed to the margins of social life, avoiding contact with people, reserve, shyness, poor contact with reality, avoiding new situations, and problems with their own "self" (schizophrenia; P = 0.003); decreased level of excitability (hypomania; P = 0.006), and the need to show themselves in a better light and hide their psychological problems in comparison with healthy controls (L – lie; P < 0.0001) (**Table 4**). In the masculinity/femininity scale, CPHD patients also showed lower scores in comparison with controls (P < 0.0001).

The principle finding of the IPP study (**Table 5**) was that not one of the examined patients with combined pituitary hormone deficiency was psychologically androgynous (the combination of "male" and "female" in the mental processes of individual creativity) with both stereotypical masculine and feminine traits. More than half of all investigated individuals (61%) were undifferentiated (those with low scores on both masculine and feminine scales), almost 18% among 28 subjects with CPHD were identified as "sex-typed" and 21% as "cross-sex". It also appeared that these scores were very similar in both males and females.

uals have poor self-esteem and tend to avoid aggressiveness [3, 17–20]. It is already known that lack of any hormone can potentially result in negative outcomes for the individual; psychological problems in the emotional, motivational, and cognitive processes have all been noted [3]. It has also been found that patients with hormonal deficits tend to retire early [21]. This could perhaps be due to the inability for these patients to cope with the stress of the workforce as they age. Further studies are needed to examine how GH plays a role in the elderly population (> 65 years old).

The individual reaction from hormonal deficiency is additionally connected with personal predispositions, including psychological traits [18, 22]. These personal predispositions play a big role in determining how certain factors can impact social functioning, especially factors that cannot be controlled. For example, people with chronic illness are at a greater risk for psychiatric disturbances and social adjustment problems than those without disease [23]. Chronic conditions are associated with increased psychological distress, functional limitations, and may affect specific personality development [24].

Table 4. The results of validation scales in CPHD patients and controls

Groups	Lying (L)	Infrequency (F)	Defensiveness (K)
CPHD patients	57.0 ± 6.1	61.0 ± 12.0	59.0 ± 8.3
Controls	50.0 ± 5.5	66.0 ± 6.6	55.0 ± 6.6
P value	< 0.0001	0.182	< 0.069

Table 5. Sex-role inventory results in patients with CPHD expressed in % (numbers)

Biological sex	Psychological sex role			
	Sex-typed	Cross-sex	Undifferentiated	Androgynous
Male	13% (2)	31% (5)	56% (9)	0
Female	25% (3)	8% (1)	67% (8)	0
All	18% (5)	21% (6)	61% (17)	0

Discussion

Growth hormone is one of the most important pituitary hormones necessary for proper growth and development of children showing unfavorable results when deficient. Sufficient physiological GH level and function are also necessary to maintain proper mental functioning and cardiovascular status. Adult patients with hypopituitarism and growth hormone-deficiency have shown many social disturbances such as: isolation, problems in rates of employment and marriage, decreased psychological well-being in terms of energy, and sex life disturbances compared with healthy people. It has been also shown that many of these individ-

Furthermore, both the sex role schema and some of the examined personality traits seem to predispose childhood-onset CPHD patients to problems with social adaptation and susceptibility to situational stressors. In our study, we were interested to see the relationship between these two factors and the outcome it has on patients. Following the MMPI scale interpretation, patients presented with a range of social and psychological implications. Our results showed some of the disturbances that patients presented with include: neurotic concern over bodily functioning (Hd), poor awareness of problems and vulnerability and tendency for hysterical reactions in stressful situa-

tions (Hy), social alienation, bizarre thought processes, peculiar perceptions, specific familial relationships, difficulties in concentration and impulse control, lack of deep interests, disturbing questions of self-worth and self-identity (Sc), irresponsibility, impulsivity, failure to conform to social norms and deceit (Pd), low level of excitability (Ma), tendency to stereotypic perception or schematic behavior, and a lack of flexibility involving problems with independent decision making, stress management, and social adjustment (IPP). These negative outcomes range to cover many different aspects of family life. It was suggested that the poor coping mechanisms and social adaptations are due to bigger underlying issues. Hathaway and McKinley stated that the tendency for patients to present themselves in a favourable light, reject shortcomings, and contain unfavourable characteristics (L) should be seen as a defence mechanism and a factor hindering the identification of their deeper psychological problems [25]. Perhaps these patients need more support and guidance when it comes to dealing with these defense mechanisms to reach a better understanding of themselves. Further study in therapeutic methods of these patients is needed.

When focusing on gender-schema as an internal cognitive network within each person, which is shaped by culture and society, it is inevitable that certain individual perceptions will be influenced by social interactions. Society has a great impact on what people consider to be stereotypical gender roles. These important factors should not be underestimated when it comes to the personal dimensions influencing social competence, proficiency, and psychical reactions [12]. Gender may even affect the stress process by determining whether a person perceives a situation as stressful and therefore influences different coping mechanisms and the final health implication of this stress. This could potentially lead to long-term health issues; however, further study in this area is needed. This could be why it is found that androgynous people, who have both masculine and feminine traits, have beneficial outcomes for behavior and stress management [11]. According to Bem, androgynous people could be more adaptable to the demands of modern life because of an expanded behavioral repertoire and superior sex-role adaptability in comparison to sex-typed individuals. This supports S. Bem's theory that androgyny is manifested as situational flexibility [26]. Examining the specifics of how men and women differ in stress management and the reasons for these differences could shed more light onto this topic. There were no androgynous patients in studied group found that could suggest poor adaptation skills, lack of stress resistance, and low flexibility of examined CPHD patients.

Our study used the short version of BSRI (which was also applied to a past study in patients with pituitary disease [27]) and showed the increased prevalence of undifferentiated sex-roles among examined patients with CPHD. This means that their personal and behavioral self-concept was not based on socially interpreted sex-role schemas. These undifferentiated patients can have more psychological problems, especially with adaptation and they often suffer from more distress. Therefore, undifferentiated people seemed to be more vulnerable when it comes to a lack of achievement and sociability. Adaptation difficulty may be one of the sources of low achievements and failures of social life among CPHD patients [28]. The short stature and biological immaturity of CPHD patients may influence the attitudes and social behaviors similar to those addressed in children. Children are not always recognized as having the characteristics consistent with the gender schema society has established. This is most likely due to the fact that children are still developing and gender identity may not set in fully until adulthood. Children generally are treated as devoid of having qualities of sexuality. In the case of childish looking people, it has been found that lower requirements are used and they are often treated condescendingly [29]. Thus, a certain personality trait in the psychological gender may result from the symptoms of the hormonal deficiency as well as the social reactions to these symptoms that affect the individual.

There is little information about previous similar studies done in such a group of patients with hormone deficiencies, but Sartorio et al. described the results of BSRI testing in eight patients with childhood-onset growth hormone deficiency before and after 6 months of recombinant GH therapy. They concluded that there were no significant changes in BSRI score after hGH treatment [30]. In another study Rekers-Mombarg et al. used MMPI to evaluate personality traits in young adults with idiopathic short stature (hormonal deficits not confirmed) and found that there was no difference between study and control group [19]. This may suggest that early hormonal deficits in childhood and the consequences that follow might affect the personality attributes in CPHD patients compared to later established hormonal deficits. It is evident that deficiencies have a greater effect during the developmental period of a child than in adulthood. Progress in medicine facilitating diagnosis and therapy earlier on in a patient

greatly benefit the quality of life one side, however may limit access to vital material on the other (hormonal therapy routinely administered in cases of hormonal deficiency reduced the sample size without substitution). Future psychological explorations are necessary for further explanation and clarification on social problems of these patients. Studies also focusing on different treatment methods with these patients could shed a great deal of insight into understanding the complex factors pertaining to the disease and different ways patients can potentially benefit.

Conclusions

The sex-role schema and certain personality traits seem to predispose childhood-onset CPHD patients not treated with growth hormone to problems with social adaptation and greater susceptibility to situational stressors. Neurotic reactions, tendency for social alienation, and lack of flexibility have all been observed in these patients. The tendency for stereotypic perception or schematic behavior may involve problems with independent decision-making, stress management, and social adjustment. Therefore, CPHD patients may more often need special support when it comes to coping with disease.

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Development and evaluation of simulation based neurosurgery curriculum. Pilot study at the Poznan University of Medical Sciences

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ABSTRACT

Introduction. Neurosurgical emergencies are complex tasks. The current learning environment limits students' ability to manage acute neurosurgical emergencies due to legal and safety concerns. Simulation provides an opportunity to participate in the care of neurosurgical emergencies and develop clinical decision making skills.
Aim. We aim to determine whether neuroscience simulation curriculum improves student ability to: manage a critically ill patient, recognize neurosurgical emergencies, to assess how stress tolerance affects experience during simulations and effectiveness of students performance. The third objective is to develop a tool for student assessment.
Material and Methods. The simulation was performed on SimMan 3G Human Patient Simulator (Laerdal Medical). Scenarios included common neurosurgical emergencies. Students were assessed before and after the course by completing a Likert type questionnaire. Response data was analysed using Cronbach's reliability for Likert-type response data and Spearman's monotonic correlation.
Results. 60 students of fifth and sixth year of medical studies attended the course. 39 students of them replied to the questionnaire. The simulated clinical experience was positive and it improved their knowledge about neurosurgical emergencies. There was an improvement in their confidence. Improvement in individual and team performance was also observed.
Conclusions. Neurosurgical simulations improve students` ability to recognize neurosurgical emergencies. The level of stress related to simulation is important factor of the education process and should be reduced to improve students' development. Our questionnaire is an effective tool for assessment of students experience during clinical simulations.

Keywords: Neurosurgery, Simulation, Education, Medical students.

Introduction

Many neurosurgical emergencies are complex tasks which require considerable repertoire of knowledge and skill for effective performance. The early diagnostics are performed in Accident and Emergency Departments. Those tasks are rapid and occur in turmoil and stressful environment and can result with serious consequences. It reflects the complexity of patient care in these clinical environments as well as the challenging demand for high-quality teamwork. Important objective of simulation based medical education is to contribute to the reduction of error occurrences during medical

treatment. Preventable medical errors result in more than 400,000 deaths each year in the United States and are the third cause of death in this country, followed by cardiovascular diseases and cancer [1] Simulation based medical education in its widest sense can be defined as any educational activity that utilizes simulative aids to replicate clinical scenarios [2]. Simulation mistakes in order to enhance patient safety and improve medical care are a central goal of simulation based medical education [3]. It provides a safe, controlled environment in which problem-based learning is developed and competences are practiced in high-standards [1]. It is especially important for neurosurgical patients where urgent operations performed within few hours of onset have much better prognosis compared to mortality if surgery is delayed [4]. High-performance environments which are characteristic of neurosurgical emergencies involve complex, multicomponent decisions; rapidly evolving, ambiguous cases; information overload; severe time pressure; severe consequences for error; adverse physical conditions; sustained fatigue; and extensive team interactions. Therefore there exist demands on medical education to prepare young professionals to practice in the 21st century emergency medicine paradigm. It is also important to focus attention on what constitutes effective scenario-based training so that medical professionals can practice and receive feedback on crucial skills. For example the current standard of surgical evacuation of all haematomas within 4 hours is not being met in Europe. Most of the time it is not related to the inability to diagnose a haematoma but to the problems with patient transfer. The efficiency of management of all other neurosurgical emergencies also requires improvement in emergency departments. Delays were identified at every stage of the management of these patients and no single step was identified as the major cause. The mean time to surgical decompression was 5.0 h and 32% performed with in 4h. Patients who initially presented to a district hospital and required transfer for neurosurgery were decompressed in 5.4 h vs 3.7 hr for those admitted directly. There may be time savings from improvement of initial treatment in district hospitals [5, 6]. This is the field where more thematic programmes of simulation based medical education are needed. In the Simulation Centre at Poznan University of Medical Sciences we developed a neuroscience simulation curriculum which is used to improve the student's ability to recognize neurosurgical emergencies. This is the first paper describing neurosurgical themed simulation scenarios for medical students [7–9].

Material and Methods

The study was approved by the Poznan University of Medical Sciences' bioethics committee. All students provided written informed consent. 60 students of fifth and sixth year of medical studies attended the course. Those students were exposed to scenarios which were created at the Simulation Centre at Poznan University of Medical Sciences. The groups consisted of twelve students who were divided on three subgroups with four students each. The Simulation was performed on SimMan 3G Human Patient Simulator at the Center for Medical Simulation in Poznan. Students had to manage scenarios of patients with subarachnoid haemorrhage, acute subdural haematoma, acute epidural haematoma, polytrauma patient, gunshot head injury, status epilepticus, ventriculoperitoneal shunt infection, vasospasm secondary to subarachnoid haemorrhage, ischemic stroke and spinal cord injury. Although those cases are relatively rare in emergency departments they constitute core of neurosurgical emergencies. The scenarios were designed by our team and undergo continuous quality improvement. We used deidentified data of real clinical cases from our Emergency Department. Prior to the simulation program each group was sent a set of questions concerning their self-confidence (**Table 1**). The questionnaire was prepared using Likert-type scale [10] and send using Google Forms tool. Participants were asked to indicate their level of agreement with an item by choosing one of four categories ranging from: 1 – strongly agree, 2 – somewhat agree, 3 – do not agree to 4 – not applicable. Each group of students consisted of 12 students who were divided into three subgroups. While one group was managing the neurosurgical case, the remaining two groups were watching the scenario live in a separate room. Each scenario lasted approximately 10–11 minutes and then was followed by the debriefing. All

Table 1. Questions sent before the course

Do you think that the negative emotions associated with failure during the simulation improve memorizing a particular material?
I can deal with people who are arrogant at work
I easily get nervous and confused and lose confidence in stressful situations
I don`t mind when someone points out my mistakes

sessions were videotaped to review during the debrief and for QI and research. Following those simulations all students were send a questionnaire which is based on the Simulation Effectiveness Tool [11] Questions were subdivided in three groups: simulated clinical experience (**Table 2),** learning subscale (**Table 3**) and confidence subscale (**Table 4**) [11]. Response data was analysed using Cronbach's reliability for Likert-type response data [12]. PQStat software version 1.4.8 for statistical analysis was used. Analysis of correlation with questions asked before the course was performed using Spearman's monotonic correlation.

Results and statistical analysis

We received 41 complete responses. From 28 to 112 points could be obtained in the scale The higher score indicate higher level of disagreement with asked question. Mean of scale was 39.27, standard deviation of scale was 7.79. Scale reliability was measured by Cronbach Alpha and was as high as 0,896 (**Table 5**). Scores in the individual questions and the individual subscales: (SCE – simulated clinical experience, L – learning and C – confidence) are shown in **Figure 1**. The lowest values equal to one indicated that students agreed with asked question predominated in the respondents obtained subscale SCE indicating that students enjoyed working with the simulator and that time and size of the group were right. On a scale of learning, the median of the results also was 1, although there were also some higher values. The greatest diversity of the results presented subscale C concerning students` confidence. Spearman`s monotonic correlation analysis of questions asked before the course demonstrated that negative emotions are positively correlated with confidence scale, whereas simulation clinical experience ques-

Table 2. Questions concerning simulated clinical experience in post course questionnaire

I enjoyed working with the simulator
The group was the right size to facilitate my learning
The time allotted for this activity was adequate
I had fun while I was learning

Table 3. Questions concerning learning in post course questionnaire

The instructor's questions helped me to think critically
Completing the simulation helped me understand classroom information better
I feel better prepared to care for real patients
I developed a better understanding of the pathophysiology of the conditions in the simulation
I developed a better understanding of the medications that were in the simulation
My assessment skills improved
I am able to better predict what changes may occur with my real patients
I was challenged in my thinking and decision-making skills
I learned as much from observing my peers as I did when I was actively involved in caring for the simulated patient
Debriefing and group discussion were valuable
I would attend simulation again

Table 4. Questions concerning confidence in post course questionnaire

I felt like it was ok to make a mistake
I felt stressed when the simulator's condition worsened
I feel more confident in my decision-making skills
My communication skills have improved a lot
My ability to deal with arrogant people have improved a lot
Do you think that the stress associated with simulation improves remembering the material?
Do you think that negative emotions are associated with the failure to remember the material?
During the simulation I experienced feelings of nervousness, confusion and I lost confidence.
Classes of medical simulation helped me to control the feeling of nervousness, confusion, and improved self-confidence
I feel more confident that I will be able to recognize changes in my real patient's condition
The simulator and the environment were realistic
Did you have sense that the atmosphere of trust and transparency was created during error analysis?
Have medical simulations taught you a more constructive approach to your own mistakes?

tions were negatively correlated among those who felt stressed in difficult situations (**Table 6**). Significantly negative and only one positive correlations of the question: "I easily get nervous and confused and lose confidence in stressful situations" with post simulations questions are demonstrated in **Table 7**.

