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70 Bukowska Street, room 104
60-812 Poznan, Poland
phone/fax: +48 61 854 72 74
email: jms@ump.edu.pl
www.jms.ump.edu.pl

**DISTRIBUTION AND SUBSCRIPTIONS**
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phone/fax: +48 61 854 74 14
email: sprzedazwydawnictw@ump.edu.pl

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60-812 Poznań, ul. Bukowska 70
tel./fax: +48 61 854 71 51
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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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ORIGINAL PAPER

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# Physical activity and the risk of breast cancer development in women

Katarzyna Plagens-Rotman[1], Karolina Chmaj-Wierzchowska[3], Małgorzata Piskorz-Szymendera[1], Aleksandra Zielińska[2], Maciej Wilczak[3], Pięta Beata[1]

[1] Department of Midwifery, Department of Mother and Child Healthcare, Faculty of Public Health Poznan University of Medical Sciences, Poland
[2] Department of Preventive Healthcare, Faculty of Public Health, Poznan University of Medical Sciences, Poland
[3] Department of Mother and Child Healthcare, Faculty of Public Health, Poznan University of Medical Sciences, Poland

ABSTRACT

**Introduction.** The role of physical activity in preventive healthcare constitutes a subject matter of numerous research. In fact, it was proven that physical effort has an impact on lowering the risk of some neoplasms.
**Aim.** The aim of the paper was to assess the influence of physical activity on the increase or a decrease of odds ratio for developing breast cancer in women.
**Material and Methods.** The research included healthy women and women diagnosed with breast cancer on the basis of biopsy material or surgical intervention. The research involved 850 women, aged 21–84.
**Results.** Increased physical effort during household duties and physical activity in patients with breast cancer presented as follows: 1102.61 MET (Metabolic Equivalent of Task) for passive rest, 3803.47 MET for household duties, and 1971.54 MET for physical activity. On the other hand, in subjects with no change in the breasts the study indicated the following results: 1024.05 MET for passive rest, 4150.97 MET for domestic activities and 1651.46 MET for sports activities.
**Conclusion.** Medium and high physical activity associated with household duties decreases the risk of breast cancer development. In order to lower the risk of developing breast cancer in women, active lifestyle should be promoted in terms of physical effort within medium physical activity, i.e. 600–1500 MET.

**Keywords:** breast cancer, physical activity, professional work.

## Introduction

The role of physical activity in malignant tumour aetiology has been the topic of many research studies. In fact, the research indicates that physical effort may contribute to the decrease in the development of breast, colon, prostate and endometrial cancer. What is more, the benefits stemming from an active lifestyle involve lowering the risk of chronic diseases, such as cardiovascular diseases, diabetes, osteoporosis and hypertension [1–4].

Additionally, minimizing the risk of malignant tumours is directly proportional to the intensity of physical activity, although intensive form of exercise is not indicated for patients with cardiovascular disorders [5].

Furthermore, regular and moderate physical activity influences proper weight and BMI within 18.5–25 kg/m$^2$. In fact, it is recommended to involve in physical exercise 3 times a week for 30 minutes [5].

The advantages of a healthy lifestyle involving a balanced diet, appropriate physical activity and maintaining proper body weight may contribute to a decrease in the incidence of cancer.

## Aim

The aim of the paper was to assess the influence of physical activity associated with domestic duties, pro-

fessional work, as well as with the recreational activities on an increase or a decrease in breast cancer odds ratio in women.

## Material and Methods

The research was conducted among the patients of the Gynaecology and Maternity Teaching Hospital at Poznan University of Medical Sciences between 2011 and 2013. It involved healthy subjects (n = 683), not diagnosed with breast cancer, as well as patients with breast cancer (n = 167) diagnosed on the basis of the histopathological examination. The research in total included 850 women aged 21–84.

The questionnaire was based on questions assessing physical activity in professional work and leisure time. The patients were asked to choose forms of physical activity which they had been involved in prior to the breast cancer diagnosis. A given unit of physical effort was assigned to a physical activity form, whereas in order to assess the intensity of the activity, a metabolic equivalent in MET units (Metabolic Equivalent of Task) was attributed to it.

Estimated physical activity was presented in MET units, as a value of the following parameters: MET value, number of days in a week when the activity was performed, and the activity duration in minutes per day. Additionally, MET coefficient facilitated the division of patients into 3 groups in terms of physical activity: low (under 600 MET), moderate (600–1500 MET) and high (more than 1500–3000 MET) [6].

The assessment of physical activity in professional work was attempted on the basis of a modified Freidenreich's questionnaire [7].

$$\sum \frac{\left[(final\ age - initial\ age) * \left(\frac{months}{year}\right) * (4{,}33) * \left(\frac{number\ of\ days}{week}\right) * \left(\frac{hours}{day}\right)\right] / 52}{age}$$

The intensity of the professional activity was defined as follows:

1. Profession involving only sedentary work with minimal walking.
2. Profession involving little physical effort, without increased breathing rate and without slightly increased heart rate.
3. Profession involving carrying light load (2.2–4.5 kg) with increased heart rate.
4. Profession involving carrying heavy load above 4.5 kg, quick pace walk, mainly in the fresh air, with increased heart and breathing rate.

**Table 1.** Odds ratio was calculated for each risk factor.

| Risk factor | Present | Absent | Total |
|---|---|---|---|
| Research group | a | b | a + b |
| Controls | c | d | c + d |
| Total | a + c | b + d | a + b + c + d |

The odds for developing breast cancer were calculated when the risk factor was present:

$$Odds\ ratio_{positive} = \frac{\frac{a}{a+c}}{1 - \frac{b}{b+d}}$$

In addition, it was also calculated when it was absent:

$$Odds\ ratio_{negative} = \frac{\frac{b}{b+d}}{1 - \frac{b}{b+d}}$$

By means of logistic regression model, odds ratio (OR) as a relative riskwas calculated withits confidence intervals (CI) at 95%.

$$OR = \frac{a * d}{c * b}$$

## Statistical analysis

The calculations were performed using StatSoft, Inc. STATISTICA Version 10.

Odds ratio (OR) with confidence intervals at 95% was established by means of logistic regression model. The odds ratio relevance was verified with a test where statistical hypotheses were the following $H_0$: $OR_i = 1$, $H_1$: $OR_i \neq 1$. Moreover, Wald test statistics was established which is characterized by asymptotic distribution $\chi^2$ with first degree freedom. On the basis of p value compared with relevance level $\alpha = 0{,}05$ the following decision was made: if $p \leq \alpha$,$H_0$ was rejected, whereas $H_1$ was accepted. On the other hand, if $p > \alpha$ there was no ground to reject $H_0$.

The research was approved by the Poznan University of Medical Sciences Ethical Board.

## Results

35.4% of the subjects diagnosed with breast cancer went for a walk daily, 11.4% took a stroll once a week,

and 17.7% did not undertake it at all. The majority of the patients (81%) did not go to the swimming pool, 11.4% went to the swimming pool less frequently than once a month, whereas 3.8% went for a swim once a week. More than a half of subjects (50.6%) did not ride a bicycle at all, 16.4% rode a bike 3 times a week, and 10.1% participated in this activity every day.