Table 5. Statistical Analysis

Mean of scale	39.272727
Standard deviation of scale	7.79933
Cronbach Alpha for scale	0.896098
Standard error of measurement	2.514018
Average correlation between pairs of items	0.266078

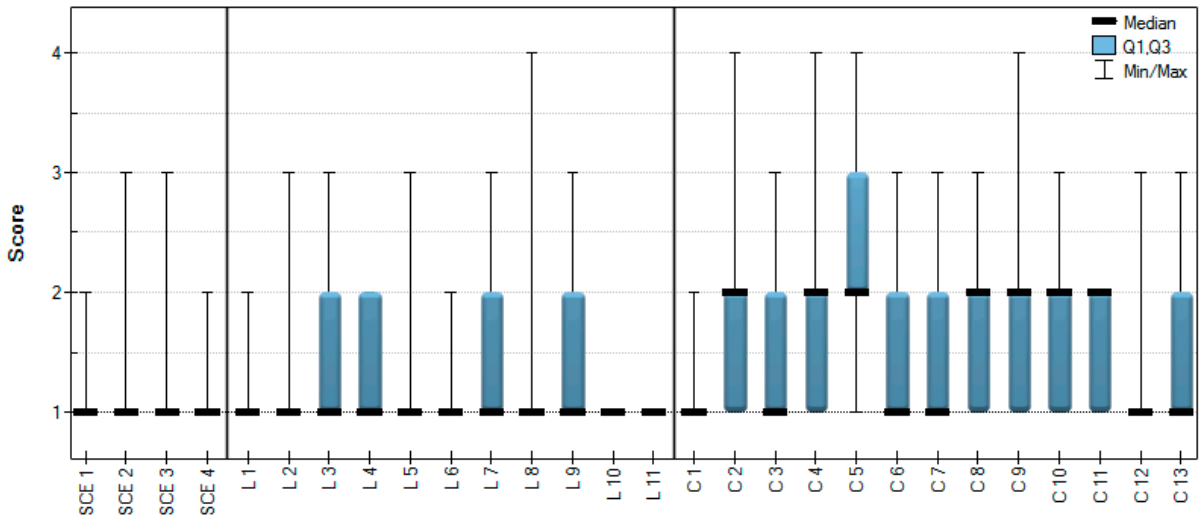

Figure 1. Responses obtained using simulation effectiveness tool. SCE 1, 2, 3, 4 – simulated clinical experience questions (details in **Table 2**), L 1–11 learning subscale questions (details in **Table 3**), C1–13 – confidence subscale questions (details in **Table 4**). Figure demonstrates obtained score for each question. 1 – strongly agree, 2 – somewhat agree, 3 – do not agree, 4 – not applicable

Table 6. Spearman's monotonic correlation of questions asked before the course with subscales in questionnaire sent after simulation. SCE – simulated clinical experience, L – learning subscale, C – confidence subscale

Scale	Do you think that the negative emotions associated with failure during the simulation improve memorizing a particular material? vs Scales		I can deal with people who are arrogant at work vs Scale		I easily get nervous and confused and lose confidence in stressful situations vs Scales		I don`t mind when someone points out my mistakes	
	p-value	r	p-value	r	p-value	r	p-value	r
SCE	0.2904	0.18	0.4628	-0.13	0.0337*	-0.35	0.9192	0.02
L	0.0653	0.32	0.0806	-0.30	0.6598	-0.08	0.8662	-0.03
C	0.0343*	0.38	0.7519	-0.06	0.1409	-0.27	0.6892	-0.07

Table 7. Statistical significance of Spearman's monotonic correlation of question: "I easily get nervous and confused and lose confidence in stressful situations" asked before the course with questions sent after simulation

Question	p-value	r
I enjoyed working with the simulator	0.0223*	-0.38
The group was the right size to facilitate my learning	0.0036*	-0.47
I learned as much from observing my peers as I did when I was actively involved in caring for the simulated patient	0.0058*	-0.45
I felt like it was ok to make a mistake	0.0055*	-0.45
I felt stressed when the simulator's condition worsened	0.0104*	0.42
Do you think that stress associated with the simulation helps to remember important information?	0.0237*	-0.38
The simulator and the environment were realistic	0.0362*	-0.35
Have you learned to take advantage of your mistakes?	0.0390*	-0.35

Discussion

The process by which experts make decisions tends to be based on experience and an increased ability to assess risk. In emergency situations the tendency is for people to want to do things faster, making them more error prone. More experienced and technically competent individuals make more effective and quicker decisions introducing the greater structure to their behavior. It has been argued that intuition alone is not sufficient to lead to a decision but rather requires a cognitive continuum which is a series of decision strategies dependent on the situation and expertise [13, 14]. A recognition primed decision model describes how people use their experience based on a repertoire of patterns. It blends intuition and analysis. A purely intuitive strategy relying only on pattern recognition would be too risky and could generate flawed options whereas a completely analytical strategy would be to slow. Weighing options generally makes sense for novices, who need a decision-making framework to help them think their way through a problem. But the way to get people past the novice stage is to accelerate their experiences so that they can rapidly accumulate the memories and cues that will enable them to make better decisions faster [13]. It is extremely important at Emergency Department where neurological emergency has to be managed in the time and efficient manner. Simulation, and the use of simulators to educate healthcare practitioners, has been shown to be effective in transferring knowledge to both trainees and practicing healthcare professionals [15].

We designed simulation course which covers purely neurosurgical emergencies. We assessed participants` experiences regarding learning, confidence improvement and clinical scenarios reality. The information obtained from the participants indicates that the course was well received and the groups of 4 students are an appropriate size [16]. The ability to not only participate in the simulation but to observe colleagues performance in simulation scenarios also proved to be of educational benefit. It was considered by students as an effective tool to learn how to manage a critically ill patient and recognize neurosurgical emergencies. We also analysed the influence of self-confidence assessed before the simulation with answers obtained in the post simulation questionnaire. Obtained answers suggest that student who have positive approach towards stress felt that simulations helped them to remember material, control emotions and easier recognize changes in patients condition. Students who felt that they easily get nervous had much less positive clinical experience during simulation. Those observations are confirmed by the fact that there was only one positive correlation with the question: "I felt stressed when the simulator's condition worsened" and students who felt they get easily nervous had negative rho correlation with the remaining questions. The literature indicates that stress is a factor in learning and performance [17, 18]. The Inverted U graph was initially presented by Robert Yerkes and John Dodson in 1908. They indicated that the performance at any task varies with stress in a predictable parabolic curve [19]. A study by Demaria demonstrated that emotional stress in simulated cardiac arrest simulation scenarios improved performance [20]. The evaluation of stress on performance was not a primary endpoint of this study but should be evaluated further in the future to improve simulation scenarios and longitudinal education for medical students. Simulation scenarios allows students to obtain a better understanding of how to utilize the complex neurological knowledge and pathophysiology from their neurosurgical rotation. Simulation also provided them an opportunity to manage patients they typically would not be allowed to manage in an acute setting during their studies. During this pilot curriculum the faculty also assessed the longitudinal progression of the students during the course. Notes and interviews with the faculty demonstrated a perception of improved individual and team performance over the course of the 5 days. Other studies support the use of deliberate practice and use of simulation to improve team performance [21, 22]. It was apparent to all the instructors that over the course of the 5 days the general approach to the critically ill patient became more structured and the speed in assessment, ordering tests and requesting neurosurgery consult improved. This improvement in performance followed constructive feedback provided in the debriefings [23, 24]. This study demonstrated that the deliberate practice model is an important property of simulation based medical education as is immediate informative feedback. In the future we intend to incorporate a Global Rating Scale in addition to checklist evaluation as part of the student evaluation process. We also plan to evaluate the retention of performance after simulated training which was not addressed in our current study.

Conclusions

1. Neurosurgical simulations curriculum improves student ability to manage a critically ill patient and recognize neurosurgical emergencies.
2. Neurosurgical simulations expose and accustom students to stress associated with clinical practice therefore improving students stress tolerance.
3. Our questionnaire is an effective tool for assessment of students experience during clinical simulations.

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Authors' contributions
Study design: BS, LG; data collection and analysis: BS, BW, RJ, MC, LG, manuscript preparation BS, RJ, MC, LG. All authors read and approved the final manuscript.

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Availability of data and material
All data generated or analysed during this study are included in this published article.

Conflict of interest statement
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The influence of alendronate therapy on the quality of life in postmenopausal women with reduced bone mineral density

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ABSTRACT

Aim. The aim of the study was to evaluate the effect of treatment on the quality of life (QoL) in postmenopausal women characterized by the reduced BMD.
Material and Methods. Postmenopausal women (n = 102), mean age (65.09 ± 5.6 years) were included in this study. The participants were divided into two groups, depending on the treatment or lack of treatment. For assessment of their QoL, QUALEFFO-41 scale and WHOQOL-100 scale were used.
Results. Mean values of the QUALEFFO-41 scale of women who used alendronate were significantly lower than those found in subjects not treated with this drug in the following areas: pain (p = 0.03), ADL (p = 0.03), jobs around the house (p = 0.01), mobility (p = 0.01), health perception (p = 0.03), emotional function (p = 0.007) and total QoL (p = 0.005). The mean values of the WHOQOL-100 scale almost did not differ significantly between both groups of studied patients. An exception was the level of independence, with mean values of women not receiving bisphosphonates being significantly higher than those of patients using bisphosphonates therapy (p = 0.04).
Conclusions. Quality of life assessment of women with osteoporosis and osteopenia using a specific scale and general scale can be a valuable clue in the planning of treatment, nursing care and psychological care.

Keywords: HRQoL; alendronate therapy; postmenopausal women; osteoporosis; QUALEFFO-41; WHOQOL-100;

Introduction

Reduced bone mineral density (BMD) and tissue degradation is one of the major public health problem at the present time. Postmenopausal women with reduced BMD are particularly vulnerable to fractures because of fragility, especially when they have poor balance which increases their risk of falling. With age, these problems are becoming increasingly important and more and more strongly deteriorate quality of life (QoL). Fracture sufferers require comprehensive and long-term customised treatment, considerable expenditure, comprehensive social support as well as nursing and rehabilitative care due to their disability and impaired QoL. Approximately 20% of patients with osteoporotic hip fractures die within one year, most of the deaths occurring within the first six months after a fracture [1]. Among these patients, 30–50% never regain their the previous functional status [2]. Numerous studies have demonstrated that the use of bisphosphonates reduces bone turnover, increases BMD and decreases the risk of fractures in patients with reduced BMD [3–9]. Currently, it is believed that bone resorption is inhibited not only directly by exerting effect on osteoclasts, but also indirectly through osteoblasts. The effect of bisphosphonates on osteoblasts consists in inhibiting certain cytokines, which may result in suppressing recruitment of osteoclast precursors and inhibiting the process of the precursors maturing into polynuclear osteo-

clasts. Moreover, a low molecular mass agent, whose release from osteoblasts is induced by bisphosphonates, inhibits the activity of mature osteoclasts and osteoclastogenesis. Furthermore, bisphosphonates stimulate synthesis of proteins and type I collagen as well as increase the activity of alkaline phosphatase and the amount of formed bone tissue [10–11]. Currently, the use of the medicines for therapy of women with postmenopausal osteoporosis has been increasing.

Numerous studies have confirmed that bisphosphonates not only reduce the incidence of fractures and increase BMD but also improve QoL [12–17]. According to the assumptions of the WHO, the concept of the quality of life determined by the state of health (Health-Related Quality of Life – HRQOL) covers the functionality in fundamental domains: physical, psychological, social and subjective assessment of the patient. This concept includes both objective and subjective evaluation. Furthermore, it is most often used to evaluate the effect of treatment [18].

Aim

This study aimed to assess the objective and subjective quality of life of postmenopausal women with reduced BMD and compare the short-term results on the QoL for a group treated with alendronate and a group not receiving such therapy. In addition, the aim of the study was to identify factors associated with the total quality of life of women with reduced BMD.

Material and Methods

The study was approved by the Ethical Review Committee at the Poznan University of Medical Sciences.

Study group

The study group consisted of 102 postmenopausal women treated in the Menopause and Osteoporosis Outpatient Clinic of the Obstetric and Gynaecological Hospital of Poznan University of Medical Sciences.

They were enrolled to this study on the basis of their densitometry results. The main inclusion criterion was BMD expressed as T-score below or equal to -1.0 standard deviation (SD). Regarding to BMD results, on the basis of the World Health Organisation definition of osteoporosis [19] women were classified as osteoporotic if their T-score was below or equal to -2.5 SD and osteopenic if its value was above -2.5 SD or below or equal to -1.0 SD in at least one of the measured areas (either the lumbar spine, or femoral, or both). The exclusion criteria were as follows: secondary osteoporosis, metabolic bone disease, malignant bone metastasis, hypogonadal states, osteogenesis imperfecta and treatment with glucocorticoids or any other disease which are known to significantly reduce quality of life such gastrointestinal tract disease, rheumatoid arthritis, severe osteoarthritis, hematological and endocrine disorders. Additional exclusion criteria were: currently bone fracture and the existence of the other diseases influencing the functioning of the locomotor system of the women.

BMD measurement

In all the women included in the study, BMD in the lumbar spine (L_1–L_4) and femoral neck was measured by dual energy X-ray absorptiometry (DXA) using a LUNAR device. The DXA method involves a very low radiation dose similar to that of natural background radiation (~7μSv/Day) [20]. Measurements of bone mineral content (gram) and area (cm^2) are provided for each measurement site. BMD results are expressed as an areal density in g/cm^2. The coefficient of variation (CV) is 0.7% at the lumbar spine and 1.0% at the hip [19]. BMD was compared with an appropriate ethnic and gender matched reference database, and was expressed as a standard deviation score (SD) from the mean of either young adult (T-score) or age matched (Z-score) [21].

Clinical parameters and sociodemographic factors

On the day of BMD examination, the body mass and height were measured. The Body Mass Index (BMI) was calculated according to the following formula: BMI = body mass/height^2 (kg/m^2). The subjects also responded to questions about sociodemographic and clinical parameters: history of previous fragility fractures, family history of fractures, current smoking, current alcohol consumption, physical activity, date of the last menstruation in the patient's life.

FRAX based assessment of the risk of fractures

The FRAX method [4, 5] has been used to assess the 10-year probability of fracture for individual study groups. The average BMD values, evaluated for the femoral neck and clinical risk factors were calculated.

Therapy

Patients with a history of previous fragility fractures, with vertebral deformities as well as those with family history of osteoporosis fractures received bisphosphonates therapy. Sixty seven of them (65.7%) were administered weekly doses of 70 mg alendronate.

Thirty five patients (34.3%) did not use the therapy. All subject had been receiving daily doses of 500 mg calcium and 400 IU vitamin D.

It should be emphasized that the patients prior to the start of the study did not receive any medication for osteoporosis.

Based on whether the participants received alendronate or not, the study group has been divided into two groups.

Evaluation of quality of life

The quality of life (QoL) was evaluated twice, i.e. before the bisphosphonates therapy and 3 months after the first survey. We used two scales, i.e. QUALEFFO-41 scale (as an objective quality of life scale) and the scale of WHOQOL-100 (as a subjective assessment of quality of life).

The women responded to questions contained in questionnaires used for assessment of quality of life – the QUALEFFO-41 scale (for objective assessment of life quality). Psychometric properties of the Polish version of the QUALEFFO-41 scale were assessed by a research team headed by Bączyk [22]. The QUALEFFO-41 scale is used for overall assessment of the quality of life as well as evaluation of the quality of life with respect to physical, social and emotional function and pain. In our study we have employed the QUALEFFO-41 scale for assessment of life quality of persons with reduced BMD without vertebral fractures as well as those with vertebral fractures, whose BMD was measured for the lumbar spine. The Polish version of QUALEFFO-41 scale, like the original version, consists of 41 question divided into five domains: pain, physical function, social function, general health perception and emotional function. The physical function domain was divided into: activities of daily living, jobs around the house and mobility. Domain scores were assessed according to the algorithm proposed in 1999 by Lips et al [23], where 0 represents the best and 100 the worst quality of life.

WHOQOL-100 Polish scale serving for subjective evaluation of quality of life and contains the following domains: physical, psychological, independence, social, environmental and spiritual (religion, personal beliefs). The scale is designed in such a manner that patients may respond to questions on their own using a five point Likert response scale, with the points range for each domain being 4–20. The higher the scores, the better quality of life [24].