Nearly half of the subjects with no change in the reproductive organs (40.6%) did not ride a bicycle, 11.2% participated in this activity once a week, and 9.3% took part in it six and more times a week. However, 15.8% of the patients went for a walk every day, 13.4% did so once a week, whereas 10.4% went for a walk less frequently than once a month. 23.8% of patients did not take part in such an activity at all.

Increased physical effort during household duties and physical activity in patients with breast cancer presented as follows: 1102.61 MET for passive rest, 3803.47 MET for household duties, and 1971.54 MET for physical activity. However, in the patients with no change in breasts the results were: 1024.05 MET for passive rest at home, 4150.97 MET for household duties, and 1651.46 MET for sports activities.

Professional work analysis in the studied groups, revealed the following results: the average number of hours per week in the breast cancer patients was estimated at 19.9 hours. On the other hand, in subjects with no change in breast it was 31.9 hours.

The average MET value during household duties was the following: the highest value of 1297.5 MET was attributed to patients with no change in breast in the course of preparing meals, whereas in subjects diagnosed with breast cancer this value was 799.4 MET. Detailed data is presented in **Figure 1**.

What is more, the influence of physical activity on an increase or a decrease in developing breast cancer odds ratio was also analysed.

Subjects assessing their sports activities between 600–1500 MET daily have 1.29 times higher odds ratio for developing breast cancer, where OR = 1.29; 95% CI 0.68–2.44. On the other hand, participating in sports activities above 1500 MET daily indicated a 1.72 increase in the risk of developing cancer, where OR = 1,72; 95% CI 0,99–2,98, as compared to patients undertaking little physical activity. The results are presented in **Table 2**.

**Table 2.** Odds ratio for breast cancer development on the basis of physical activity

| Sports activities | Odds Ratio OR | Confidence Intervals 95% | Level of significiance |
|---|---|---|---|
| 600–1500 MET | 1.29 | 0.68–2.44 | p = 0.4378 |
| ≥ 1500 MET | 1.72 | 0.99–2.98 | p = 0.0537 |

Moderate physical effort during household duties decreases the risk of breast cancer development. The odds ratio equals to OR = 0.52; 95% CI 0.06–4.53 in comparison with low physical effort.

On the other hand, in subjects participating in passive rest of 600–1500MET daily the risk is increased. Odds ratio for developing breast cancer is OR = 1.51; 95% CI 0.81–2.81, whereas in patients characterized by passive rest higher than 1500MET the odd ratio was elevated to OR = 1.33; 95% CI 0.65–2.72. The data is shown in **Table 3**.

What is more, the influence of physical effort associated with professional works on the odds ratio increase was also analysed. In these calculations the

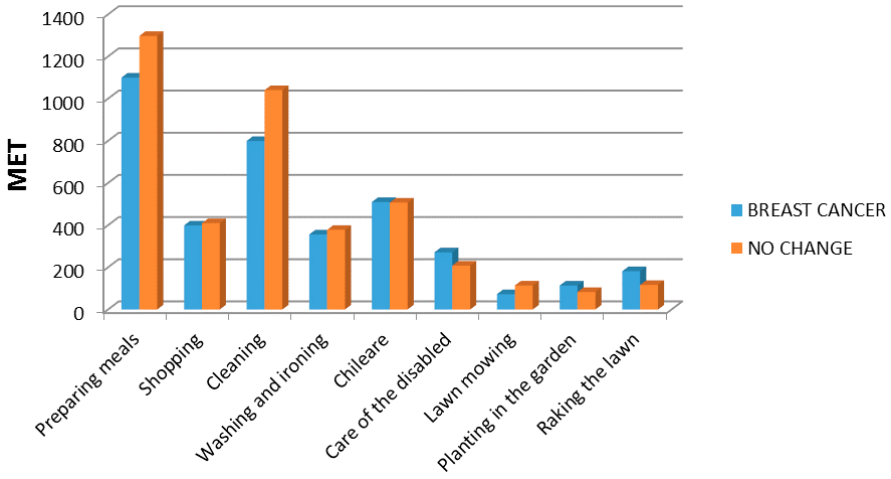


**Figure 1.** Average MET value during household duties in the research group

following time spans were established: up to 10 hours of physical effort a week, 20–30 hours per week, and more than 30 hours per week.

**Table 3.** Odds Ratio for the breast cancer development on the basis of the declared passive rest

| Passive rest | Odds Ratio OR | Confidence Intervals 95% | Level of significiance |
|---|---|---|---|
| 600–1500 MET | 1.51 | 0.81–2.81 | p = 0.1968 |
| ≥ 1500 MET | 1.33 | 0.65–2.72 | p = 0.4416 |

## Discussion

The role of physical activity in the cancer aetiology has been a subject of numerous research. In fact, it was proven in a number of analyses that regular participation in physical exercise has a substantial influence on lowering morbidity rates due to chronic diseases and cancer [8–14].

Furthermore, there are more data suggesting that in order to lower the risk of breast and colon cancer development, physical effort is optimal when it is performed 45–60 minutes at least 5 times a week. In addition, physical activity may reduce the risk of breast cancer by decreasing the time endogenous steroids affect breast gland epithelial cells, as well as by controlling a woman's weight throughout her life [8].

What is more, Henderson et al. suggest that physical activity presents beneficial influence on breast cancer development also in terms of decreasing insulin and insulin-like growth factor (IGF-1) concentration level. It is the IGF which stimulates cell division, slows cell death and decreases glucose level, at the same time increasing hormone binding globulin concentration. Another physical effort defensive mechanism type is enhancing the immune system where regular and moderate physical activity may decrease the risk of breast cancer development by active enzyme regulation, which possess the properties of free radicals inhibitors, as well as by an increase in biogenic antioxidants [9].

The majority of research papers indicates a decrease in the risk of breast cancer development reaching 10–60% in women who are physically active as compared to those who rarely participate in physical effort [15–17].

In our research, moderate physical effort during household duties decreased the risk of developing breast cancer. The odds ratio was OR = 0.52; 95% CI 0.06–4.53 as compared to low physical activity.

Similar results were obtained by Kruk J. who observed a decrease in breast cancer development in women declaring moderate physical effort associated with household duties and work in the garden. Additionally, the research indicated that 50% decrease in developing of breast cancer was presented in women participating in moderate physical activity in comparison to those who remained inactive [16].

In the course of analysis, it is clear that not all of the authors present the protective influence of physical activity on the development of malignant tumours. Research by Dosemeci et al. is a suitable example where the protective influence of increased physical effort on the relative risk of breast cancer development was not observed. In the group of women with high activity, the relative risk was estimated at 1.4 as compared with patients characterised by low physical activity which was confirmed in our study. An increase in breast cancer development is visible in patients with physical activity established at 1500 MET when compared to subjects with low physical activity [18, 19].

Regular physical effort contributes to a decrease in the risk of breast cancer development by means of hormonal regulations, and an increase in the immune system function. However, intense physical activity may contribute to a delayed first menstruation, as well as primary or secondary amenorrhoea. Furthermore, the production of steroid hormone binding globulin increases, thus decreasing oestrogen function [11, 20–22].

As far as prevention is concerned, three 30-minute intensive units of training are sufficient to reduce the risk of breast cancer development by half [12].

Therefore, physical effort should be one of the basic elements of a healthy lifestyle. What is more, in the course of health education, the importance of positive health behaviours should be stressed, particularly in terms of a proper diet, stimulants avoidance, as well as participation in regular physical activity.

## Conclusion

1. In order to decrease the risk of breast cancer development in women, active lifestyle should be emphasised which can be expressed by participating in physical effort within moderate physical activity of 600–1500 MET.
2. Moderate and high physical effort associated with household duties decreases the risk of breast cancer development.

3. The promotion of increasing physical activity should be aimed at women presenting low physical activity, i.e. below 600 MET, especially in their spare time.

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

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**Correspondence address:**
Katarzyna Plagens-Rotman
Department of Midwifery, Department of Mother and Child Healthcare, Faculty of Public Health
Poznan University of Medical Sciences, Poland
email: plagens.rotman@gmail.com

ORIGINAL PAPER

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# Violation of the regulation of cytokine in chronic catarrhal gingivitis in overweight children

Viktoria Kostura, Elvira Bezvushko, Khrystyna Musij-Sementsiv

Department of Orthodontics, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

ABSTRACT

**Introduction.** The development of inflammation in the periodontium is inextricably linked to the system processes in the organism, including an accompanying pathology, which is often parallel. The WHO particularly emphasizes the dissemination of overweight among children. Against the background of excessive weight gain in children there are the series metabolic disorders that provoke chronic diseases, including chronic catarrhal gingivitis.
**Aim.** The aim of this study was to identify the dynamics of cytokines (IL-4, IL-6) in oral fluid and characterize the immune system of the oral cavity in children with overweight and obesity.
**Material and Methods.** This study presents the results of examination of 80 children with overweight and obese patients with chronic catarrhal gingivitis (CCG), aged 12–15 years. Based on anthropometric surveys body mass index (BMI) was calculated. Also the obtained measurements and calculations were evaluated by percentile tables that were designed by the WHO in 2007 for children and adolescents 5 to 19 years for both sexes separately (WHO, 2007). The content of interleukin (IL-4; IL-6) in oral fluid in children was determined by using kits of reagents of company "Vector-Best" (Russia), based on solid-phase "sandwich" variant of immunoenzyme analysis.
**Results.** The level of IL-4 in the oral fluid of children with chronic catarrhal gingivitis decreases (1.8 times) with increasing of age and with the deepening of violations of fat metabolism. The level of IL-6 in the oral fluid of children with chronic catarrhal gingivitis increases (2.9 times) with increasing of age and the presence of excess body weight and obesity.
**Conclusions.** Further study of interleukin imbalance in the oral fluid of patients with chronic catarrhal gingivitis and overweight is a promising area of research to develop methods of prevention and pathogenic therapy.

**Keywords:** cytokine, chronic catarrhal gingivitis, overweight children, oral fluid.

## Introduction

The problem of the origin and development of the diseases of periodontal tissues is relevant not only for adults but also for children. The significant place in the structure of periodontal tissue diseases in children is occupied by chronic catarrhal gingivitis [4–6]. It is known that the development of inflammation in the periodontium is inextricably linked to the system processes in the organism, including an accompanying pathology, which is often parallel.

The WHO particularly emphasizes the dissemination of overweight among children. Against the background of excessive weight gain in children there are the series metabolic disorders that provoke chronic diseases [7]. It is known that adipocytes of the fat tissue secrete over 50 biologically active substances – adipokines, which have different biological effects that may cause the development of obesity related metabolic abnormalities, including insulin resistance and dyslipidemia. The increased expression of adipokines in children with excessive fat deposition is associated not only with increased volume of fat, but also with elevating their synthesis per unit of mass of fatty tissue. Chronic increase in local and / or systemic concentrations of adipokines makes a significant contribution in the development of metabolic syndrome. Thus,

TNF-α, interleukin-6 (IL-6) and resistin play a key role in the development of chronic inflammation. It is also known that TNF-α is the main factor that determines the development of insulin resistance in obesity [1].

Therefore, research content and activity of cytokines in oral fluid in children with overweight and obesity may be considered relevant and appropriate.

The aim of this study was to identify the dynamics of cytokines (IL-4, IL-6) in oral fluid and characterize the immune system of the oral cavity in children with overweight and obesity.

## Material and Methods

This study presents the results of examination of 80 children with overweight and obese patients with chronic catarrhal gingivitis (CCG), aged 12–15 years. 40 children of the group were 12 years old (20 overweight, 20 obese) and 40 children were 15 years old (20 overweight, 20 obese) from Lviv schools № 4, 28, 53. In comparative terms and in full methodological volume there were examined 30 somatically healthy children (aged 12–15 years) with normal body weight (a comparison group) and chronic catarrhal gingivitis.

Dental diagnosis was set on the totality of clinical manifestations of the disease, according to the classification by M.F. Danilevsky. Evaluation of the physical development of children was conducted according to anthropometric measurements. Based on anthropometric surveys body mass index (BMI) was calculated. Also the obtained measurements and calculations were evaluated by percentile tables that were designed by the WHO in 2007 for children and adolescents 5 to 19 years for both sexes separately [12].

The collection of the oral fluid for laboratory tests was carried out in the morning on an empty stomach by spitting into the measuring centrifuge tubes volume of 5 ml. The content of interleukin (IL-4; IL-6) in oral fluid in children was determined by using kits of reagents of company "Vector-Best" (Russia), based on solid-phase "sandwich" variant of immunoenzyme analysis. Interleukin in oral fluid of children with overweight and obese patients with chronic catarrhal gingivitis (CCG) was analysed and compared with healthy children with normal weight and with chronic catarrhal gingivitis (CCG).

The research was approved by the Human Research Ethics Committee of the Danylo Halytsky Lviv National Medical University on 20.01.2015, protocol № 1.

## Results and Discussion

As it is shown in **Table 1**, the investigated levels of interleukin in oral fluid were significantly different in the examined groups of children. The content of IL-4 in the group of 12-year-old overweight children with CCG decreased 1.14 times comparing with the group of children with normal weight and CCG (the comparison group). In 12-year-old children with obesity a marked reduction in the concentration of IL-4; 1.3 times less than in the comparison group and 1.15 times less than in the peer group of overweight children was observed. The 15-year-old children with similar disorders and overweight also experienced a reduction of IL-4 in the oral fluid; 1.4 times less than in the comparison group and 1.23 times less than in the 12-year-olds who were overweight. The lowest level of IL-4 was observed in the group of 15-year-old obese children, 1.8 times than in the comparison group and 1.4 times than in the group of 12-year-olds with obesity.