Statistical analysis

The statistical description uses numbers, percentages, mean values and SD. Comparison of group results on the QUALEFFO-41 and WHOQOL-100 was performed after converting values for the purposes of specific tools. Differences between groups with regard to QUALEFFO-41 and WHOQOL-100 were analysed using the t-Student test and ANOVA analysis of variance for independent and dependent data. For groups unevenly numerous test results verified the corresponding non-parametric tests, Welch test was used.

A determination of predictive factors for total QoL was performed using stepwise logistic regression analysis and Akaike Information Criterion (AIC) for model assessment. The cut-off for the total QUALEFFO-41 scale was set at the median the overall score. Score equal to the median, or lower, indicated a high QoL, while score higher than the median pointed to a low QoL. The cut-off for the WHOOL-100 scale was set at the median the overall score. Score equal to the median, or lower, indicated a low QoL, while score higher than the median pointed to a high QoL.

The regression analysis model used the quantitative continuous variables: age, BMI (kg/m^2), the other variables were considered as categorical (0–1): education, previous fractures, reduced height, physical activity and use of bisphosphonates.

The significance level was accepted as $p < 0.05$. The statistical analysis was performed using the SPSS Windows package, Version 20 (SPSS Inc., Chicago, IL, USA).

Results

Demographic and clinical characteristics of the study group and BMD results

The mean age of the studied postmenopausal women was 65.09 ± 5.6. Most women (73.5%) included in this study lived with their families and had a secondary education (56.9%). Forty percentage of women led a sedentary lifestyle and almost 16% patients were current cigarette smokers.

At the time of enrollment, among 78.4% of women the osteoporosis was diagnosed, whereas osteopenia was revealed in 21.6%. Twenty seven women (26.5%) had a history of previous fragility fractures. None of them sustained a femoral neck fracture. On the other hand, wrist or forearm fractures occurred in almost 13.7% of osteoporotic patients. Parental history of fracture was reported by 36.3% of women. Hip fracture was recorded in 1 parent of women. Moreover, wrist or forearm fractures were reported in 24.5% of parents of studied participants. BMD was evaluated based on the T-score for L_1-L_4 and for femoral neck:

For lumbar spine, the mean BMD was 0.82 ± 0.07 g/cm² and the mean T-score was -2.99 ± 0.34, while the values for the femoral neck amounted to 0.61 ± 0.07 g/cm² (BMD) and -2.98 ± 0.34 (T-score). For detailed information see in **Table 1**.

Quality of life of the study group

Objective quality of life of all participants was assessed using the QUALEFFO -41 scale and subjective quality of life was assessed using the WHOQOL-100 upon the first measurement (before alendronate therapy) and upon the second measurement after 3 months.

The results concerning the QoL with regard to specific domains of the QUALEFFO-41 scale were presented based on mean values (SD). In both the first and the second survey, the patients obtained high mean scores for pain (42.12 vs. 41.32), social function (46.40 vs. 46.45), health perception (60.88 vs. 62.51) and emotional function (41.11 vs. 41.42). Statistically significant differences between the measurements were noted for pain ($p < 0.01$) and health perception ($p < 0.01$) (**Table 2**).

Moreover, mean values of women who used bisphosphonates were significantly lower than those of subjects not treated with bisphosphonates in the following areas: pain ($p = 0.03$), activities of daily living (ADL) ($p = 0.03$), jobs around the house ($p = 0.01$), mobility ($p = 0.01$), health perception ($p = 0.03$), emotional function ($p = 0.007$) and general quality of life ($p = 0.005$). A statistically significant difference was not observed for social function (**Table 3**). Similarly, the subjective quality of life is presented as mean values

Table 1. Demographic and clinical characteristics of studied subjects (n = 102)

Parameter	Value
Age, mean (SD) [years]	65.09 (5.6)
Age at menopause, mean (SD) [years]	50.70 (4.6)
Body Mass Index (BMI), mean (SD) [kg/m^2]	21.90 (3.2)
Education, n (%)	
Basic	3 (2.9)
Work-related	14 (13.7)
Secondary	58 (56.9)
University level	27 (26.5)
With family or with another, n (%)	75 (73.5)
Paid work, n (%)	26 (25.5)
Current physical activity, n (%)	61 (59.8)
Current smoking, n (%)	16 (15.7)
BMD L_1-L_4 (g/cm²), mean (SD)	0.82 (0.07)
T-score L_1-L_4, mean (SD)	-2.99 (0.34)
BMD femoral neck (g/cm²), mean (SD)	0.61 (0.07)
T-score femoral neck, mean (SD)	-2.98 (0.34)
Previous non-vertebral fractures, n (%)	27 (26.5)
Parental history of fracture, n (%)	37 (36.3)
Osteoporosis, n (%)	80 (78.4)
10-year probability of fracture risk for women with osteoporosis and with 1 factor for 67 women (%)	14
Osteopenia, n (%)	22 (21.6)
10-year probability of fracture risk for women with 1 factor for 35 women (%)	5.9
Osteoporosis treatment, n (%)	
Weekly alendronate	67 (65.7)
Without bisphosphonates therapy	35 (34.3)

Table 2. Quality of life of postmenopausal women with reduced bone mineral density (n = 102). Measurement I - assessment at inclusion; Measurement II – assessment after three months of study. Data are presented as means (SD)

QUALEFFO-41	Measurement I	Measurement II	p
Pain (back pain, sleep disturbance)	42.12 (29.98)	41.32 (31.11)	< 0.01
ADL (activities of daily living)	19.2(12.21)	19.22(12.85)	N.S
Jobs around the house	28.3(13.43)	28.32(13.55)	N.S
Mobility (standing up, bending, kneeling, stairs, walking, body image)	23.37 (15.31)	23.42 (15.30)	N.S.
Social function (sport, gardening, hobby, friends)	46.42 (22.32)	46.45 (22.32)	N.S.
General health perception	60.88 (24.33)	62.51 (22.84)	< 0.01
Emotional function (fatigue, depression, loneliness, energy, cheerfulness, hope, fear)	41.11 (13.78)	41.42 (13.03)	N.S.
Total QUALEFFO-41 score	28.89 (11.81)	29.02 (11.66)	N.S.

Higher scores indicate poorer QoL; N.S. – not significant

for specific areas of the WHOQOL-100 scale. In both the first and the second survey, high mean values were noted for the social domain (14.16 vs. 14.15) and overall subjective assessment of quality of life (15.46 vs. 15.45). A statistically significant difference between the measurements was observed for the mental function (**Table 4**).

The mean values in individual domains of the WHOQOL-100 scale did not significantly differ between patients treated with bisphosphonates and women not receiving such therapy. An exception was the level of independence, with mean values of osteoporotic women not receiving bisphosphonates being significantly higher than those of patients using bisphosphonates therapy (p = 0.04) (**Table 5**).

Table 6 shows the factors associated with total QoL for women by logistic regression analysis, using the QUALEFFO-41 and WHOQOL-100. For the total QUALEFFO-41 score, the associated factors were: age (OR = 1.56; 95% CI 1.39–1.45) secondary and higher education (OR = 0.59; 95% CI 0.4–0.85), physical activity (OR = 0.55; 95%CI 0.32–0.97), bisphosphonates therapy (OR = 0.41; 95% CI 0.13–0.71). For the total WHOQOL-100 score the associated factors were: age (OR = 0.43; 95% CI 0.23–0.98) and BMI ≥ 25 (kg/m^2), (OR = 0.85; 95% CI 0.73–0.99).

Table 3. Comparison of quality of life after three months of treatment with bisphosphonates in postmenopausal women and among those not receiving such therapy. Data are presented as means (SD)

QUALEFFO-41	Women treated with bisphosphonates (n = 67)	Women not treated with bisphosphonates (n= 35)	p
Pain (back pain, sleep disturbance)	34.31 (29.87)	46.16 (29.50)	F = 4.71; *p* = 0.03
ADL (activities of daily living)	15.09 (12.11)	21.32 (12.32)	F = 4.94; *p* = 0.03
Jobs around the house	20.2 (18.44)	31.61(20.01)	F = 6.57; *p* = 0.01
Mobility (standing up, bending, kneeling, stairs, walking, body image)	17.53 (11.86)	26.39 (16.09)	F = 6.85; *p* = 0.01
Social function (sport, gardening, hobby, friends)	41.60 (19.80)	48.90 (23.27)	N.S.
General health perception	52.80 (26.72)	65.03 (22.2)	F = 4.99; *p* = 0.03
Emotional function (fatigue, depression, loneliness, energy, cheerfulness, hope, fear)	52.90 (26.66)	65.03 (22.16)	F = 7.78; *p* = 0.007
Total QUALEFFO-41 score	23.97 (10.90)	31.44 (11.52)	F= 8.31; *p* = 0.005

Higher scores indicate poorer QoL, N.S. – not significant

Table 4. Quality of life of postmenopausal women with osteoporosis (n = 102). Measurement I – assessment at inclusion; Measurement II – assessment at 3 months. Data are presented as means (SD)

WHOQOL-100	Measurement I	Measurement II	p
Physical function	12.44 (1.13)	12.43 (1.75)	N.S.
Mental function	13.18 (1.24)	13.04 (1.11)	N.S.
Level of independence	13.72 (1.66)	13.72 (1.51)	N.S.
Social function	14.16 (2.17)	14.15 (1.92)	N.S.
Environment	13.55 (1.36)	13.55 (1.25)	N.S.
Spirituality	13.67 (3.55)	13.64 (3.67)	N.S.
Total WHOQOL-100 score	14.78 (2.62)	14.78 (2.19)	N.S.

Higher scores indicate better QoL, the points range: 4–20

Table 5. Comparison of subjective quality of life of women treated with bisphosphonates and those not receiving such therapy. Data are presented as means (SD)

WHOQOL-100	Women treated with bisphosphonates n = 67	Women not treated with bisphosphonates n = 35	p
Physical function	12.41 (1.17)	12.45 (1.12)	N.S.
Mental function	13.12 (1.02)	13.20 (1.60)	N.S.
Level of independence	13.45 (1.59)	14.24 (1.71)	F = 4.57; *p = 0.04*
Social function	13.85 (1.99)	14.76 (2.40)	N.S.
Environment	13.38 (1.15)	13.38 (1.11)	N.S.
Spirituality	13.32 (3.25)	13.34 (4.04)	N.S.
Total WHOQOL-100 score	15.46 (3.52)	15.45 (3.52)	N.S.

Higher scores indicate better QoL, the points range: 4–20

Table 6. Variables associated with total QUALEFFO-41 and total WHOOQL-100 in women with reduced BMD evaluated by stepwise multiple logistic regression analysis (n = 102)

	Variables	*p* -Value	OR	95% CI
Total QUALEFFO-41 score > 25.0	Age	0.02	1.56	1.39 1.45
	Secondary and higher education	0.005	0.59	0.4 0.85
	Physical activity	0.037	0.55	0.32 0.97
	Bisphosphonates therapy	0.01	0.41	0.23 0.71
Cox i Snell R^2 = 0.44 Nagelkerke R^2 = 0.51				
Total WHOOQL-100 score < 17.0	Age	0.04	0.43	0.23 0.98
	BMI ≥ 25 (kg/m^2)	0.03	0.85	0.73 0.99
Cox i Snell R^2 = 0.14 Nagelkerke R^2 = 0.34				

Discussion

Assessing the quality of health life has been recognized as an important determinant of the clinical evolution of patients with reduced BMD and its serious consequences, such as osteoporotic fractures.

That is why in this study we analyzed changes in the quality of life in postmenopausal women with reduced BMD observed during the three-months therapy of bisphosphonates. For this purpose, we used QUALEFFO--41 questionnaire, consisting of 41 grouped questions, that have already been prepared for use in Poland and WHOQOL-100 consisting of 100 grouped questions [22, 24].

Analysis of data from two measurements showed that the objective quality of life in pain in women improved (change to the significance level p < 0.01). The observed improvement in the perception of pain may be the result of therapeutic effects. In the second measurement of quality of life compared with the results and the measurement has not changed in terms of physical and social functioning. Also, no changes were observed in the evaluation of emotional state, unlike in the studies Dennison et al [25], who reported deterioration in emotional functioning in women with postmenopausal osteoporosis. The observed differences in the assessments may result from the length of observation. In studies conducted by Dennison et al [26] study was repeated after four years while ours after 3 months.

A disturbing fact is that in the second measurement of the subjects obtained a lower quality of life in terms of the perception of health. The reason for this require additional research. Reduced perception of health status could result from other aspects of the postmenopausal period. An analysis of data from the two measurements showed that the subjective quality of life assessed on the basis of the scale WHOQOL-100 in all areas of the scale has not changed.

In our studies in the assessment of the impact of alendronate therapy demonstrated a significant reduction in back pain (p = 0.03), as in the reports Iwamoto et al. [27] which inform about the positive effects of alendronate sodium therapy on back pain. Like Panico et al. [28] in our study we observed a significant improvement in physical functioning. Panico et al. well as getting good grades emphasize the quality of life in terms of activities of daily living, mobility and range of household activities compared to the patients treated with alendronate [28].

In addition, women who used alendronate were characterized by significantly higher quality of life in the area of emotional, better perception of their health and higher overall quality of life. Similarly, a Brazilian cross-sectional study by Ferreiro et al. [29] and studies conducted by Iwamoto et al. [27] showed that antiosteoporotic therapy significantly improved quality of life of the participants.

Subjective quality of life in all areas of the scale of women treated with bisphosphonates did not differ significantly in comparison with assessments of quality of life among untreated women. The exception here is the independence of participants, in which the subjective quality of life was lower in women who took bisphosphonates, compared to a group of women not taking these drugs.

Perhaps this is related to the need to comply with very favorable rules for the application of drugs. Bisphosphonates therapy entail the receiving the drug on an empty stomach (half hour before eating, drinking boiled water, with the recommendation of walking, do not go to bed). This procedure creates inconvenience for the patient. Perhaps that is why in the assessment of subjective quality of life in the area of independence was statistically significantly lower compared to the quality of life of women who were not taking bisphosphonates.

Our results showed the quality of life of patients treated with bisphosphonates was superior to that of women not receiving such therapy. The participants using alendronate scored significantly better with regard to pain,

their physical and social function was significantly superior as well as health perception and overall quality of life. The present study, as well as that of Iwamoto et al. [27], showed a positive correlation between bisphosphonates therapy and physical activity and QoL. According to Iwamoto et al. [27] alendronate and physical activity rapidly decreased back pain and improved quality of life in postmenopausal women with osteoporosis. Flood et al. [13] evaluated satisfaction with bisphosphonates therapy among osteoporotic and osteopenic patients using The Osteoporosis Patient Satisfaction Questionnaire (OPSAT-Q). Approximately 60% of subjects used alendronate sodium once a week and 13% of patients took this drug once a day. Moreover, 20% of participants received risendronate sodium once a week and 6% of subjects took risendronate sodium once a day. While evaluating satisfaction with bisphosphonates therapy, the patients referred to benefits from the treatment, such as effectiveness of the therapy ("noticeable effects", "disease progress has been stopped"), absence of adverse effects and ease of the medicine use. On the other hand, the respondents were dissatisfied with difficulty to observe an improvement in the state of bones and problems with memorising the name of the medicine.

Different conclusions may be drawn from a study by Sezer et al. [30], who did not find a correlation between the quality of life measured using the QUALEFFO-41 scale and the manner of osteoporosis treatment.

While assessing satisfaction with bisphosphonates therapy, patients stress inconvenience associated with the rules of the medicine administration. Patients who follow recommendations concerning the bisphosphonates use show good tolerance of the therapeutic agents. However, bisphosphonates may cause local irritation of the mucosa in the upper gastrointestinal tract, nausea, dyspepsia or diarrhoea. There have been cases of oesophagitis, ulcers or oesophageal erosion. Such complications affect patients who do not follow recommendations concerning the bisphosphonates use. The main reasons for discontinued treatment were digestive events, problem with receiving prescriptions within the first 3 months of treatment, dissatisfaction with the clinical condition. Patients on bisphosphonates may not be adherent to the therapy due to complex dosing regimens and a slightly decreased gastrointestinal tolerance, affecting the patients' quality of life. Several studies reported increased patient adherence related to a decreased frequency of bisphosphonates dosing [31]. Moreover, data showed that a decreased dosing frequency was more convenient and for the majority of patients [32].