The level of IL-6 in the oral fluid of the examined groups of children on the contrary increased. So in a group of 12-year-old overweight children with CCG upward trend in the concentration of IL-6 ($p > 0.05$) was observed. But in oral liquid of children of the same age, but with obesity content of IL-6 was 1.9 times higher than in the comparison group and 1.8 times higher than in the 12-year-old overweight children. In oral liquid of 15-year-old overweigh children with CCG the increase of the concentration of IL-6 in 2.5 times compared with the group of children with CCG but with normal body

**Table 1.** Levels of IL-4 and IL-6 in the oral fluid of 12- and 15-year-old children with chronic catarrhal gingivitis (CCG) with normal weight (comparison group), overweight and obesity

| The examined groups | IL-4pg / ml | IL-6pg / ml | IL-6/IL-4 |
|---|---|---|---|
| The comparison group | 2,34 ± 0,12 | 6,72 ± 0,48 | 2,8 |
| 12-year-old overweight children with CCG | 2,05 ± 0,07$^{*}$ | 7,04 ± 0,50 | 3,4 |
| 12-year-old obese children with CCG | 1,78 ± 0,06$^{*\Delta}$ | 13,05 ± 0,65$^{*\Delta}$ | 7,3 |
| 15-year-old overweight children with CCG | 1,67 ± 0,05$^{*\#}$ | 16,6 ± 0,75$^{*\#}$ | 9,9 |
| 15-year-old obese children with CCG | 1,27 ± 0,05$^{*\Delta\#}$ | 19,4 ± 0,85$^{*\Delta\#}$ | 15,3 |

Notes:
* – The likelihood of differences compared to those of controls ($p < 0.05$).
Δ – probability differences compared with those in a group of children the same age who are overweight ($p < 0.05$).
# – the likelihood of differences compared with those in the group of children with some metabolic disorders, but different age ($p < 0.05$).

weight and 2.3 times compared with a similar group of 12-year-oldchildren was observed. The most pronounced increase in the content of IL-6 in the oral fluid was observed in the 15-year-old obese children: 2.9 times more than in the comparison group, at 1.17 times than in their peers who are overweight and 1.5 times compared to the group of 12-year-old children with obesity.

The imbalance between the levels of IL-4 and IL-6 in the oral fluid of groups of surveyed children is most noticeable when analyzing the ratio of these cytokines. As can be seen in **Table 1**, the value of the index increases with the age of children and the deepening of the disruption of lipid metabolism. Adipose tissue, according to researchers [3, 8] is an important endocrine organ with a number of effects, including immune system and cytokine profile. In particular, adipose tissue secretion is the source of a number of proinflammatory mediators. At the same time, obesity inhibited the synthesis of proinflammatory cytokines [10]. As a result of our research, we saw the most pronounced decrease in IL-4 in the oral fluid of 15-year-olds with obesity (1.8 times compared to the level in children with normal body weight). Several studies [2, 11] found that IL-4 inhibits destructive-inflammation in periodontal and reduces osteoporosis. Reduction of IL-4 in the oral fluid of our examined children may be associated with the presence of chronic catarrhal gingivitis.

IL-6 is one of the key mediators of inflammation in obesity. It is known that about 30% of circulating IL-6 is synthesized in adipose tissue. Rising concentrations of proinflammatory cytokines (IL-1β, IL-6) and reducing anti-inflammatory IL-4 for obesity was noted by researchers [9] in patients with deforming osteoarthritis. Our research showed that in the 15-year-old children with obesity and CCG the level of IL-6 was higher than in other groups of the surveyed children.

The study of cytokine levels in oral fluid for gingivitis allows to find out the changes of the immune regulation in the inflammation and it is not invasive and safe method for the patient.

Therefore the level of IL-4 in the oral fluid of children with chronic catarrhal gingivitis decreases (1.8 times) with increasing of age and with the deepening of violations of fat metabolism. The level of IL-6 in the oral fluid of children with chronic catarrhal gingivitis increases (2.9 times) with increasing of age and the presence of excess body weight and obesity. Further study of interleukin imbalance in the oral fluid of patients with chronic catarrhal gingivitis and overweight is a promising area of research to develop methods of prevention and pathogenetic therapy.


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**Correspondence address:**
Viktoria Kostura
Department of Therapeutic Dentistry
Danylo Halytsky Lviv National Medical University
69 Pekarska Street, Lviv, Ukraine, 79010
phone: +38 097 159 52 79
email: toria1max@yahoo.co.uk

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# Clinical features of gastroesophageal reflux disease in children with different genotypes of C825T polymorphic loci of GNB3 gene

Marta Dats-Opoka[1], Halyna Makukh[2]

[1] Department of Propedeutics of Pediatrics and Medical Genetics, Danylo Halytsky Lviv National Medical University, Ukraine
[2] State Institution "Institute of Hereditary Pathology Ukrainian National Academy of Medical Sciences", Ukraine

ABSTRACT

**Introduction.** Considering the steady growth of the gastroesophageal reflux disease (GERD) in children in recent decades, the difficulty of GERD diagnosing in children, the variety of GERD clinical and morphological features as well as the factors that cause it, including genetic predisposition, a detailed analysis of each of them remains relevant.
**Aim.** To analyze the peculiarities of nutritional status in children with GERD and its correlation with the different genotypes of C825T polymorphic loci of *GNB3* gene as well as its association with different GERD clinical manifestations.
**Material and Methods.** The analysis of GERD clinical features was carried out and the nutritional status in 100 children of school age was estimated. Molecular and genetic research of C825T loci of *GNB3* gene using PCR method (rs5443) was carried out in the studied group (100 children) and in 40 healthy children that formed the control group.
**Results.** The distribution of the genotypes of C825T polymorphic loci of the *GNB3* gene in children with GERD and healthy children in the control group did not have any statistically significant difference ($\chi 2 = 0.27$, $p = 0.87$). Among more than a half of the children in both groups, the *GNB3* 825ST heterozygous genotype were detected (54.0% of the experimental group and 57.5% of the control group), according to de Vries et al. data is a factor of GERD increased risk. The association between the genotype of C825T locus of *GNB3* gene and the data of intragastric endoscopy with pH monitoring was found: in patients with hyperacidic GERD the genotype 825CT was predominantly revealed, and in children with normal and hypoacidic GERD a higher frequency of the 825TT genotype was found. In children with GERD having a lack of the nutritional status (61%), the genotype 825CT (61.82%, $p = 0.013$) and 825TT (100%, $p = 0.005$) of the *GNB3* gene were detected significantly more often.
**Conclusions.** The distribution of the genotypes of C825T polymorphic loci of the *GNB3* gene in children with GERD was determined. Differences in GERD development depending on the different *GNB3* genotypes were not detected. The distribution of the genotypes of C825T loci of the *GNB3* gene remained unchanged at different GERD clinical manifestations. The presence of 825CT and 825TT genotypes of *GNB3* gene in patients with GERD is associated with a decrease in physical development signs. The association between genotype of C825T loci of *GNB3* gene and pH intragastric endoscopy data was identified: in patients with hyperacidity GERD 825CC genotype was usually found, and in children with normal- and hypoacidity GERD 825TT genotype was usually found.

**Keywords:** gastroesophageal reflux disease, children, physical development, polymorphism C825T, *GNB3*.

## Introduction

Nowadays, it is well-known that gastroesophageal reflux disease (GERD) has a multifactor etiology [1–4]. Patients with GERD may have a normal esophageal acid exposure, but their esophagus mucous membrane may be more sensitive to acid reflux, which leads

to heartburn and erosive esophagitis by visceral and neural dysfunction [5, 6].

Studies showing a higher predisposition to this disease in monozygotic twins, in contrast to the dizygotic twin pairs, point to the leading role of genetically determined factors in the pathogenesis of this disease [7, 8]. Recent studies show that the course of inflammatory reactions of the organism, drug metabolism, DNA reparative processes, mutagenesis, cell cycle regulation processes, and alternative splicing are associated with the risk of GERD and its complications – Barrett's esophagus and adenocarcinoma of the esophagus [9]. The majority of works point to the necessity of the additional research using more samples to make conclusions about the role of certain genes or mechanisms [10].