Limitations of this study. An important limitation of the study is absence of data on patients' compliance with recommendations concerning the medicine use. Information was also not collected on adverse effects of bisphosphonates treatment. Therefore, continuation of the study will take into account these aspects.

Conclusions

The objective quality of life of osteoporotic women receiving alendronate sodium was significantly superior to that of subjects without such treatment in all domains of the scale (except for the social function).

The subjective quality of life did not significantly differ between the groups, except for the level of independence, which was significantly higher among women not receiving bisphosphonates therapy.

Quality of life assessment of women with osteoporosis and osteopenia using a specific scale can be a valuable clue in the planning of treatment, nursing care and psychological care. This is the first study of Polish women treated with bisphosphonates suffering from osteoporosis and osteopenia using the scale QUALEFFO-41 and WHOQOL-100 accordance with the concept of quality of life of the conditioned state of health.

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Conflict of interest statement
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CONTACT – communication protocol for family practitioners and specialists

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ABSTRACT

Introduction. Ability to gather and process medical data serves as a basic tool of doctor's work, which can be improved by applying communication protocols. In most cases, however, instructions presented by such models are too general and do not take into account patients' preferences.
Material and Methods. The study was carried out in the form of an electronic questionairre sent to a randomly chosen group of adult patients (N = 967). It consisted of close-ended questions about the quality of communication skills of family practitioners and specialists working at outpatient clinics and health centers.
Results. Only 21% of patients claimed that the appointment started on time. 51% mentioned disruptions during the meeting, mainly by a third party (34%). A considerable majority of physicians carried out the interview in a manner that was understood by patients, nevertheless 56.6% of the respondents felt underinformed as far as the nature of their illness was concerned – these objections were mostly expressed by patients suffering from chronic diseases.
Conclusions. Our research shows that a proper organization of work as well as observing the principles of appropriate clinical communication can facilitate doctors' performance, and thus increase both the level of patients' satisfaction and the quality of medical services.

Keywords: communication and interview skills, clinical-patient communication/relationship, cultural competence/proficiency.

Introduction

Communication between physician and patient serves as an essential tool to obtain clinical information [1]. Gaining and transferring data provided by patients determines the effectiveness of therapeutic course of action [2]. Models of communication are created in order to improve the quality of this process. Their role is to establish subsequent stages of gathering, processing and conveying information in specific medical situations (e.g. informing the patient about the negative diagnosis, poor prognosis etc.). Communication protocols constitute an important educational device that can be used to teach communication skills, both in the context of training students and raising the quality of doctors' work. What follows, the competence gained on the basis of these templates effectively improves the quality of medical activities in the clinical context [3]. As proved by the research, proper communication on the part of doctors considerably increases the sense of professional effectiveness [4] and fulfillment derived from work, which has a direct impact on the level of patients' satisfaction [5], and prevents professional burnout [6, 7]. On the other hand, defective communication deteriorates the quality of treatment, at the same time increasing its costs [8]. What is more, it is a critical factor leading to filing lawsuits against physicians [9] and it increases the risk of legal claims and accusations of abuse [10]. Most of these accusations, as evidenced by research, result from doctor-patient relationship. A quarter of these claims stems from flawed conveyance of medical information [11]. Analy-

ses reveal that a patient satisfied with medical care is likely to share a positive opinion on the received services with 5 other people. In contrast, a patient who is displeased will probably share their disappointment with 15–19 different people [12].

Considering the above, as well as bearing in mind the broad impact of communication on both the quality and effectiveness of medical activities, we have undertaken to construct a tool which could help family practitioners and specialists working at health clinics to properly collect information, process it and transfer it to patients. At the same time it would guarantee a high standard of medical services and maximize patient satisfaction, while taking into account the structurally limited time of a visit.

Material and Methods

The research was carried out between June 15 and August 15, 2015 with the use of an original, self-made electronic questionnaire. The survey was published on non-commercial Internet research website (www.e-badania.pl) dedicated to professional sociological research. The respondents were informed that their participation in the study was anonymous and voluntary. The questionnaire consisted of highly standardized, close-ended questions. A random group of adult patients (n = 967) was asked to provide answers to the questions concerning their evaluation of medical services as well as their own preferences related to their contacts with family practitioners and specialists working at outpatient clinics and health centers.

For the purposes of statistical analysis a software package SPSS v. 16.0. was applied. For the analysis of correlations between discrete variables and statistical heterogeneity of the groups Pearson's chi-square test was used. Differences for $p < 0.05$ were considered statistically significant. The opinions and evaluations provided by the respondents were confronted with socio-demographic variables (age, sex), health variables (chronicity of health problems) and medical variables (the place of encounter with the physician, the form of payment for the visit).

The present research was a cross-selectional study approved by the Independent Bioethics Commission for Research at the University of Gdansk.

Results

Thanks to the use of an electronic tool we have reached a relatively numerous (n = 967) and diverse group of respondents. On the other hand, it contributed to an overrepresentation of women and young people with an academic degree, which is characteristic of this type of research. 86% of the surveyed were women, while only 14% were men. Over 50% of the participants were people below the age of 30 (58%). Moreover, half of them graduated from universities. 23.8% of the respondents were between 31 and 40, 10% were between 41 and 50, 4.9% were between 51 and 60, and only 3% were over 60. Most often their last visit took place at public health centers (50.5%), followed by public specialist clinics (21.2%), private health centers (15.8%), private doctors' offices (8.7%) and private specialist clinics (3.8%). In most cases the cost of the visit was covered by the National Health Fund (77%). Otherwise, it was paid for by the patients themselves (17.5%) or by independent health insurance agencies (4.6%). Exactly 0.9% of the respondents could not remember the method of payment.

Participants were asked to provide details concerning their latest visit at the family practitioner's or specialist's office. A common problem reported by the respondents was insufficient length of a visit, which had a negative impact on their overall evaluation. Only one in five patients (21%) claimed that the appointment started on time. The others had to wait for the meeting; one third of the respondents spent more than 20 minutes in the waiting room. Additionally, half of the surveyed mentioned interruptions during their visit, mainly caused by the appearance of a third party (34%) at the office, which was probably one of the sources of delays.

Time shortage can also be observed in the context of another question: over half of the respondents (57%) concluded that the doctor did not dedicate enough time to the conversation during the visit. The opinions discussed above were juxtaposed against health and medical variables, yet no statistically significant differences were observed among the respondents' statements. This means that, contrary to expectations, the delays within the schedule were reported by the patients provided with medical services at public health centers and clinics as well as at private offices. The method of payment also did not have any impact on the subjective evaluation concerning time dedicated by a physician to the conversation with the patient.

The next analyzed problem pertains to medical jargon used during a visit which is incomprehensible to the patients. Exactly 37% of the respondents had difficulty understanding the message conveyed by a doctor, while over a half (58%) had no such problem. The survey also contained a question concerning

impersonal forms of address used by a doctor during an interview with a patient. The occurrence of such expressions was reported by 30% of the participants. In the course of statistical analysis the influence of socio-demographic aspects (sex and age) on the frequency of using impersonal forms by a doctor [in Polish impersonal forms, which stem from specific conjugation of verbs and inflection of nouns, may be considered impolite] was observed. As far as sex is concerned, 31% of women and 20% of men were addressed in such a manner during the last visit (*chi^2* = 6.393; *p* = 0.041). Taking into account the age, it becomes clear that the younger the respondent, the more frequent the use of impersonal forms by a doctor.

At this point it is worth pointing out that orders given in an impersonal form are inacceptable for the majority of patients. 64% of the respondents are definitely against such forms, while 26% rather do not approve of them (**Table 1**). In this case an influence of socio-demographic variables (such as sex, age, the place of residence and the level of education) on patient preferences was not observed.

The study has also revealed which questions – typical for communication with a doctor – were delivered to the patient in a satisfactory manner, and which were not. It turned out that in almost half of the cases (49%) the physician acquainted the patient with a plan of therapy. A similar number of respondents claimed that the doctor explained to a sufficient degree the necessity of taking additional tests (46.5%) and consulting other specialists (45.6%). The subject of dosage and application of drugs raised the least objections: 79% of the surveyed expressed no reservations related to this aspect of communication with a physician. On the other hand, the largest group of respondents felt underinformed as far as their disease entity is concerned. Only 38% of patients received a satisfactory amount of information on this subject, while over half of the surveyed (57%) claimed otherwise (**Table 2**). It is worth noting that lack of sufficient information about the necessity of taking further tests, consulting a specialist, drug dosage and the nature of an illness was reported more frequently by patients suffering from chronic diseases. It follows that this health variable indeed has an impact on the subjective evaluation of the amount/quality of information delivered by the doctor (**Table 2**).

From the diagnostic point of view, carrying out an interview is an important element of communication with a patient (**Table 3**). Therefore, the participants were asked to evaluate this part of a conversation, again in relation to their latest visit. The majority of them expressed a positive opinion when asked whether a doctor created conditions which enable a free conversation (69%) and whether he or she used clear, comprehensible language (79%). Almost half of the respondents (49%) confirmed that during the interview the doctor was asking precise, yes/no questions. Similar number of the surveyed (50%) positively evaluated doctors' involvement in dispelling any doubts as well as answering patients' questions. In contrast, as for making sure whether the patient understood the most significant information, only one in three respondents (33%) positively evaluated this aspect of an interview,

Table 1. Elements of communication used by a doctor during the latest visit

Categories of response	Yes	No	Not applicable or don't remember
	% of *n* = 967		
Did the doctor during the latest visit...			
address you in an impersonal form?	29.6	61.4	9.0
use incomprehensible terms and phrases?	36.6	57.6	5.8
present you with a plan of therapy?	49.1	43.8	7.0
Did the doctor explain to a sufficient degree...			
the necessity of taking additional tests?	46.5	41.9	11.6
the necessity of consulting other specialists?	45.6	36.7	17.7
the method of dosing and applying drugs?	79.1	15.7	5.2
the nature of your illness?	37.7	56.6	5.8
While gathering information, did the doctor...			
aske precise questions concerning your illness?	48.8	35.1	16.1
create conditions enabling free conversation?	69.1	20.2	10.8
use clear, comprehensible language?	79.1	14.9	6.0
dispel all doubts?	49.9	41.9	8.2
make sure that all information was understood?	33.4	57.2	9.4

Table 2. Subjective sense of being underinformed and type of illness

Patients who felt underinformed about...	Are you treated for a chronic disease?	
	Yes	No
the necessity of taking additional tests (*n* = 405)	210 (45.9%)	195 (38.3%)
	chi^2 = 12.536; *p* = 0.002	
the necessity of consulting other specialists (*n* = 355)	180 (39.3%)	175 (34.4%)
	chi^2 = 17.807; *p* < 0.001	
the method of dosing and applying drugs (*n* = 152)	77 (16.8%)	75 (14.7%)
	chi^2 = 6.779; *p* = 0.034	
the nature of their illness (*n* = 546)	287 (62.7%)	259 (50.9%)
	chi^2 = 23.148; *p* < 0.001	

while 57% expressed negative opinion. In the case of assessing comprehensibility of language and dispelling doubts, the health variable – treating chronic diseases – is of considerable significance. Negative evaluation of these two aspects of communication is much more common among patients suffering from chronic illnesses than among the ones who do not cope with such ailments.

Discussion

The results of the survey reveal some discrepancies between patients' expectations and certain aspects of medical practice. The first significant problem pertains to the organization of visits. While answering the questions, participants frequently mentioned delays disturbing the schedule of appointments. The next important inconvenience stemmed from interruptions during a visit, caused mainly by third parties. This problem was also reported in other research [13]. In our opinion this sort of incidents during medical encounters have a negative impact on the evaluation of the quality of medical services. What is more, they contribute to the conviction that doctors do not dedicate enough time to conversations with their patients.

Our study has also revealed some deficiency as far as conveying medical information is concerned. Patients expect physicians to present a plan of therapy and to deliver information concerning the nature of their illness during a visit. That also expect their doctors to use comprehensible language and create atmosphere conducive to openness. Therefore, we suggest that doctors should make sure whether their patients understood the most significant information discussed during a visit.

The obtained answers together with a detailed analysis of the current results of research in the field of clinical communication published in indexed medical journals served as the basis for drawing up a medical communication procedure (**Table 3**). With the mnemonic acronym CONTACT (context, organization, niceties, taking stock, assimilation, counseling, taking care) we propose a communication pattern which takes into account the nature of work performed by family practitioners and specialists.

C – context (preparation)

A doctor, when alone in his or her office, can prepare for the meeting with a patient, carefully analyzing all the relevant information (identification of the patient, analyzing patient's medical history, checking the results of previous tests etc.). This activity does not take much time and can positively affect both the quality and the duration of the visit. A physician who knows the results of the latest tests and who remembers the ailments of his or her patients as well as doses of previously prescribed medicine etc. is more likely to be highly evaluated (i.e. regarded as a caring, emphatic and competent person). Factors hindering communication such as using a computer while having a conversation with the patient or pauses between doctor's comments, who analyzes the data during the meeting increase the likelihood of negative evaluation of the visit by the patients [13].

O – organization (work environment)

Proper organization of the workplace has a considerable influence on the quality of work. There should be enough time between visits to arrange documentation, air the office, if need be, or carry out all the necessary steps to ensure that proper hygiene is observed. Proper organization of work also concerns establishing the rules of calling patients in. The right approach is to invite them to the office by using their name. The doctor, apart from identifying the patient, can also settle the situation outside the office. Eliminating any interference from third parties during the visit significantly raises its quality [14].

Table 3. The model of the CONTACT communication procedure

Context	Preparing for the encounter with a patient	personal identification of a patient analyzing patient's medical history checking documentation checking the results of previous tests
Organization	Organization of the workplace	time for arranging documentation airing the office, if necessary activities connected with occupational hygiene
	Establishing the rules of calling patients in	breaks between visits inviting patients to the office by using their names (identification of patients)
Niceties	Greeting	calling patients in by using their full names shaking hands self-introduction (during the first visit)*
	Confirming/checking patient's personal data	
	Initiating a converstation	
Taking stock	Interview	open and close-ended questions probing questions active listening
	Explaining all actions, step by step	explaining wyjaśnienie podejmowanych czynności giving clear orders formulating clear questions
	Thanking the patient for the examination	
	Making a diagnosis or a diagnostic hypothesis	
	Noting down the results in the documentation the results	
Assimilation	Conveying the results of the examination	the cause of illness mechanisms governing the development of the illness possible consequences or complications
	Presenting a plan of the following medical/diagnostic measures	if possible, presenting alternative diagnostic and therapeutic methods
	Explaining the therapeutic process	explaining the effects of the prescribed medicines explaining the necessity of taking additional tests and/or consulting another specialist
	Obtaining patient's approval of the therapy/ permit for further tests	
	Dispelling patient's doubts	
Counseling	Explaining the method of dosing drugs/ the rules of conduct related with a particular ailment,	if necessary, providng additional information concerning diet, exercise, everyday activity and hygiene
	Referring a patient to a specialist/consultant*	providing a patient with relevant details (the place and time of such a visit)* indicating where such information can be obtained*
	Giving guidelines	indicating a source of information that the patient can use in order to expand the knowledge concerning their illness
	Handing over the documentation	prescriptions, referrals other information
Taking care	Repeating the most important information	Summing up the visit by repeating all the key information concerning a diagnosis, therapy, consultations, further tests etc.
	Providing a sense of security	mental suport*
	Providing support*	social support (informing the relatives) institutional support (social services etc)*
	End of the visit	thanking the patient for the meeting a goodbye handshake seeing the patient to the door

Note: *if applic able / if necessary

N – niceties (creating communication context)

Evaluation of both patients' satisfaction and the quality of medical services to a large extent depends on the doctor's ability to establish relationship with the patient [15]. Greeting a patient with a handshake is a simple gesture which is an excellent way to open the communication space. Haptic gestures, being strong stimuli, enhance the message, and, in a context of building up empathy, non-verbal behaviors play a significant role [16]. The abovementioned form of greet-

ing is expected by the majority of patients [17]. This moment is also a perfect opportunity to obtain permission for a third party (such as students or interns) to be present in the medical office during the visit, as well as to indicate to the patient (and/or the accompanying person) the chair which they are supposed to occupy. Initiating the conversation is another key factor. At this point it would be worthwhile to ask about the patient's mood before passing on to inquiring about the actual reason of the visit.

T – taking stock (gathering and arranging medical data)

One of the most important stages of a medical encounter is carrying out both an interview and a physical examination. Appropriate gathering and arranging of clinical data determines the accuracy of a diagnosis, the course of a therapeutic process and the evaluation of a physician's work. Asking open and close-ended questions, which enhance both active listening and empathy of a doctor, not only increases the level of trust [18], but also has a direct influence on clinical effects. In a properly functioning communication space, patients share much more clinically relevant biomedical and psychosocial information with their doctors [19]. The majority of diagnostically significant data comes from medical history, which is a part of the interview. More often than not physicians do not provide patients with sufficient amount of time to describe this part of medical interview. Therefore, while asking introductory open-ended questions, it is important to listen to the patient without any interference, which is a very common mistake [20]. When interrupted, the patient often refrains from describing all symptoms [21], which, in consequence, hampers the whole diagnostic process. After finishing a physical examination a doctor should thank the patient and only then pass on to noting down the results. When the doctor fills the papers, the patient has time to put their clothes back on.

A – assimilation (transfer of information)

Conveying the results of the examination and the absorption of the received information by the patient constitutes a significant part of the whole visit. The degree to which the doctor's recommendations are understood determines the subsequent steps taken by the patient. The deeper the understanding of the cause of illness, its mechanisms and therapeutic activities, the more consistent the observance of doctor's recommendations, the deeper involvement in the therapeutic process and the more efficient cooperation based on the patient's trust [22]. After presenting the results of the examination and making a diagnosis (or a diagnostic hypothesis which requires further verification), a physician should suggest a plan of following medical measures to be taken. Patient's involvement in the decision-making process (concerning the choice of diagnostic or therapeutic methods, e.g. preliminary consent to a medical examination, the choice of medicines) results in sharing responsibility for the therapeutic process, which in turn, as proved by the research, increases the efficiency of medical action while at the same time decreasing its costs [23–25]. Explaining particular stages of medical treatment and the necessity of taking further tests or visiting a specialist, along with justifying the choice of prescribed medicines and presenting the possible consequences of the illness, as well as the expected effects of pharmaceutical drugs, results in a patient's deeper involvement in a therapy [26, 27]. Moreover, such approach increases the level of patient's satisfaction [28]. At this point it is essential to check whether the patient has any doubts or reservations concerning the proposed treatment (e.g. to make sure that the price of a drug is acceptable) since doctors, in general, overestimate patients' ability to absorb and process medical information [29]. Accepting the therapy is the key condition for its effectiveness. Patients who did not approve of the applied treatment displayed undesirable medical effects due to their failure to comply with doctor's recommendations, which in turn led to recurrent deterioration of health condition [30].

C – counseling

This stage consists in precise explanation of the rules which are to be observed during treatment (e.g. drug dosage, rules of conduct related with a particular ailment, diet, exercise, everyday activity or hygiene). If it is necessary for a patient to consult a specialist, a doctor should provide them with relevant details (the place and time of such visit) or indicate where such information can be obtained. In certain situations it is also advisable to recommend a source of information that the patient can use in order to expand the knowledge concerning their illness. Exact therapeutic instructions should be delivered to the patient in written form (dosage, dietary restrictions etc.)

T – taking care

The last part of the visit involves summing up all the key arrangements and repeating the most significant

information. A patient should be provided with a sense of support. Signs of empathy build trust and positively affect patient's satisfaction concerning the medical encounter [31]. At the end of the visit it is advisable to thank the patient for the meeting, see them to the door and initiate a goodbye handshake.

To our mind, the above procedure is a perfect way to organize clinical space and time as well as the key verbal and non-verbal elements of the visit at the office of both family practitioners and specialists. Application of this model can effectively increase patient satisfaction, at the same time raising physicians' own evaluation of their professionalism. While preparing this template, we paid attention to the results of existing research concerning the issue of doctor-patient communication. Nevertheless, in order to verify our model of communication, it would seem indispensable to carry out randomized cohort studies, which could serve as an adequate tool to measure its effectiveness.

It needs to be pointed out that both patients' and physicians' communication preferences are always ingrained in a specific cultural context. Therefore, we suggest that they were taken into account while applying this protocol. We also believe that it is important to pay attention to structural factors such as the principles governing organization of work in outpatient clinic and health centers. Systemic limitations, the type of illness and health care context may considerably determine application of this protocol since they have direct impact on the relationship between a patient and a physician [32].

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Conflict of interest statement
The authors declare no conflict of interest.

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Informed consent and ethical approval
Informed consent was obtained from all individual participants included in the study. The research was positively evaluated and approved by the Independent Bioethics Commission for Research at the Medical University of Gdansk.

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REVIEW PAPER

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Mesotherapy – a method of facial skin rejuvenation from an interdisciplinary perspective on improving facial aesthetics

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ABSTRACT

Mesotherapy has been used in medicine since the 1950s and for aesthetic procedures since the 1970s. In the medical literature there are numerous reports about the positive effect of mesotherapy with regard to rejuvenating and improving the appearance of facial skin (reduction of fine wrinkles and discolouration, facial contour correction, improvement in skin tightness and elasticity). The variety of formulations used and the different techniques for administering them mean that the subjectively observed effects of mesotherapy cannot be objectively verified. To date, only a few studies have been published in the international literature where histopathological, ultrasound, and electron microscopy examinations were performed to confirm the efficacy of this method. Most studies, however, have been based on assessing subjective improvement. The aim of this paper was to review literature on the subject, namely 15 full-text reports on the effects of mesotherapy in the treatment of facial skin. The findings of the review show that the studies published to date are based primarily on subjective assessment methods, presenting the results of mesotherapy procedures through an analysis of "before and after" medical photographic documentation. It would seem necessary that further research should be conducted based on a unified protocol.

Keywords: mesotherapy, revitalisation/rejuvenation, aesthetic medicine, face.

Introduction

In today's world, with its cult of broadly understood youth and a beautiful face, most researchers in this area agree that the aim is to retain and, if possible, restore the skin's moisture, structure and tissue volume. In this context, it seems that there is no alternative to aesthetic medicine and anti-aging treatments. Contemporary people, who very often lead fast and stressful lives, cannot afford to wait for slow improvement or undergo time-consuming preventive treatments, but need a quick instantaneous effect. This opens up possibilities for practitioners of aesthetic medicine, who have at their disposal an increasingly wide range of treatment options and more advanced formulations, as well as a greater possibility for individualizing treatment and adjusting it to the needs of particular patients depending on the condition of their skin. In this context, mesotherapy should be regarded as one of the most important, and at the same time relatively simple, techniques [1–3].

For years, because of its safety and hydrophilic properties, non-crosslinked or, less frequently, crosslinked (or modified) hyaluronic acid has been used for intradermal administration. It is often combined with other substances such as vitamins (e.g. vitamin C, biotin); organic silica; DMAE (Dimethylaminoethanol); precursors of collagen and other structural and functional proteins; polynucleotides; minerals; or cofactors, which

can be used either individually or mixed into a cocktail. This makes it possible to create a formulation that will ensure the best results in terms of prevention or restoration of a lost function while minimizing the burden on the patient [4, 5]. However, these preparations require further research work and documentation to objectively verify their impact on young and ageing skin [6, 7].

Particular attention should be paid to the possible side effects and accompanying symptoms, especially if a patient receives several ingredients at the same time. It is essential that each patient should be correctly qualified for the treatment and that detailed medical interviews are conducted with regard to allergies, intolerances to various substances, chronic diseases and medications [8, 9, 10].

These problems can be minimized through the use of platelet-rich plasma (PRP) in mesotherapy, whose efficacy in revitalization is quite well-documented. Experts in this field appreciate its safety (no extraneous biological material is used) as well as very good results, confirmed by studies [11, 12]. These results are also appreciated by patients as this is probably the most effective hair loss treatment apart from hair transplants. For an even better therapeutic effect, PRP is often combined with the preliminary administration, also by means of mesotherapy, of medical carbon dioxide (carboxytherapy), which, among other things, promotes platelet activation [13].

In aesthetic treatment it is important not to go too far but to properly balance the number and frequency of procedures, depending on the age and needs of the patient's skin, so that the optimum therapeutic effect can be achieved while taking into account the economic burden on the patient [14]. Also, the ethical, psychological and social effects must not be ignored, being part of the definition of health formulated by the World Health Organization [1, 15].

Aim

The aim of this study is to show the influence of mesotherapy on improving the condition of ageing skin on the basis of literature reports.

Materials and Methods

The literature on the use of mesotherapy for skin revitalization, with particular attention to facial skin, was analysed. Following an analysis of the abstracts, fifteen full-text papers thematically related to the subject were selected and used in the study.

Results

Kubiak et al. [16] present mesotherapy (intradermotherapy) as a method of treating selected conditions and ailments by injecting small amounts of a drug (an active ingredient) directly at the place where the therapeutic effect is desired. The authors treat mesotherapy as a method for treating mesodermal tissue disorders. They believe that the most popular and most commonly used treatment is a "mesolift", a preventive procedure which aims to inhibit skin ageing processes and is applied using either the classic or the no-needle method. According to the authors, the cocktails of hyaluronic acid (often also used alone), embrioblasts, vitamins and polydeoxyribonucleotides which are used for biorevitalization form the basis of the aesthetic medicine. The above treatments, together with chemical peels, constitute first-line anti-ageing therapies which stimulate natural regenerative processes in the skin, thus leading to improvement in its appearance and properties. These authors, as well as others [17], also mention autologous mesotherapy, an innovative skin revitalization treatment which uses autologous platelet-rich plasma obtained from the patient's blood.

In recent years mesotherapy has become one of the most popular facial rejuvenation methods [18]. The treatment is performed by injecting a small amount of a substance into the dermis and subcutaneous tissue of the area to be treated. Mesotherapy is intended to stimulate elastic and collagen fibres by introducing active substances using microinjections. The effect of mesotherapy is the combined result of skin stimulation through microneedling and the therapeutic and regenerative properties of the administered substances [19]. The 1–2 mm deep microinjections create micro-channels through which active ingredients from individually designed high-concentration cocktails, such as organic silica, pyruvic acid, vitamin C, caffeine, penta-peptides, DMAE and hyaluronic acid, are delivered [20, 21]. Microinjection mesotherapy is performed on the face and neck to improve skin colour and enhance its elasticity, though it can also be applied to other areas of the body. Mesotherapy as a biorevitalizing and bioregenerative treatment method helps to increase the level of skin hydration and reduce facial wrinkles. The healing process stimulates the regeneration and repair of the treated skin. When qualifying a patient for the treatment it is essential to consider contraindications to mesotherapy, such as decompensated diabetes, autoimmune diseases, pregnancy or lactation. The side effects of the treatment can include local urticaria,

skin discolouration, the Koebner phenomenon, hardening of the skin (lumps) at the injection site, and minor hematomas [22, 23].

Mesotherapy is a treatment for people with dehydrated, dry, dull and grey skin in need of revitalization. It is used in the treatment of dark circles under the eyes as well as acne, wrinkles, skin discolouration and photoageing. It is worth mentioning that mesotherapy can also improve the condition of the hair and protect against hair loss by repairing and strengthening hair follicles. In addition, after an injection of low-molecular-weight peptides the skin does not need special protection from the sun. Mild inflammation with erythema or edema, or minor hematomas may appear, but these symptoms usually subside within 24 hours [24–26]. Aesthetic medicine procedures are followed and complemented by cosmetic treatments. Cooperation between the physician and cosmetologist can be of great benefit to a patient: first a patient undergoes an invasive treatment administered by a doctor, and then visits a cosmetologist to maintain the results [27]. Many cosmetic procedures performed with the use of formulations containing similar substances make it possible to continue the facial skin therapies initiated by the aesthetic medicine practitioner [28, 29]. Also, it is believed that free radicals are responsible for the ageing process. Under normal circumstances, the human body can self-regulate their levels by means of enzymatic and non-enzymatic reduction processes. Contemporary cosmetology offers patients a range of antioxidants that support the natural processes of skin regeneration and neutralize the harmful effects of free radicals [30].

Discussion

An analysis of the literature on the subject of mesotherapy shows that the studies published to date are based primarily on subjective assessment methods, presenting the results of procedures on the basis of analysing "before and after" medical photographic documentation [11]. Objective verification of the results solely on this basis is extremely difficult, especially since the photographs often differ considerably in terms of lighting or the position of the patient. As a rule, the "after" photographs are taken under more favourable conditions to enhance the outcome of the treatment [31]. Changes occurring in the skin as a result of mesotherapy require further broader research, as well as more objective research methods and tools. It seems necessary on the one hand to increase the number of patients in the study and control groups, and on the other to make the groups more uniform through an appropriate selection of the administered treatments as well as the inclusion and exclusion criteria. Currently there seems to be a shortage of prospective studies, even though such studies could be extremely interesting and informative by showing the specific effects of a specific procedure in a certain patient population over a given period of time [32]. Additionally, there appears to be a shortage of original research in Polish publications. Instead, the authors refer to a small number of foreign studies, where the number of patients also usually tends to be small when compared to research in other fields of medicine [15].

In many scientific papers the authors focus their attention on the specific action of individual substances, which translates into knowledge about the ingredients themselves and their effects on the skin [20, 21, 29, 30]. Recently, however, authors have started to put more emphasis on combination therapies as they provide a better, faster and more synergistic effect. Such therapies involve not only combining different ingredients with specific properties into a cocktail that can be administered in one syringe, but also combining mesotherapy with other techniques, such as tissue fillers, botulinum toxin (mesobotox), thread lift, or physical methods such as laser treatment, carboxytherapy and others [3, 4, 16]. Various treatment combinations can be created to achieve better results. This is intended to make it more beneficial for the patient, but it also means that it is more difficult to unambiguously evaluate the results. It is rare for a patient to have mesotherapy used alone and with only one preparation. More uniform observations have only been made with regard to platelet-rich plasma [11, 17].

It should also be noted that the ability to absorb and utilize nutrients decreases with age, and that nutritional disorders are becoming increasingly common in young people, in which case they are usually related to stress, eating highly processed foods and other environmental factors. This can lead to a quantitative and qualitative imbalance in the intestinal flora as well as the so-called leaky gut syndrome. Research confirms that following a balanced nutrient-rich diet, even supplemented with some ingredients that the body is lacking (such as vitamins C, D3, K2; group B vitamins; collagen precursors and other structural and functional proteins; coenzymes; minerals, etc.), is not always sufficient. Sometimes it is necessary to administer some nutrients locally, for example with an injection, in which case mesotherapy can be invaluable for two main rea-

sons: firstly, because the effect connected with the supplied nutrients is combined with the physical effect associated with the use of a needle, during which tissue damage occurs but at the same time the tissue is stimulated to regenerate; and secondly, because this method enables the bypassing of the gastrointestinal tract and its possible dysfunction [3, 5, 8, 19].

The literature review reveals that this seemingly simple treatment technique is not yet sufficiently researched, despite having been used in aesthetic medicine for half a century.

Final remarks

The primary task of facial aesthetic treatments is to improve a person's appearance in the context of the quality of life, comfort, well-being and self-esteem. Treatment options are extensive, both in terms of the range of biologically active substances that can be applied and the techniques for improving their absorption. Thus they provide many opportunities for improving the condition of mature skin and preventing the signs of ageing. It is essential, however, that aesthetic medicine practitioners, in addition to interpersonal skills, should possess a knowledge of anatomy, physiology, dermatology, stomatology, chemistry, microbiology, allergology and other disciplines, supported by reliable scientific research.

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REVIEW PAPER

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Fatty acid binding proteins (FABPs) – a new laboratory biomarker for kidney diseases

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ABSTRACT

Fatty acid binding proteins (FABPs) are a family of intracellular proteins involved in metabolism of lipids. By regulating the intracellular level of free fatty acids, those proteins indirectly influence intracellular signaling pathways. FABPs also play a role in regulation of cell growth and differentiation, and exert a significant antioxidant effect. Some reports suggest that FABPs are released by various organs (heart, liver, intestines, kidneys) in response to tissue damage. The article briefly summarizes the structure, classification and function of FABPs and discusses the applicability of liver- (L-FABP) and heart-type (H-FABP) FABPs in laboratory diagnosis of selected clinical entities, particularly kidney diseases.

Keywords: fatty acid binding protein (FABP), biomarker, kidney diseases.

Introduction. Classification, structure and function of fatty acid binding proteins

Fatty Acids (FAs) are compounds possessing various important biological functions. First of all, along with phospholipids and cholesterol they build biological membranes. They also form triglycerides (TGs) – esters of glycerol, the main energetic material. Some FAs are also source of eicosanoids – compounds that modulate many physiological functions in auto- and paracrine mechanisms [1, 2]. Fatty acids as exogenous compounds are delivered with a diet mainly in form of TGs. In the gastrointestinal tract, in the presence of bile, triglycerides are emulsified to micelles and subjected to enzymatic degradation by lipase. That results in TGs breakdown into free FAs and glycerol absorbed by enterocytes. In enterocytes those compounds are reassembled into TGs and, when combined with the corresponding apoproteins, released into the lymphatic fluid as one of lipoproteins – chylomicrons.