Heterotrimeric guanine nucleotide binding proteins (G proteins) transmit signals between receptors and effector proteins. Many surface-cell receptors of the neurotransmitters use G-proteins for the transduction of intracellular signaling pathways [11]. The subunits of these proteins are encoded by the entire genetic G family. Beta subunits (encoded by the *GNB3* gene) are important regulators of alpha subunits as well as some signal transductor receptors and effector. Polymorphism in the 10 exons of the *GNB3* 825C/T gene is rather interesting [12]. The single-nucleotide polymorphism of the *GNB3* gene is due to the replacement of cytosine (allele 825C) with thymine (allele 825T). In the presence of the 825T allele there is an alternative splicing: the shorter version of the mRNA called Gb3s is being expressed [13]. In this case, 123 nucleotides are absent in the given mRNA, and as a result, the protein with the deletion of 41 amino acids must be synthesized. Using RT-PCR experiments, the expression of this alternative splicing type of mRNA was confirmed in B-lymphoblasts [12], neutrophils [14], and T-lymphocytes [15].

Recent studies show that the genetic polymorphism of the *GNB3* C825T gene became a factor involved in the chain of mechanisms that cause reflux [16]. The Gb3s type, which is synthesized in the presence of the 825T allele, as reported is associated with depression [17], hypertension [12], obesity [18], medically induced weight gain [19, 20], although some contradictions remain.

## Aim

The aim of the research was to study the peculiarities of distribution of the genotypes of C825T polymorphic loci of *GNB3* gene in children with GERD at its different clinical manifestations and depending on the nutritional status markers.

## Material and methods

100 children of school age formed the experimental group (mean age 10.8 ± 3.07 years), being cured of GERD in Lviv Regional Children's Clinical Hospital (MI LRCCH "OHMATDYT"). The children included 56 girls (56% ± 9.6%) and 44 boys (44% ± 9.6%).

The protocol of the study was approved by the Ethics committee of the Danylo Halytsky Lviv National Medical University (№ 10 dated December 15, 2014). All patients were informed about the study and provided written informed consent to participate in the research. All children were generally examined, all children had a somatometry with body mass index (BMI) and a standardized index (Z-score), endoscopic examination of the esophagus, stomach and duodenum, using Fujinon WG 88 FP video gastroscope, 21 children had intragastric endoscopy with pH monitoring using acid gastrographer (AG TU U 33.1-13300318-002: 2007, the manufacturer of Start Ltd., Vinnitsa) as well as genetic analysis.

The criteria for inclusion in the research group are as follows:

1) complaints: pain syndrome (rebound pain that is not associated with damage of other organs and systems, and epigastric pain), dyspeptic syndrome (heartburn, nausea, vomiting, eructation), irritable bowel syndrome (constipation, flatulence);
2) the presence of GERD was confirmed endoscopically, according to the Savari-Miller classification in Tytgat GNJ et al., 1990 modification;
3) voluntary informed consent of the parents for the genetic analysis.

Exclusion criteria: refusal to participate in any stage of the study.

As a control, a group of 40 healthy children of the given age (19 boys and 21 girls) was formed. The experimental materials were 40 DNA samples isolated from buccal epithelial cells.

Molecular and genetic study of the polymorphic loci C825T of *GNB3* gene (the number of polymorphism in NCBI database is rs5443) was performed. The gene fragment of the 268 b.p. size was amplified using primers GP-1 (sense, 5-TGACCCACTTGCCACCCGTGC-3') and GP-2 (antisense, 5'-GCAGCAGCCAGGGCTGGC-3'). In result of the PCR product proceeding by endonuclease of BseD1 restriction, fragments sized 268 b.p. – genotype 825TT, 268 b.p., 152 b.p. and 116 b.p. – gen-

otype 825CT, 152 b.p. and 116 b.p. – genotype 825CC, respectively, are visualized on the electrophoregram.

## Results and discussion

In result of the study of molecular and genetic analysis of DNA in 100 people of the given group with GERD and 40 people of the control group genotype of the polymorphic loci C825T of *GNB3* gene was determined. More than a half of the children in both groups, the *GNB3* 825CT heterozygous genotype were detected, which according to de Vries et al. data is a factor of GERD increased risk. The results of the carried out molecular and genetic study and statistical analysis are shown in **Table 1.**

Distribution of genotypes of the polymorphic loci C825T of GNB3 gene in children with GERD was similar to the determined in the control group ($\chi 2 = 0,27$, $p = 0,87$). The calculated GERD odds ratios depending on genotype of loci C825T of *GNB3* gene are shown in **Table 1**; all of them are within the insignificant limits. Frequencies indicators of alleles of loci C825T of *GNB3* gene in the studied groups of the children are presented in **Table 2.**

As it is shown in **Table 2,** frequencies of the normal C (69%) and highly functional T (31%) alleles of loci C825T of *GNB3* gene were practically identical both in the studied and in the control groups. It should be mentioned that the determined distribution coincided with the data of other studies in the Caucasian population. Frequency of T allele *GNB3* 825C/T of polymorphism is higher in Asian populations (42–53%), than in Caucasian population (27–42%) [19, 21]. It is known that *GNB3* 825TT is associated with functional dyspepsia in the Japanese [22], in contrast to the western studies. In the Caucasian population genotype of *GNB3* 825CC is associated with functional dyspepsia [23]. In de Vries et al. studies it is shown that GERD is connected to CT genotype genotype of *GNB3* C825T polymorphism compared with CC genotype [16]. Genetic studies of the association for functional dyspepsia have shown results similar to irritable bowel syndrome (IBS), namely: *GNB3* 825T allele can be connected with IBS and constipation in the Koreans [24]. The obtained results coincide with Tadayuki Oshima et al. data: there was no connection between *GNB3* C825T genotype and GERD symptoms [25]. These results point to a significant variations in the genetic effect of *GNB3*, depending on ethnicity.

Besides, we analyzed the frequency of genotypes of loci C825T of *GNB3* gene in children with different GERD stages (**Table 3**).

**Table 1.** Distribution of genotypes of the polymorphic loci C825T of *GNB3* gene in the studied groups

| *GNB3* genotype | Studied group, % | Control group, % | χ2 | p | OR | |
|---|---|---|---|---|---|---|
| | n = 100 | n = 40 | | | Value | 95% CI |
| *GNB3* 825CC | 42.0 | 40.0 | 0.27 | 0.87 | 1.09 | 0.51–2.29 |
| *GNB3* 825CT | 54.0 | 57.5 | | | 0.87 | 0.41–1.82 |
| *GNB3* 825TT | 4.0 | 2.5 | | | 1.63 | 0.18–15.00 |

Note: n – number of subjects, P – the significance of the differences in distribution of genotypes between the control and the studied groups, OR – odds ratio

**Table 2.** Frequency of the alleles of polymorphic loci C825T of *GNB3* gene in the studied groups

| *GNB3* gene alleles | Frequency, % | | χ2 | p | OR | |
|---|---|---|---|---|---|---|
| | Studied group, n = 100 | Control group, n = 40 | | | value | 95% CI |
| *GNB3* 825C | 69.0 | 68.8 | 0.00 | 0.97 | 1.01 | 0.58–1.77 |
| *GNB3* 825T | 31.0 | 31.3 | | | 0.99 | 0.56–1.73 |

Note: n – number of subjects, P – the significance of the differences between the control and the studied groups, OR – odds ratio

**Table 3.** Distribution of genotypes of polymorphic loci C825T of *GNB3* gene depending on the patients' gender

| Genotype *GNB3* | Girls | | Boys | |
|---|---|---|---|---|
| | Control group, % (*n = 21*) | Studied group, % (*n = 56*) | Control group, % (*n = 19*) | Studied group, % (*n = 44*) |
| *GNB3* 825CC | 47.6 | 35.7 | 31.6 | 47.7 |
| *GNB3* 825CT | 47.6 | 60.7 | 68.4 | 47.7 |
| *GNB3* 825TT | 4.8 | 3.6 | 0.00 | 4.5 |
| $\chi 2 = 1.07$, P = 0.59 | | | $\chi 2 = 2.72$ , P = 0.26 | |

Note: n – number of subjects, P – the significance of the differences in genotypes distribution, between the control and the studied groups: * – statistically significant value.

Results of the statistical calculations point to the absence of significant differences in polymorphic loci C825T genotype distribution both between boys and girls of the studied and the control groups, and between the groups ($p > 0.05$).

The distribution of genotypes of the C825T loci of *GNB3* gene in the groups of patients with different GERD clinical manifestations was analyzed. The distribution of genotypes in the groups of patients with esophagitis (I–II stage according Tytgat et al. 1990), without esophagitis, in patients with dyspeptic symptoms and in the presence of an irritable bowel syndrome with constipation (Roman criteria IV) did not differ both from the control group data and among itself (**Table 4**). At comparing the patients with GERD by pH-monitoring results, significant differences in the distribution of C825T loci genotypes of *GNB3* gene ($p < 0.05$) were found. Among the patients with hyperacidic GERD (pH 0.86–1.59), one third had a CC genotype and there was no patients found with a TT genotype, at the same time in a group of the children with normal and hypoacidic GERD (pH 1.60–3.59) 25% of patients had a *GNB3 825*TT genotype. The obtained data indicate the association of the loci C825T genotype of *GNB3* gene with the pH-monitoring data. The probable mechanism of such action requires additional discussion.

In accordance with a number of publications, the presence of T allele is a predisposition to obesity and hypertension factor [26, 27]. It has been shown that in the presence of T allele of C825T loci of *GNB3* gene, there is an alternative splicing and increased expression of m-RNA [28]. However, Ruiz-Velasco V and Ikeda SR in their studies have shown that Gb3s protein, which is expressed from the truncated version, is functionally inactive [29].

According to Tadayuki Oshima et al., the frequency of TT genotype was significantly higher in patients with epigastric pain syndrome. The 825TT genotype, which causes increased transduction of the signal through Gb3s, can be involved in gastroduodenal motility change and dyspeptic symptoms development [25].

Considering a significant number of publications devoted to the association of C825T polymorphism with obesity and insulin resistance, the next aim of the study was to analyze the physical development markers in children with GERD. Determination of BMI (body mass index) and z-score, which displays a deviation from the mean values (**Table 5**) was carried out.

Standardized deviation index (z-score) can be positive or negative. As the data from the **Table 5** show, in all genotype groups z-score mean value is negative that points to a lower than the corresponding normal markers of the nutritional status in children with GERD. BMI and z-score indexes in the groups of GERD patients with different genotype by C825T loci of *GNB3* gene were similar ($p > 0.05$). The difference of BMI mean value between the groups with 825TT and 825CC genotypes approached a meaningful value ($p = 0.08$).

Considering that the spread of the standardized z-score deviation in children with GERD ranged from -4.36 to 1.94, it became important to analyze the proportion of patients with negative (less than average norm) and positive (normal or higher than average

**Table 4.** Distribution of genotypes of the C825T locus of the *GNB3* gene in the groups of patients with different clinical manifestations of GERD

| Group | N (men ) | Age mean (s.d.) | BMI mean (s.d.) | Allele distribution | | | HWE |
|---|---|---|---|---|---|---|---|
| | | | | CC, N(%) | CT, N (%) | TT, N (%) | |
| Healthy controls | 40 (19) | 9.3 ± 3.86 | 16.12 ± 2.04 | 16 (40.0%) | 24 (57.5%) | 1 (2.5%) | no |
| All GERD patients | 100 (44) | 10.8 ± 3.07 | 17.11 ± 2.96 | 41 (41.0%) | 55 (55.0%) | 4 (4.0%) | no |
| hyperacidic GERD | 13 (10) | 11.7 ± 3.04 | 16.86 ± 2.07 | 4 (30.8%) | 9(69.2%) | 0* | yes |
| normal and hypoacidic GERD | 8 (3) | 11.3 ± 3.24 | 17.25 ± 3.44 | 0 | 6(75.0%) | 2 (25.0%)* | yes |
| with esophagitis | 64 (34) | 11.04 ± 3.03 | 17.67 ± 3.03 | 24 (37.5%) | 37(57.8%) | 3 (4.7%) | yes |
| without esophagitis | 36 (10) | 10.31 ± 3.08 | 16.11 ± 2.55 | 17 (47.2%) | 18 (50.0%) | 1 (2.8%) | no |
| Only GERD symptoms | 8 (5) | 12.36 ± 2.87 | 18.69 ± 3.10 | 1 (12.5%) | 7 (87.5%) | 0 | no |
| Concomitant FD symptoms | 48 (21) | 10.76 ± 3.06 | 17.36 ± 2.60 | 23 (47.9%) | 23 (47.9%) | 2 (4.2%) | yes |
| Concomitant IBS symptoms | 17 (6) | 10.55 ± 3.21 | 18.33 ± 3.46 | 6 (35.3%) | 10 (58.8%) | 1 (5.9%) | yes |
| EPS | 77 (34) | 11.16 ± 3.00 | 17.41 ± 3.05 | 34 (44.2%) | 40 (51.9%) | 3 (3.9%) | no |

Note: N – numbe r of subjects; BMI – body mass index; HWE – Hardy-Weinberg equilibrium; yes/no – Hardy-Weinberg $\chi 2 < 3.84$; * – statistically significant value.

Hyperacidic GERD – intragastric pH 0,86–1,59; normal and hypoacidic GERD – intragastric pH 1,60–3,59 (n = 21). Only GERD symptoms: patients with GERD without symptoms of FD or/and IBS. Concomitant FD/IBS symptoms: GERD patients with concomitant symptoms of FD or IBS, respectively. EPS – Epigastric pain syndrome. When added, these groups contain more than the total 100 patients; thisis due to the overlap between FD and IBS: 40 patients had both symptoms of FD and IBS.