Finally, chylomicrons reach the systemic circulation and they are subjected to the action of lipoprotein lipase present in the endothelium of blood vessels, muscles, and in the adipose tissue. The action of that enzyme results in release of FAs, that are then absorbed by adipocytes, muscle cells and the liver and stored in those tissues again assembled into TGs. In turn, the TGs lipolysis process, or *de novo* synthesis of new FAs from glucose, provides endogenous FAs that are transported in blood as VLDL lipoproteins, that undergo catabolic changes similar to those of chylomicrons, releasing FAs necessary for various cells functions [1, 3].

In the abovementioned briefly described FAs metabolism, an important role is also played by fatty acid binding proteins (Fatty Acid Binding Proteins; FABPs), described in 1972 by Mishkin et al. [4], as a low molecular weight cytoplasmic agent isolated from the liver of laboratory rats, binding long chain fatty acids (LCFAs). In the same year Ockner et al. [5] also described the protein demonstrating the same properties and confirmed its presence in other tissues.

FAs intracellular influx from plasma takes place by both passive diffusion and an active transport with transmembrane protein carriers. One of the proteins that carry FAs through the cell membrane are FABP associated with plasma membrane – $FABP_{PM}$. Those proteins are low molecular weight translocases, found in different cells characterized by high lipid-turnover: hepatocytes, adipocytes, enterocytes and cardiomyocytes [2].

After entering the cell, FAs are linked to cytosolic FABPs. Those proteins are also low molecular weight (14–15 kDa), widely distributed compounds. They form a family of proteins, classified according to their localizations and are products of specific genes. Moreover, the FABPs family belongs to a super-family of other proteins that also bind fatty acids (e.g. $FABP_{PM}$ mentioned above, fetuin, heat shock protein, S-transferase). The superfamily also includes some less characterized compounds that appear to be structurally related to cytosolic FABPs: cellular retinoid binding proteins (CRBPs), cellular retinoid acid binding proteins (CRABPs), mammary-derived growth inhibitor (MDGI), fibroblast growth regulator (FGR) and gastrotropin binding bile salts and bilirubin in the ileal mucosa. The currently distinguished FABPs and FABPs-related compounds are listed in the **Table 1** [1, 6–8].

All FABPs demonstrate a similar tertiary structure associated with a twisted-barrel hydrophobic core. They are composed of 10 anti-parallel β-strands organized into two β-sheets forming an elliptical β-barrel. Inside the barrel there is a water-filled cavity (the bound ligand place) with polar and hydrophobic amino acids and different amino acids probably determine the volume of the cavity and the binding specificity of various types of FABPs [1, 2, 6]. All FABPs mainly bind both long chain, saturated and unsaturated FAs with no specificity for a particular fatty acid and the binding affinities of FABPs correlate with FAs hydrophobicity. Derivatives of LCFAs – acyl-CoA units are also ligands for FABPs. Moreover, L-FABP and I-FABP bind other hydrophobic agents such as lysophospholipids, phosphatidylserine, prostaglandins E1 and other eicosanoids (lipoxygenase metabolites), bile acids and some drugs [1, 6, 9].

FABPs intracellular concentration is variable and depends on environmental (e.g dietary), physiological and pharmacological conditions. The H-FABP level is higher in women compared to men, increases during pregnancy and lactation and decreases with age. The gender dependency of the FABPs amount is a result of influence of sex hormones – some experimental studies indicated that testosterone decreases, whereas estrogen increases FABPs levels in rats. High fat and high carbohydrate diets increase FABPs in the liver and intestine. A similar effect was observed during chronic alcohol intake, even at low dose [1, 10].

FABPs have several functions, mostly related to transport and metabolism of FAs. As mentioned above, the intracellular uptake of FAs is a protein-mediated phenomenon, based on membrane $FABP_{PM}$, and the process is parallel to a simple transmembrane diffusion. The finding that incubation of hepatocytes with an anti-$FABP_{PM}$ antibody decreased the saturable oleate uptake by 70% provided an evidence for the $FABP_{PM}$ role in FAs uptake, and a similar observation was made for cardiomyocytes, enterocytes and adipocytes [1]. After entering cells, FAs are bound to cytosolic FABPs that mediate the transport to or from various intracel-

Table 1. Cytosolic fatty acid binding proteins (FABPs) and FABPs-related proteins

Name and abbreviation	Previous / alternative names	Gene name	Tissue localization
Liver FABP; L-FABP	Z-protein, heme-binding protein	FABP1	liver, small intestine, kidney, stomach
Intestinal FABP; I-FABP	gut FABP	FABP2	small intestine (proximal), stomach
Heart FABP; H-FABP	Muscle FABP	FABP3	heart, aorta, vascular endothelium, skeletal muscle, brain, mammary gland, kidney, ovaries, testis
Adipocyte FABP; A-FABP	aP2	FABP4	adipose, monocytes
Epidermal FABP; E-FABP	psoriasis-associated FABP (PAFABP), KFABP, skin FABP	FABP5	epidermis, adipose, mammary tissue, testis, lens, retina
Ileal FABP; Il-FABP	gastrotropin	FABP6	small intestine (distal)
Brain FABP; B-FABP		FABP7	central nervous system
Myelin FABP; M-FABP	myelin P2, MP2	FABP8	peripheral nervous system
Testis FABP; T-FABP		FABP9	
Cellular retinol binding protein I; CRBP I			liver, kidney, testis, lung
Cellular retinol binding protein II; CRBP II			small intestine
Cellular retinoic acid binding protein; CRABP			brain, skin, testis, epidermis, adrenal

Note: the indication of the given FABP in the listed tissue is not equivalent to the presence of the protein in all cells of the listed tissue – FABP may be present only in selected cells or in selected developmental periods of the mentioned tissue

lular organelles (e.g. mitochondria, peroxisomes), thus enabling their translocation to enzymes involved in lipid metabolism. FABPs also stimulate activities of those enzymes, that catalyze both synthesis, oxidation and esterification of FAs [1, 6].

In addition to the influence on transport and metabolism of FAs, FABPs also perform other functions associated with modulation of intracellular FAs as important regulators of many cellular metabolic processes. FAs and other lipid derivatives (such as eicosanoids) regulate some processes by affecting membrane ion channels, cellular receptors or genes. Unsaturated FAs are considered to be secondary messengers responsible for signal transduction inside the cell. Experimental studies indicated that FAs inhibit growth factor-induced diacyloglicerol kinase α activation in vascular smooth muscle cells and may contribute to chronic protein kinase C activation observed in diabetes. Moreover, FAs are ligands of nuclear peroxisome proliferator-activated receptors – PPAR-α (expressed in the liver and adipose tissue, mediating FAs catabolism) and of PPAR-γ (present in adipocytes, regulating adipose differentiation and adipogenesis) [1, 6].

Therefore, by regulating the FAs intracellular level, FABPs indirectly control the mentioned above processes influenced by FAs.

Furthermore, FABPs are thought to be factors implicated in regulation of growth and differentiation of other cells besides adipocytes. In experimental studies, bovine mammary-derived growth inhibitor (MDGI) that was later identified as a mixture of both H-FABP and A-FABP, caused inhibition of growth and proliferation of bovine, murine and human mammary epithelial cells. Those findings led to the conclusion that H-FABP should be considered a tumor suppressor.

Another important feature of FABPs is their antioxidant properties. It is hypothesized that FABPs provide protection against toxic effect on cell membranes induced by high concentration of FAs and other lipid derivatives. An undesired accumulation of LCFAs and acyl-CoA is noted in the myocardium during prolonged ischemia, and the disturbed oxidation of those compounds leads to the generation of free oxygen radicals that oxidize membraneous macromolecules. By binding LCFAs, FABPs reduce oxidative stress and they also act as direct scavengers of free radicals [1, 6].

To sum up, in addition to their primary role in transport and metabolism of FAs, FABPs have also some important functions as regulatory compounds of cell growth and differentiation, and exert a significant antioxidant effect reducing the ischemic cell damage.

FABPs as diagnostic marker for tissue damage

Taking into account some features of FABPs (small size, solubility in water and body fluids, tissue specificity), those compounds seem to be attractive candidates for laboratory biomarkers for tissue damage. There are premises to consider H-FABP as a diagnostic tool of a heart failure. Some reports suggest that plasma H-FABP may be considered a marker for the estimation of infarction size because the protein is released into circulation by dying cardiomyocytes in prolonged ischemic conditions [11–13]. H-FABP also seemed to be a more sensitive parameter of myocardial injury compared to myoglobin. In patients undergoing a thrombolytic therapy, plasma peak of H-FABP was observed in about 4 hours after the first symptoms and returns to normal ranges within 24 hours after an infarction. When no thrombolytic treatment was applied, the plasma H-FABP peak was observed after 8 hours and reached normal values in 36 hours after the infarction [14]. On the other hand, however, some studies brought contradictory results, indicating that H-FABP cannot be used as a laboratory marker due to its unsatisfactory specificity and sensitivity compared to currently used parameters [15]. A transient, elevated plasma level of H-FABP was also reported in about half of patients with unstable angina. Some studies suggest that H-FABP is a promising laboratory marker of progressive deterioration of the heart function and of inferior prognosis in patients with congestive heart failure. Due to the fact that H-FABP is also (to a lesser extent) expressed in skeletal muscles, interpretation of recognized heart/muscle damage must be based on the myoglobin to H-FABP ratio assessment to avoid the risk of false positive results (the myoglobin/H-FABP ratio of 2–10 suggests a heart injury, whereas the result of 20–70 suggests injury of skeletal muscles) [14].

Similarly, increased I-FABP plasma level was found in early period of acute phase of intestinal ischemia [14, 16, 17]. However, some studies brought different results [18, 19]. In turn, there are preliminary studies revealing that L-FABP is released into plasma from the liver in diseases of that organ, which makes the protein a candidate for a novel, biochemical marker of diseases of the liver [14, 20]. The most recent attempt of application of FABPs as diagnostic parameters is the estimation of B-FABP and H-FABP as markers of brain injury. In healthy people, B-FABP is not found in the plasma. In mild traumatic brain injury, an increase in serum B-FABP and H-FABP was observed in 68% and 70% of

patients, respectively. The electroconvulsive therapy led to elevated plasma B-FABP levels in 6% while H-FABP in 17% of the patients. Hence, it seems that B-FABP and H-FABP may be used for laboratory evaluation of brain trauma and it is expected that those proteins will widen the laboratory diagnostic capabilities, currently based primarily on the S100B protein, neuron-specific enolase (NSE) or the myelin basic protein [14].

FABPs as another "troponin-like" biomarker of kidney diseases

Plasma and/or urinary H-FABP and L-FABP estimation is also considered in the context of diagnostics of kidney diseases. In the early 1990's Maatman et al. [21] demonstrated presence of two types of FABPs in the kidneys, primarily labelled as A and B, that at further stages of experiments were identified as L-FABPs and H-FABPs, respectively. Moreover, the same researchers confirmed the presence of both FABPs isoforms in their rat kidney experimental studies [22]. There is some specificity regarding the location of individual FABPs isoforms in the kidney – H-FABPs are expressed mostly in distal tubular cells whereas L-FABP is found in proximal ones [21, 22].

Some of the known pathophysiological factors responsible for kidney damage are external toxic factors, ischemia and endogenous noxious factors. Among endogenous toxic factors, proteins (such as the complement system, LDL lipoproteins or FAs that are transported with blood in association with lipoproteins and albumin), filtered in the glomerulus, should be mentioned. FAs are then re-absorbed into proximal tubules and subjected to FABP-mediated pathways [23]. When FAs are overloaded in the glomerulus and tubules, those compounds initiate both cellular inflammatory reactions and inflammatory mediators overproduction contributing to tubulointestinal damage. The developing inflammatory response also leads to up-regulation of FABPs in kidney tubules and their final damage with release of those proteins into urine and circulating blood [24–27]. FABPs found in kidneys play the same roles as those already mentioned in the general description of FABPs. Taking the pathophysiology of kidney damage into account, oxidative stress plays an important role in initiation and maintenance of the inflammation. FAs, especially unsaturated LCFAs, are also a direct source of reactive oxygen species. FABPs are antioxidant compounds, so they are overproduced in the renal tubules as part of a compensatory anti-inflammatory response, and that explains their increased levels observed during kidney damage. Moreover, FABPs are also responsible for the maintenance of low FAs inside tubular cells by carrying them into the mitochondria and peroxisomes for accelerated metabolic breakdown. Therefore, FABPs play a strong cytoprotective role in the development of kidney injury [23].

One of the most important clinical entities that may be diagnosed based on the analysis of plasma or urinary concentration of FABPs is acute kidney injury (AKI).

AKI is defined as a rapid deterioration of kidney function resulting in retention of nitrogenous waste (compounds that would normally be excreted with urine, such as urea and creatinine) [28]. The disorder is characterized by a wide spectrum of symptoms – from transient elevation of biochemical indicators of kidney damage (e.g. creatinine) with moderately intensified symptoms usually resulting from oliguria, to total anuria, severe overhydration and electrolyte and metabolic disturbances taking the form of acute renal failure requiring the renal replacement therapy.

The detailed diagnostic criteria for AKI are based on the Acute Dialysis Quality Initiative Guidelines (ADQI) Group (2004) [29], Acute Kidney Injury Network (2007) [30], and recently published Kidney Disease Improving Global Guidelines (KDIGO) Clinical Practice Guidelines for Acute Kidney Injury (2012) [31]. These guidelines are based on the assessment of elevated plasma creatinine level, diminished diuresis per hour and the estimated decrease in glomerular filtration rate (eGFR).

Pathophysiologically, AKI is a consequence of impairment of kidneys function due to noxious, toxic agents affecting the kidney, or develops as a result of some co-existing pre- or post-renal disturbances. Pre-renal etiological AKI factors, such as hypovolemia, generalized vasodilatation with hypotension, or rapid reduction in cardiac output, finally lead to reduction of renal blood flow and consequently to decrease of the effective glomerular filtration pressure. Similarly, post-renal factors cause an increase in hydrostatic pressure in the urinary tract that exceeds the glomerular pressure. The mechanism also contributes to decrease of the effective filtering pressure and to development of oliguria/anuria. The final pathophysiological consequence, independently of the nature of the etiologic AKI factor, is the development of Acute Tubular Necrosis (ATN). The condition is defined as an irreversible, morphological destruction of renal tubules manifesting in kidney failure [28].

As mentioned above, progressive damage of kidney tubules is responsible for the excessive FABPs

release, as well as for an excessive synthesis and disturbed renal excretion of the other proteins that may be considered as laboratory markers of kidney damage. According to current reports [32–35], cystatin C, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin-1 (NGAL-1), interleukine-18, N-acetyl-β-glucosaminidase (NAG) and FABPs should be mostly listed among the novel AKI biomarkers ("renal troponins"), for they are characterized by a better sensitivity and specificity in the early diagnosis of AKI during its asymptomatic period, compared to the currently used ones (e.g. creatinine, urea, electrolytes). The clinical entities associated with AKI development, in which the FABPs increase was observed are listed in the **Table 2**.

FABPs are also considered in the context of their suitability for the laboratory diagnosis of chronic kidney disease (CKD) – a clinical entity ultimately leading to chronic renal failure (CRF). CKD may be a consequence of all diseases that primarily affect kidneys (e.g. chronic glomerulonephritis), as well as of currently commonly occurring civilization diseases: diabetes and hypertension. Moreover, in some cases, CKD develops as a result of AKI [36]. According to current guidelines, chronic kidney disease is a multi-symptomatic syndrome resulting from permanent and progressive damage of the kidney structure and abnormalities of all of the kidney functions (excretory, endocrine, metabolic), and conditioned by a variety of pathological processes in the glomeruli and tubulointerstitial compartment [36]. The general CKD definition was established by a team of experts in the *Kidney Disease Outcome Quality Initiative (KDOQI)* in 2002 [37], and then confirmed by the *Kidney Disease Improving Global Outcome (KDIGO)* [38]. CKD is defined as a kidney disease that lasts for at least 3 months and associated with signs of kidney damage (such as albuminuria at least 30 mg/24h, electrolyte disturbances, pathological findings in kidney histological assessment, abnormalities revealed in imaging studies), or a GFR decrease to < 60 ml/min./1.73 m^2, with or without the abovementioned disturbances, and with a negative effect on health. Depending on the eGFR value, the degree of progression of CKD is gradated (grades G1-G5), with uremia as a CKD descent recognized in G5 grade [39].