**Table 5.** Nutritional status indexes in children with GERD

| Genotype group | BMI | | | z-score | | |
|---|---|---|---|---|---|---|
| | M ± m | min | max | M ± m | min | max |
| 825 CC n = 41 | 17.11 ± 2.99 | 12.6 | 23.0 | -0.49 ± 1.28 | - 3.41 | 1.60 |
| 825 CT n = 55 | 17.20 ± 3.06 | 11.5 | 24.5 | -0.55 ± 1.37 | - 4.36 | 1.94 |
| 825 TT n = 4 | 15.78 ± 1.35 | 14.7 | 17.6 | -0.48 ± 0.30 | - 0.92 | -0.28 |
| Total (n = 100) | 17.11 ± 2.96 | 11.5 | 24.5 | -0.52 ± 1.29 | -4.36 | 1.94 |

n – number; BMI – body mass index; M ± m – mean value and its deviation; min – the minimum value; max – the maximum value; z-score – standardized deviation

**Table 6.** Z-score in patients with GERD and different genotypes of C825T loci of *GNB3* gene

| Genotype group | z-score value | | | | χ2 | p |
|---|---|---|---|---|---|---|
| | ≥ 0 | | < 0 | | | |
| | n | % | n | % | | |
| 825 CC (n = 41) | 19 | 46.34 | 22 | 53.66 | 0.44 | 0.508 |
| 825 TC (n = 55) | 21 | 38.18 | 34 | 61.82 | 6.15 | 0.013* |
| 825 TT (n = 4) | 0 | 0 | 4 | 100 | 8.00 | 0.005* |

n – number; z-score – standard deviation z; * – statistically significant value

norm) values of z-score in patients with different genotypes of *GNB3* gene (**Table 6**).

In the groups of GERD patients with 825TC and 825TT genotypes, the children having lag in physical development were predominant, 62% and 100% of them, respectively. A statistically significant difference concerning the ratio of the patients with negative and positive z-score values in patients with genotypes 825CT and 825TT of *GNB3* gene was found (**Table 6**). Thus, in patients with GERD, the presence of 825CT and 825TT genotype of *GNB3* gene can be considered as a factor of propensity to lowered physical development rates. The obtained results correlate with the found associations concerning pH-monitoring results in patients with GERD.

## Conclusions

1) Distribution of the genotypes and the alleles frequency of polymorphic C825T loci of *GNB3* gene in children with GERD, similar to the determined in the control group. The frequency of the allele 825T of *GNB3* gene coincided in the studied group of children with GERD and the control group, and it was 31%.
2) Association between genotype of C825T loci of *GNB3* gene and pH-monitoring data was found: genotype 825CC was determined mostly in the patients with hyperacidic GERD, and in the children with normal and hypoacidic GERD 825TT genotype was found more frequently.
3) Distribution of C825T loci of *GNB3* gene in the groups of patients with esophagitis, without esophagitis, in patients with dyspepsia, and in case of irritable bowel syndrome with constipation did not differ from both the control group data and between itself.
4) In 39% of the patients with GERD a normal value of the nutritional status was determined, while 61% of them characterized by its delay. The presence of 825CT and 825TT genotype of *GNB3* gene in patients with GERD can be considered as a propensity factor to lowered physical development rates.


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**Correspondence address:**
Marta Dats-Opoka
Department of Propedeutics of Pediatrics
and Medical Genetics
Danylo Halytsky Lviv National Medical University
69 Pekarska Street, Lviv, Ukraine, 79010
email: martadats@gmail.com

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# Evaluation of using dietary supplements among polish adult people below and over 60 years of age

Katarzyna Suliga[1], Teresa Grzelak[1], Joanna Grupińska[1, 2], Marta Pelczyńska[1], Marcelina Sperling[1], Krystyna Czyżewska[1]

[1] Division of Biology of Civilization-Related Diseases, Poznan University of Medical Sciences, Poland
[2] Departament of General Chemistry, Poznan University of Medical Sciences, Poland

ABSTRACT

**Introduction.** The population of elderly people is exposed to the development of disorders related to physiological ageing, as well as relatively common diseases occurring in the old age period. The gastrointestinal diseases, which reduce the absorption of many nutrients, are more frequent in the elderly compared to younger population.
**Material and Methods.** In the study was attempted the assess of conditions of dietary supplementation by 60 years old people or older (60+). The comparisons of results were obtained with relation to young adults and middle aged persons (18–58 years). The survey was conducted with 368 adult, including 123 respondents over 60 years old and 245 younger people.
**Results.** The study has been shown that dietary supplements were used by 64.2% of elderly people and 59.6% of younger respondents. The most common source of knowledge about dietary supplements in the elderly was a physician (40.7%) in contrast to younger population, where dominated the Internet (60.0%). The main reason of supplementation in the 60+ group was an enhancement the immune system, while in younger population it was important to improve the appearance of skin, hair and nails. The most common components of supplements, which were used in the elderly population, were vitamin C (32.5%) and magnesium (28.5%) compared to younger group where the most popular were vitamin D (22.5%) and magnesium (19.2%).
**Conclusions.** The overuse of supplements, especially among the elderly, can lead to disability, therefore it is important to expand the education about side effects the improperly using of dietary supplements.

**Keywords:** dietary supplements, vitamins, minerals, senility.

## Introduction

Older people belong to the population, which is especially exposed to the nutrients deficiencies. They are the result of decreased basal metabolic rate, which is a reason of reduced food intake while the request for macro- and micronutrients is constant. Frequent occurrence of diseases of the digestive system (including gastroesophageal reflux disease, chronic inflammation of the stomach, periodontitis, Helicobacter pylori infection, tumours or dyspepsia), as well as many drugs taken is also a problem. Deficiencies of vitamin D, calcium, and vitamin $B_{12}$ are particularly common in this population [1–4]. The increase of ageing population, who despite the risk of malnutrition, is exposed on the development of immune response disorders leading to degenerative disease it has been observed. Dietary supplements intended for this part of population are becoming more popular [3, 5, 6]. These products are often used by patients in an uncontrolled way, without consultation with any specialist. The oral products, rich in nutrients, intended to complement a daily diet are called dietary supplements. They may contain vitamins, minerals, herbals, amino acids or other substances, such as enzymes, metabolites, probiotics, bee products [7]. According to the Act on the Safety of Food and Nutrition, "dietary supplements are

foodstuffs, which purpose is to supplement the normal diet and which are concentrated sources of vitamins or minerals, or other substance with a nutritional or physiological effect [8]". In many cases the supplementation in the elderly is totally justified, especially when diet is not able to cover the requirements of the macro- and micronutrients. However, it does not mean that dietary supplements should be used without any restrictions. The supplementation of many nutrients should be justified by an unbalanced diet, healthy state, recent and current diseases, drugs taken or other factors, e.g. psychological or economic [1, 7]. The purpose of the study was to evaluate the conditions of using dietary supplements by 60 years old and elder people and to compare the results with population of young adults and middle-aged respondents (between 18–58 years).

## Material and methods

The studies were conducted among 368 Poles both gender between months: February–May 2015 and November–December of the same year. After the preliminary analysis of obtained data, all respondents were divided into two groups. The first population consisting 60 years old and elder people (EP) counted 123 respondents. The second group included 245 adult respondents aged 18–58 (GP). The average age in the EP was 67.3 ± 5.4 years, whereas in the GP 28.8 ± 9 years. The Body Mass Index (BMI) was used to evaluate the body weight. The mean (± SD) value of BMI was 26.4 ± 3.7 kg/m$^2$ in the EP and 22.9 ± 4.3 kg/m$^2$ in the GP. Women predominated in both populations, respectively 68.3% of people in the elderly and 90.2% of younger respondents. The information needed to complete the study was obtained by using an anonymous questionnaire, which was available to fill in senior clubs, universities of the third age, and by the medical clinic patients. The questionnaire mostly contained single-choice questions. It was also a few multiple-choice questions and one question describing accepted dietary supplementation. Further issues concerned the details associated with intake of dietary supplements (e.g. type, frequency of using, causes of supplementation, place of purchase). In the survey were questions about gender, age, weight and growth of the body, education, place of residents, health status, and socioeconomic situation. The obtained results were statistically calculated using PQStat (Poznan, Poland) and GraphPad Prism 6 (San Diego, U.S.A.). Normality of the distribution of data was checked using Shapiro-Wilk test and homogeneity of variance was tested using Levene's test. There was not the normal distribution, therefore in the current analyses of statistical differences were used non-parametric tests (Mann-Whitney test, chi-squared test and Fisher test). Statistically significant level of error was established at $p < 0.05$.