Current data demonstrate that about 4 million people in Poland suffer from CKD, but it appears that the disease is much more common than previously thought, being unrecognized for long periods of time due to its extensive latent course in grades G1 and G2 [36]. Hence, the thorough and accurate diagnosis of renal dysfunction plays an important role, because only a careful monitoring of the patient allows efficient diagnosis of early, clinically asymptomatic CKD. However, despite the fact that measurement of serum creatinine concentration remains at present the simplest and most commonly used method for the assessment of renal function, there is an ongoing search for new biomarkers of CKD, just as it is in case of AKI. Those parameters should be characterized by superior laboratory features (sensitivity, specificity, dynamic change already at an early stage of the estimated disorder) compared to creatinine used currently.

Cystatin C, markers of fibrosis and Klotho-FGF23 axis are most likely reported to be novel biomarkers for CKD. [40–43]. Noticeably, some biomarkers (NGAL, KIM-1, NAG and FABPs) may be used for both diagnosis and monitoring of AKI and CKD [44, 45]. Therefore it can be concluded that FABPs may be useful in recognition of various kidney diseases, including both acute and chronic kidney damage. The detailed information is provided in the **Table 2**. The table also lists other

Table 2. The clinical entities associated with increased plasma / urinary FABPs levels as a potential diagnostic value

Kidney or urinary tract disease	A closer description of the disease	Reference number
Acute kidney injury (AKI)	kidney transplantation	46
	cardiac surgery	47, 48
	contrast induced	49, 50
	cis-platin induced	51
	sepsis	52
	AKI of various etiology	53
Chronic kidney disease (CKD)	non-diabetic CKD	54, 55
	diabetic nephropathy	56, 57
Glomerulonephritis	newly diagnosed, biopsy-proven primary chronic glomerulonephritis	58
Unilateral ureteral obstruction	unilateral ureteral obstruction experimental model in mice	59
Vesicoureteral reflux	children with primary vesicoureteral reflux confirmed by voiding cystourethrography	60
Renal toxicology	cyclooxygenase-inhibitor-induced renal injury	61

nephrological and urological disorders associated with increased FABPs levels, therefore it is considered as a marker of those clinical entities.

Conclusions

To sum up, FABPs appear to be proteins that meet the requirements for biomarkers of kidney damage in the course of various diseases of the organ. It is expected that the new panel of proteins, including L-FABP and H-FABP (currently the most commonly used from FABPs family), NGAL-1, KIM-1 or interleukin-18 will be soon introduced to common practice in the laboratory diagnosis of kidney diseases.

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The molecular basis of non-syndromic orofacial clefts and tooth agenesis

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ABSTRACT

Non-syndromic orofacial clefts and tooth agenesis are two of the most common craniofacial birth defects. Both of them have a complex etiology, with genetic and environmental factors involved. Additionally, the epigenetic modifications have been implicated in the pathogenesis of these structural malformations. Despite an increasing number of research studies, using a variety of methodological approaches, the role of genetic factors in the etiology of orofacial clefts and tooth agenesis is still not well elucidated. The most consistent findings across studies concerning the genetic factors influencing the risk to orofacial clefts include the association of polymorphic variants of the *IRF6* gene and the chromosomal locus 8q24.21. The major candidate gene for tooth agenesis in the European populations is *WNT10A*; its pathogenic mutations are present in more than 50% of patients with this dental anomaly. It has been found that both orofacial clefts and tooth agenesis, which co-occurrence is often reported, may share common candidate genes.

Keywords: orofacial clefts, tooth agenesis, etiology, candidate genes.

Introduction

Non-syndromic orofacial clefts (OFC) and tooth agenesis (TA) are two of the most common craniofacial birth defects [1, 2]. OFC affect 1 per 700 live births in the global population [1]. According to the *Polish Registry of Congenital Malformations, the prevalence of orofacial clefts in Poland ranges from 1/500 to 1/1000 births* [www.rejestrwad.pl]. OFC are divided into two main forms: non-syndromic cleft lip with or without cleft palate and cleft palate only [3]. The incidence of TA, excluding the lack of the third molars, varies from 1.6 to 9.6% depending on ethnic background [2]. TA can be classified based on the number of missing teeth into hypodontia (the lack of one to five teeth), oligodontia (the lack of 6 or more teeth) and anodontia (the complete absence of teeth). In this classification, the third molars are not taken into account since their absence is highly prevalent [2]. The co-occurrence of OFC and TA is often reported [4, 5]. Patients with OFC have an increased risk of dental anomalies, including alteration in tooth number, size, shape, a timing of formation and eruption comparing to the general population [6]. It has been shown that dental anomalies appear primarily in the cleft area and their prevalence is higher in left-sided OFC [7]. Additionally, in patients with OFC the agenesis of teeth *outside* the *cleft area have* also been reported to be more frequent [8]. This observation may indicate that the same molecular mechanisms may be shared in the development of the teeth, palate, and lip [8].

The etiology of non-syndromic OFC and TA is complex with genetic and environmental components [3, 9]. Additionally, the epigenetic modifications have been implicated in the pathogenesis of these structural malformations [10]. Genetic studies using a variety of research approaches, including linkage studies, candidate gene analyses, and genome-wide association studies, have identified a number of genes and chromosomal regions underlying these craniofacial anomalies [9, 11]. However, nucleotide variants of identified

candidate genes and chromosomal loci can still explain only a fraction of the predicted heritability. It has been demonstrated that both OFC and TA have a number of common candidate genes, which nucleotide variants can influence their risk [4, 5].

Across OFC' studies conducted in various populations, including the Polish population, the most consistent results were observed for nucleotide variants located in the *IRF6* gene (OMIM *607199) and the chromosomal region 8q24.21 [12–14]. The *IRF6* gene encodes a transcription factor, which is involved in the regulation of the keratinocyte proliferation-differentiation switch and formation of oral periderm [15]. It is worth noting that in a study conducted in the Latvian population, the *IRF6* variant (rs642961) located in the promoter region was found to be more frequent in individuals presenting OFC associated with tooth agenesis when compared to healthy individuals [16]. Moreover, Vieira *et al.* have demonstrated that this functional variant, disrupting an AP-2α binding site in the *IRF6* enhancer, is associated with the risk of isolated TA [12, 17, 18]. The 8q24.21 risk locus, identified by the first genome-wide association study conducted for OFC and further confirmed by a number of post-GWAS replication studies, is a gene-poor region devoid of protein-coding genes [19, 20]. Studies using mice as model organisms have demonstrated that this chromosomal locus contains very distant *cis*-acting enhancers controlling the expression of the *Myc* gene during craniofacial development [21]. Mice homozygous for the deletion including this medionasal enhancer region show mild alterations in the face morphology and *occasionally* cleft lip and palate [21]. Within the 8q24.21 chromosomal region, which nowadays is considered as a key susceptibility locus for non-syndromic OFC, the top marker associated with the risk of this anomaly is rs987525 [19]. A significant association between this intragenic variant and the co-occurrence of OFC and TA outside the cleft region was also observed [22].

The major candidate genes for non-syndromic TA include *WNT10A* (OMIM *606268), *MSX1* (OMIM *142983), *PAX9* (OMIM *167416), *AXIN2* (OMIM *604025), *EDA* (OMIM *300451) and *EDAR* (OMIM *604095). Van den Boogaard *et al.* [23] have demonstrated that the *WNT10A* mutations are present in more than 50% isolated TA cases. Pathogenic mutations within the coding region of *WNT10A* have also been identified in 62% of tooth agenesis patients from the Polish population [24]. The *WNT10A* gene is a member of the Wnt family, which consists of genes encoding secreted signaling proteins involved in a number of developmental processes during embryogenesis [25]. Interestingly, the missense mutation of the *WNT10A* gene has been associated with the increased risk of non-syndromic OFC in the Chinese population [26]. Moreover, nucleotide variants in *WNT3* (OMIM *165330), *WNT3A* (OMIM *606359), *WNT5A* (OMIM *164975), *WNT9A* (OMIM *602863), and *WNT11* (OMIM *603699) have been found to be significantly associated with non-syndromic orofacial clefts in various populations [27, 28]. Similarly, polymorphisms and mutations in the *MSX1* gene are known factors increasing the risk of non-syndromic OFC [29]. In addition, *MSX1* and two other major TA candidate genes, *PAX9* and *AXIN2*, have been associated with the co-occurrence of cleft anomalies and TA [5]. The *MSX1* and *PAX9* genes encode transcription factors that play an essential role during embryogenesis [9]. It has been demonstrated that these genes are co-expressed during craniofacial development, and the genetic interactions between their protein products are involved in the regulation of the lip formation and tooth morphogenesis [30]. *Msx1* and *Pax9* deficient mice lack all teeth, which development is arrested at the bud stage, and exhibit a number of craniofacial defects, including cleft palate [31, 32]. The *AXIN2* gene encodes a protein which is a negative regulator of the Wnt-signalling pathway [33].

Besides the genes described above, there are a number of other candidate genes and chromosomal loci underlying the co-occurrence of OFC and TA. The systemic review conducted by Phan *et al.* [5] revealed that they include among others the TGF pathway genes and the cancer predisposing gene *CDH1* (OMIM *192090).

In summary, OFC and TA are one of the most common craniofacial anomalies that share a number of common candidate genes. There is growing evidence suggesting that tooth agenesis should be considered as an extended phenotype for oral clefts [34].

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The importance of epigenome research in the diagnosis and treatment of endometriosis

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ABSTRACT

The causes of endometriosis remain unexplained. Studying the molecular mechanisms at the origin of the lesions leads to conclusions about the important role of the epigenome. This mini-review is a summary of the current state of knowledge about the processes of epigenetic control of gene expression involved in the pathogenesis of endometriosis.

Keywords: endometriosis, gene expression, methylation.

Endometriosis, defined as the presence of endometrial cells outside the uterine cavity (ectopic endometrium), affects up to 10% of women of reproductive age and is one of the most common causes of infertility, and gynecological hospital ward admission and one of the main causes of hysterectomy [1]. In endometriosis, the ectopic endometrium occurring intraperitoneally and extraperitoneally is sensitive to hormonal changes characteristic of the sexual cycle and maintains its secretory activity, resulting in a chronic inflammatory reaction. This process leads to internal bleeding, the development of painful nodules, inflammation, scar formation and adhesions and anatomical changes in the pelvic [2]. Endometriosis is most frequently found in women suffering from pelvic pain and/or infertility. Due to its non-specific symptoms, early diagnosis of endometriosis is difficult, and often incidental. Laparoscopic examination is standard in the diagnosis and treatment of endometriosis [3]. The etiopathogenesis of endometriosis is also not fully explained; however amongresearchers, the most popular hypothesis concerns retrograde transport of menstrual blood through the fallopian tubes and into the peritoneal cavity [4]. However, hereditary, environmental, autoimmune, allergic and epigenetic factors are a key influence in the implantation of endometrial cells, and the ectopic formation, metastasis and recurrence of the disease [5]. Among the mentioned factors, epigenetic ones are the subject of intense research aimed at elucidating the etiopathogenesis of the disease.

Epigenetics can be defined as a branch of science investigating inherited traits introduced into the genome through methylation of the nucleotides in the DNA sequence, histone modification, and interaction between microRNA (miRNA). Such modifications alter the expression of genes without interfering in the nucleotide sequences in the DNA strand. Characteristics included within the study of epigenome affected prenatal development, hormonal factors, age, gender, and environmental factors such as diet, exposure to chemical and physical factors. The etiology of many diseases correlates with specific patterns of the epigenome, which are manifested in disorders of gene expression, and thus in impaired function of cells and tissues [6, 7].

The most widely investigated element of epigenetic gene modifications is methylation of the cytosine residues of cytosine-guanine dinucleotides (CpG). CpG

dinucleotide present throughout the genome once per 80 base pairs. However, in some areas of the genome specifically related to gene promoter sequences, CpG appear sequentially in lengths of approx. 200 base pairs. These places are called CpG islands. The entire genome outside the CpG island cytosines in dinucleotides is constitutively methylated. However, CpG islands located in the promoter site of a gene are generally not the targets of methylation. In this way, they are accessible to transcription factors, initiating the process of gene expression [9].

Advances in our understanding of the methylation patterns of the genes in endometriosis occurred after the publication of the results of two projects focusing on a genome – wide DNA methylation analysis. Working independently of each other and using slightly different strategies, these authors reached similar results. Their results are consistent in terms of different methylations of promoter regions of the 21 genes in the ectopic endometrium compared to the eutopic endometrium[10, 11]. Both studies confirmed observed also in our studies, gene promoter hypermethylation of *HOXA10* and *HOXA11*, and as a result – reduced expression of these genes in the ectopic or eutopic endometrium [12]. Among genes with different methylations of CpG islands in the ectopic endometrium, which appears to be unquestionably associated with endometriosis, are genes encoding steroid hormone receptors: i.e. the *NR5A1* gene encoding transcription factor SF-1, which is responsible for the expression of genes encoding enzymes of steroidogenesis pathway; the *CYP-19* gene encoding the aromatase; and *COX-2* encoding cyclooxygenase-2, a key enzyme in the conversion of arachidonic acid to prostaglandins and one which triggers an inflammatory response.

The process of CpG methylation involves DNA methyltransferase (DNMT), while demethylation occurs with the participation of other enzymes such as TETs, AID and GAAD45. Also, the expression of these enzymes appears to have a different effect on the DNA methylation pattern in the ectopic endometrium [13].

Another mode of epigenetic gene regulation involved in the etiopathogenesis of the endometriosis is histone code post-translational modification. Histone proteins are responsible for chromatin organization. There are 130 known variants of the post-translational modification of histone proteins, of which the most important seem to be: trimethylation of the lysine 4 of histone H3 (H3K4-ME3); and acetylation of histones H3 and H4 (H3 / H4Ac). Both of these changes lead to a loosening of the chromatin structure and consequently, to the initiation of transcription. On the other hand, methylation of the lysine 27 on histone H3 leads to chromatin condensation and silencing of the gene expression associated with modified histones [14, 15]. Compared to the eutopic endometrium, which is dependent on histone deacetylase (HDAC), research into post-translational modification of the histone code in the ectopic endometrium indicates a lower level of the acetylation of histones H3 / H4 covered with promoter regions of *p16, p21, Bcl2, BclX* genes critical for apoptosis [16]. While the results of other studies into the expression and activity of HDAC compounds with endometriosis are still contradictory, the existence of differences in the histone code seems to be of indisputable importance in the etiology of endometriosis [17].

Further important regulatory molecules able to modify gene expression are miRNAs. They are single-stranded, non-coding, short (21–23 nucleotide) RNA sequences. By pairing with a homologous sequence of the mRNA, microRNAs can silence geneits expression [18]. Specific miRNA also plays a role in transcriptional gene silencing by inducing structural changes in a complementary locus of the chromatin [19]. A detailed review of published results of research into miRNA expression in endometriosis was published by Borghese *et al*. (2016) [20]. Similarly to the previously discussed mechanisms, miRNA acts in a multi-dimensional manner, also by modification of the expression of genes responsible for other epigenetic mechanisms: DNA methylation and histone modification [21, 22].

The mechanisms for the epigenetic control of gene expression associated with endometriosis presented in this review require further research to answer many questions. There is still no clear answer as to whether the many instances of methylation observed in the ectopic endometrium are an effect or a cause of endometriosis. Another problem is the patchy research methodology, which often precludes a comparison of any obtained results. Despite these difficulties, the development of our understanding of the functioning of the epigenome allows us to be optimistic about the prospects for the diagnosis and treatment of endometriosis. While the repair of the genetic code is still a matter for the distant future, epigenome modifications are still possible. There have been successful results from therapy with DNMT inhibitors [23]. Promising results have also been provided by HADAC inhibitors [24]. However, the greatest hope is offered by the therapeutic use of miRNAs [25]. The obtained results probably can be used to develop and introduce new patterns of diagnosis and treatment for endometriosis.

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The authors declare no conflict of interest.

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Evaluation of efficacy and mechanisms of action of Cannabis sativa extracts with analgesic, anti-inflammatory and antiemetic properties in an *in vivo* model

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ABSTRACT

University of Medical Sciences participates in the realization of the project titled: „Development of the technology of producing cannabinoids from low THC hemp for use as preparations supporting treatment in oncological patients" awarded by the National Centre for Research and Development under project number: INNOMED/I/11/NCBR/2014. The duration of the grant is 36 months, and the total value of the grant is 28011845 PLN. The project is run by University of Life Sciences in Poznan.
Laboratory of Experimental Pharmacogenetics at the Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences (PUMS) is realizing the task number 4 titled "Evaluation of efficacy and mechanisms of action of Cannabis sativa extracts with analgesic, anti-inflammatory and antiemetic properties in an in vivo model." The aim of this project is the development of cannabinoid extract with reduced psychoactive component (THC), which due to its high content of cannabidiol (CBD) is meant to provide analgesic properties, and at the same time to reduce the risk of addiction and overdose. University of Medical Sciences is evaluating the analgesic, anti-inflammatory and antiemetic properties of the extract of *Cannabis sativa* in animal models coupled with neuropathic pain. Pharmacodynamic effects of plant extracts will be later assessed taking into account the level of selected genes and proteins expression..

Keywords: pharmacogenomics, gene expression level, neuropathic pain, cannabinoid.

General information

Research task No. 4 entitled "Evaluation of efficacy and mechanisms of action of Cannabis sativa extracts with analgesic, anti-inflammatory and antiemetic properties in an in vivo model" is realized within the framework of the research project entitled "Development of the technology of producing cannabinoids from low THC hemp for use as preparations supporting treatment in oncological patients" awarded by the National Centre for Research and Development under project number: INNOMED/I/11/NCBR/2014. The duration of the grant is 36 months, from 2014-09-01 to 2017-08-31 and the total value of the grant is 28,011,845 PLN, the total cost of the task No 4 is 880,000 PLN. The project is run by University of Life Sciences in Poznan with the head of the project, prof. Ryszard Słomski. The project titled: „Development of the technology of producing cannabinoids from low THC hemp for use as preparations supporting treatment in oncological patients" will be based on cooperation between partners that have established long term cooperation i.e. Poznan University of Life Sciences (PULS), Department of Biochem-

istry and Biotechnology, Institute of Natural Fibres and Medicinal Plants (INF&MP), Poznan University of Medical Sciences (Poznań Laboratory of Experimental Pharmacogenetics, Department of Clinical Pharmacy and Biopharmacy) (PUMS), Institute of Human Genetics PAS (IHG), Laboratory of Molecular Genetics (LMG), and PozLab Ltd.

Establishing of the consortium composed of the above mentioned organizations aims at timely completion of the project tasks and reaching the implementation stage for a standardized hemp extract prepared for pre-clinical trials according to the registration procedures for a medical product. The main project team realized task No 4 consists of: head of the task No 4 dr hab. Agnieszka Bienert and co-investigators: prof. Przemysław Mikołajczak, prof. Edmund Grześkowiak and dr Joanna Bartkowiak-Wieczorek. Local Ethics Committee of the Use of Laboratory Animals in Poznań permission number is nr 42/2015, 3/2017 and 66/2017.

Research Project Objectives

Cancer is a growing health and economical problem of the global population. In Poland there are about 200,000 people needing treatment for cancer pain, and every year comes around 65,000 patients more. 30–50% of patients in the early and 70–90% in the advanced stage of the disease suffer from cancer pain, 42% suffer from poor control of the aliment; 23% experiencing moderate or severe pain does not get any pain treatment, 31% of patients suffer from this pain for more than one year and 64% of patients experience side effects of analgesic drugs [1]. Neurophatic pain (NP) occurs in as many as 90% of patients during cancer. The International Association for the Study of Pain defines neurophatic pain as pain initiated or caused by a primary lesion or dysfunction in the nervous system, thus NP may be caused by any disease or injury to the nervous system [2]. The basis of treatment of cancer pain remains pharmacological treatment. Recent clinical trials have demonstrated the current role of cannabinoids in refractory chronic and cancer pain [3]. Cannabinoids may be a useful addition to current analgesic treatments [4]. The project is in full accordance with the INNOMED program as it focuses on innovative production of a preparation for medical use, including cancer patients. It also meets the needs of society in this respect.

The main objective of this project is preparation of the cannabinoid extract with decreased content of the psychoactive ingredient (THC), having at the same time high levels of cannabidiol (CBD). Such extract would have analgesic characteristics, but simultaneously the risk of addiction and overdosage would be significantly decreased. Products available so far on the market, including both the cannabis extracts and synthetic medicines, display high content of THC, since they are dedicated to mitigate symptoms other than neuropathic pain – therefore the presence of psychoactive compound is desired [5]. As a result of innovative approach suggested in this project, cannabinoid extract with low concentration of THC could be applied in the treatment of cancer patients.

A major contribution to science will be brought by the study of expression of genes involved in the metabolism of cannabinoids in in vivo model. The aim of the research task No 4 is to assess an analgesic and anti-inflammatory activity of 2 varieties of *Cannabis sativa* with a low content of THC in animal models of pain. After detailed phytochemical analysis, pharmacodynamic activities of plant extracts, the gene (mRNA) and protein levels of the CB1, CB2, and the NMDAR2B receptors in hippocampus and frontal cortex as well as the Nf-kB, TNFα, COX1, COX2 in the brain, and peripheral blood lymphocytes, liver of rats will be examined. On the basis of these complex studies both the mechanisms of action of the extracts as well as their eventual use as the analgesic or antiemetic products will be proposed.

Additionally, bioavailability of cannabidiols from the examined extracts will be assessed in an animal model.

Research plan

There are some evidence provided both from animal and clinical studies on the efficacy of analgesic effect of different cannabinoid compounds, both hallucinogenic (delta9-tetrahydrocannabinol – THC) and non-hallucinogenic (cannabidiol, delta9- tetrahydrocannabivarin, beta-caryophyllene) [6]. In the project, some aspects of the analgesic and anti-inflammatory effects of *Canabis sativa* extracts containing non-psychotropic plant-derived cannabinoids seem to be interesting in context of discovery and development of drug for the treatment of neuropathic pain. At first the acute toxicity after oral administration of the extracts (according to OECD directions) will be performed with starting dose of the extract 2 g / kg b.w. intragastrically (i.g.) in mice. Moreover a conventional 28-day repeat dose toxicity test will be also assessed in mice

Animals will receive extracts with different concentrations of cannabidiol after induction of neuropathic pain by vincristine and acute inflammation by carra-

geenan. Next, the tail flick test and von Fray test will be performed in order to assess both typical central-mediated analgesic activity and peripheral response. The biological material derived from the animals will be examined in molecular and biochemical tests. This step of study will be focused on both, the relative CB1, CB2, and the NMDAR2B receptors mRNA and protein level changes analyses in hippocampus and frontal cortex as well as the Nf-kB, TNFα, COX1, COX2 mRNA and protein level changes analyses in peripheral blood lymphocytes of rats. The obtained results will give the answer which of the active compounds dominates in extracts and whether their concentration is coupled with the expression of above mentioned genes and proteins levels with interrelationship to pharmacodynamic activities.

Basic Concept

Clinical studies largely affirm that neuropathic pain patients derive benefits from cannabinoids treatment. Cannabinoids exert their antinociceptive effects by complex mechanisms involving also effects on the central nervous system. This is consistent with the anatomical location of CB1 receptors in areas relevant to pain in the brain. There is recent evidence implicating CB2 receptors in the antihyperalgesic activity of cannabinoids in models of acute and chronic neuropathic pain, especially of inflammatory origin. Moreover, there is considerable evidence that activation of NMDAR contributes to the mechanism of pathological pain, especially NR2B-containing NMDA receptor is one of the best potential targets for neuropathic pain. Also, it has been proved that inflammation process plays a crucial role in pain induction by activation of selected factors such as TNF, Nf-kB, COX1, COX2. Taking into account the neuropatic and inflammatory background of the pain the evaluation of analgesic, anti-inflammatory and antiemetic properties of Cannabis sativa extract in in vivo model will be analyzed. In addition, CB1, CB2, NMDAR2B, Nf-kB, TNFα, COX1, COX2 genes expression and proteins levels in hippocampus, frontal cortex, lymphocytes and liver of rats will be studied. On the basis of these studies the mechanisms of action and eventual use of the extracts as an analgesic or anti-inflammatory product will be proposed.

Research Methodology

Male Wistar rats (200–220 g) will be housed five per cage at constant temperature (22 ± 2°C), with a 12:12 h light/dark cycle, and free access to food and water at all times for at least a week before starting of the study. The experiments will be carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals. Acute inflammation will be induced by i.pl. injection of 0.1 ml carrageenan (1% w/v in saline) into the right paw. Extracts' doses calculated in relation to their contents of cannabidiol (5, 7.5, 10, 20, and 40 mg/kg, p.o.) or an appropriate volume of vehicle, will be administered orally after the induction of acute inflammation: 2 h after carrageenan on the 1st day and then on the 2nd and 3rd days at the same time as the first injection. Control animals will receive an i.p. injection of saline (0.1 ml) and oral doses of drug vehicle.

Neuropathic pain: To induce neuropathic pain, the animals will be administered for 5 days with anintraperitoneally (i.p.) dose of vincristine of 0.1 mg/kg m.c, followed by two days with saline by the same route in the corresponding volume (Aley et al. 1996, Bhalla et al. 2015). This cycle will be repeated twice. After induction of neuropathic pain, it will be treated over 5 days with the substances (examined extracts or standard substances).

Carrageenan-induced edema: The paw volume will be measured with a plethysmometer (Ugo Basile, Varese, Italy). On the 1st day, the volume will be measured directly before the injection of carrageenan or saline and then after 3, 5, 6, and 7 h. On the next 2 days, paw volumes will be recorded just before drug or vehicle injection, and on the last day (the 4th after carrageenan) just before euthanasia. Data will be expressed as changes of edema (difference in volume between the right and left paws).

Tail flick test

The analgesic effect of the substances (extracts and standard drugs) will be assessed by the tail-flick test using the apparatus Ugo Basile Tail Flick Test Apparatus. In this method the light beam will be directed to the rat's tail 2 cm of its end. The animals will be immobilized at a special cage with a tail protruding outside the cage and placed on a plastic pad. The time since the beginning of light stimulus until the tail withdrawal from the place of exposition on the light will be measured (latency or reaction time in seconds). In all groups immediately prior to the measurement substance administration time t = 0 will be determined. Then measurements will be obtained at 1, 2, 3, and 6 hours after administration of the substance. The maximal time of a tale light exposure after drug administration was established as 60 seconds.

Von Fray test

The test is used to evaluate sensory sensitivity. The test assesses a time of pain response to peripheral mechanical stimuli. The animal will be placed in a cage made of a transparent plastic material, wherein the substrate is a wire mesh having a mesh size of 0.5 cm. To adapt to conditions in the unit the animals will be placed in it three minutes before the measurement. At this time, the measurement will be performed (3 to 6) with a gradual increase in the force of the metal filament having a diameter of 0.5 mm at the plantar rear right paw of rat. The increase in force filament will be from 0 to 50 grams per 10 seconds. Endpoint measurement will be a paw withdrawal by the animal. In each group, six measurements will be made for (all of which arithmetic mean) in each rat – immediately before administration of the substance (time t = 0) and 1, 2, 3, and 6 hours after administration.

Antiemetic activity (Wang et al. 2005, Tatsushima et al. 2011, Shi 2014)

It is known that kaolin intake is a good preclinical screen for drugs that are antiemetic, therefore its consumption will be used as an indicator of pro- and anti-emetic activity (by Wang et al. 2005, Shi 2014). The mesh container of 30 g kaolin pellets will be placed on the wire mesh floor of the cage, along with 70 g normal feed for 3 days before the experiment, and the animals will allow to adapt to the presence of both containers. To measure the kaolin consumption during a 24-h period, the remaining kaolin in the container and kaolin spilled in the cage will be collected, dried, and weighed at 10:00 h every day. The amount of normal feed and water intake will be measured in the same manner as for kaolin intake. Fresh kaolin, water and normal feed pellets will be placed in these containers every day.. The rats with kaolin intake <1.0 g/day on the last of 3 days adaptation will be used for substance tests. The animals will be injected i.p. with vehicle (saline) or cisplatin (3 mg/kg) 60 min before the placement of new pellets and kaolin after adaptation (day 0). Ondansetron (reference substance, 2 mg/kg, i.p.) will be administered 1 h before (on day 0, 1 and 2 – three times in total) before administration of cisplatin. Extract in doses calculated in relation to the contents of cannabidiol (2.5, 5, 7.5, 10, 20, and 40 mg/kg, p.o.) or an appropriate volume of vehicle, will be administered acutely 1 h before cisplatin injection. The studies will be carried out only using the extract of Cannabis obtained from one set and one part of the plant – the most effective in studies of analgesic activity.

Biochemical studies: Four days after carrageenan injection, animals will be decapitated, brain (hippocamp, frontal cortex), peripheral blood, liver tissues will be collected, rapidly frozen in liquid nitrogen and stored at -80°C.

Locomotor activity

Locomotor activity will be evaluated using a Ugo-Basile apparatus (Varese, Italy) by placing animals in the centre of the apparatus and recording their horizontal and vertical activity (Mikolajczak et al., 2002). The data obtained will be expressed as signals corresponding to spontaneous movements for 5 minutes after previous 20 minutes habituation to the activity meter cage. Locomotor activity will be measured: 60 minutes after a single dose of the extract. Control groups will be treated according to the appropriate treatment schedule.

Motor coordination

The effect of extracts on motor impairment will be quantified with chimney test. In this test rats will able to climb backward up to the plastic tube. Motor impairment will be indicated by the inability of animals to climb backwards up the tube within 60 sec (Borowicz et al. 2002).

Molecular investigations on CB1, CB2, NMDAR2B, COX1, COX2, TNF α, and Nf-kB mRNA and protein level changes in the isolated biological material RNA.

Total RNA isolation from the rats' hind paw, brain (hippocamp, frontal cortex) and blood lymphocytes will be carried out using TriPure Isolation Reagent (Roche) according to manufacturer's protocol. The 1–2 µg of total RNA from all samples will be used for the reverse transcribed into cDNA using SuperScript First-Strand Synthesis System (Roche) according to manufacturer's protocol. The CB1, CB2, and the NMDAR2B receptors genes in hippocampus and frontal cortex as well as the Nf-kB, TNFα, COX1, COX2 mRNA levels in the paw and peripheral blood lymphocytes of rats will be analyzed by quantitative real-time PCR (qPCR) reaction using a LightCycler TM Instrument (Roche, Germany) and a LightCycler Fast Start DNA Master SYBR Green I kit (Roche Applied Science, Germany) according to the instructions of the manufacturer. An GAPDH and/or PBGD gene will be used as a housekeeping gene (endogenous internal standard) for normalizationof qPCR reaction.

Changes of proteins levels (Nf-kB, TNFα, COX1, COX2, NMDAR2B, CB1 and CB2) in a rat samples will be determined with immunoenzymatic technique (ELISA method) and/or Western-Blot analysis using com-

mercial kits, buffers and antibodies. In the experiment homogenized and lysed (heart, aorta and blood lymphocytes) samples taken from animals immediately after decapitation will be used, followed by centrifugation at 2000 rpm for 10 minutes to obtain a supernatant. On the basis of these data a precise correlation between the observed quantitative changes in mRNA and proteins will be made. Knowing these relations can significantly the mechanism of action of studied plant extracts.

Measurable Effects

The proposed project is closely innovative. The very idea is innovative, because Cannabis extract with reduced THC content intended for biomedical purposes has not yet been developed, what provides applicants with a possibility to enter the market with a wide offer of a global scope. Results of pharmacological and pharmacogenetic studies will broaden the knowledge about content of nonpsychotropic cannabinoids in the Canabis sativa extracts and their analgesic and anti-inflammatory activities in animal models coupled with gene expression and protein level of CB1, CB2 and NMDAR2B receptors and Nf-kB, TNFα, COX1, COX2 in rats. On the basis of these complex studies the mechanisms of action will be proposed. The results would be a starting point for planning a future medicinal product used for alleviation of harmful pain (e.g. neuropathic pain).

Expected Results

The results obtained during the realization of the task No 4 will allow understand the molecular mechanism of the influence of Canabis sativa on neuropatic pain thorough examination of the animal behavior and different gene expression level of selected genes and proteins: CB1, CB2 receptors and NfkB, TNFα, COX1, COX2 in rats. Taking into account a number of scientific data showing the occurrence of cannabinoid receptors in high density in many areas related to pain [8; 9] and suggesting that cannabinoids inhibit cyclooxygenase enzymes COX1 and COX2 [10]. We expect that different doses of Canabis interact with examined receptors in brain, limphocytes, paw and liver in different manner.

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Conflict of interest statement
The authors declare no conflict of interest.

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