## Results

The most of respondents came from the cities above 100,000 (47.1% of the EP and 51.8% of the GP) and the other extent from small towns and villages. Older people usually had a secondary education (44.7%), while among the people from population below sixty years old, dominated higher education (56.7%). Statistically significant difference was found in the level of education between the EP and GP ($p < 0.000001$). Health and financial situation of the elderly respondents were worse than in younger population ($p = 0.001$).

Among people of the EP 64.2% and 59.6% of the GP, declared using dietary supplements regularly or occasionally. Statistically significant differences were not observed between groups in this aspect ($p = 0.428$). The percentage of frequency distribution of using dietary supplements among women and men was shown in the **Table 1**. Vitamin preparations were the most common type of dietary supplements in both populations (78.5% of the EP and 74.7% of the GP, no significant difference). Minerals were consumed less often, however the elderly people (67.1%) chose them more frequently, than the population under sixty years old (48.6%, $p = 0.0079$). The intake of other supplements was at the similar level (32.9% and 39.7%). Significant differ-

**Table 1.** The percentage of people both gender using (or not) dietary supplements in EP and GP groups

| Groups | People 60+ (60–89 years) | | People aged 18–58 | |
|---|---|---|---|---|
| | Men (n = 39) | Women (n = 84) | Men (n = 24) | Women (n = 221) |
| The use of dietary supplements by respondents – **regularly** | 25.6% | 33.3% | 20.8% | 33.5% |
| The use of dietary supplements by respondents – **occasionally** | 28.2% | 35.7% | 33.3% | 26.7% |
| Not using dietary supplements | 46.2% | 31.0% | 45.9% | 39.8% |

EP – group 60+; GR – adult persons aged 18-58; n – number of persons

ences were not obtained in the EP and GP between the dietary supplements consumption and state of health, drugs taken, excess body weight (according BMI) and also economic situation. In younger group of people with higher education than others (p = 0.007) and living in the big cities (p = 0.015) compared to the residents of small town and villages more often reached for dietary supplements (lack of discussed dependence in the elderly population).

In the 60+ population the supplementation was mostly practiced 1–2 times per week (39.2%) and every third person (32.9%) declared daily intake discussed products. In the GP, dietary supplements were generally consumed each day (49.3%). Between studied groups were not statistically significant differences for the above information. Funds allocated for preparations were below twenty zlotys per month in both populations. The most popular form of dietary supplements was tablets, usually purchased in pharmacies, both by the elderly and younger people. Water was a liquid used to sipping the supplements (respectively 92.4% of the EP and 95.2% of the GP).

Among people aged 60 and older 55.7% and 41.1% of the younger group declared a slight health improvement after using dietary supplements (statistically significant difference p = 0.036), while the EP (11.4%) compared to the GP (34.9%) less often noticed the significant benefits in general health (p = 0.00013). Elderly people more often (43.9%) than representatives of second population (29.0%) ascertained that the dietary supplements are rather beneficial in the health. The doctor turned out to be the most common source of information about dietary supplements (p = 0.018) among the elderly (40.6%) compared with younger (19.6%). In the EP (22.8%) significantly less often (p = 0.0001) than in the GP (60.0%) indicated that a knowledge is obtained from the Internet. In the elderly population dietary supplements were used more often, if the knowledge about products was confirmed by the doctor (p = 0.00066), but in the GP if it was obtained from the Internet (p = 0.00036). The study has also shown that older people more frequently (p = 0.010) than younger took dietary supplements because of the doctor recommendation. The reasons of using dietary supplements by respondents were presented in the figure (**Figure 1**).

Conducted research allowed to assess the most popular vitamins and minerals in the various groups. In both populations were usually used many supplementary preparations at the same time. In the 60+ group the most common was vitamin C and in the younger group – vitamin D. In both populations the first choice mineral was magnesium. The elderly people more frequently consumed vitamin C (p = 0.0008) and magnesium (p = 0.047) and less often chose folic acid (p = 0.01) compared to younger. Significantly differences in the frequency of taking other B vitamins, calcium, iodine, iron, zinc, and omega-3 fatty acids were not observed. The percentage of using vitamins and minerals supplements was shown in the figure (**Figure 2**).

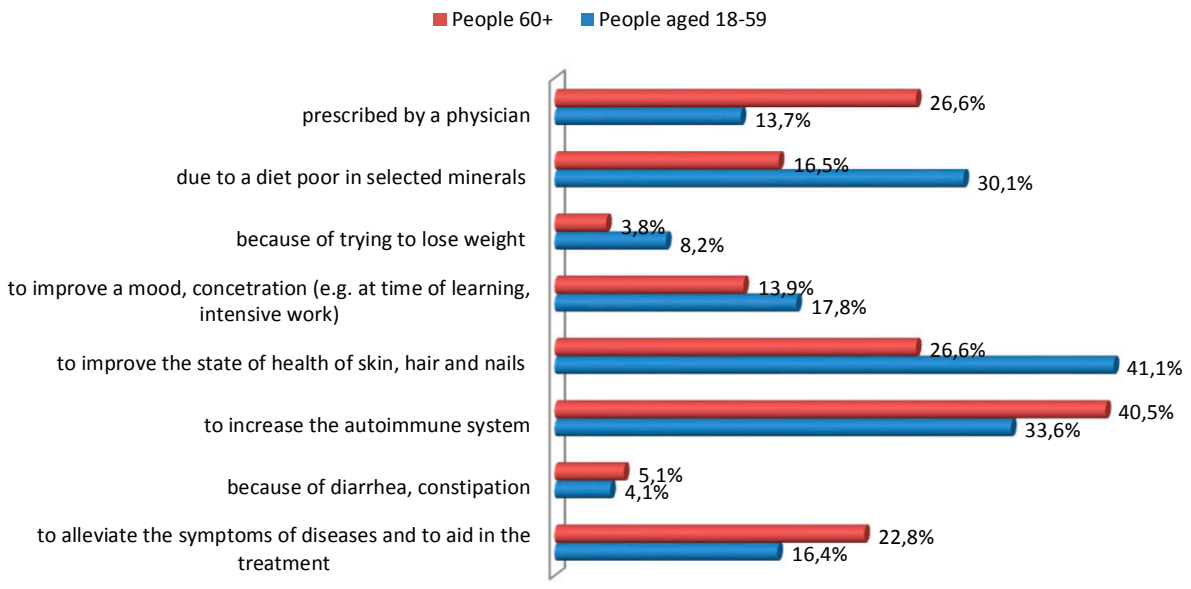


**Figure 1.** The reasons of using dietary supplements by respondents

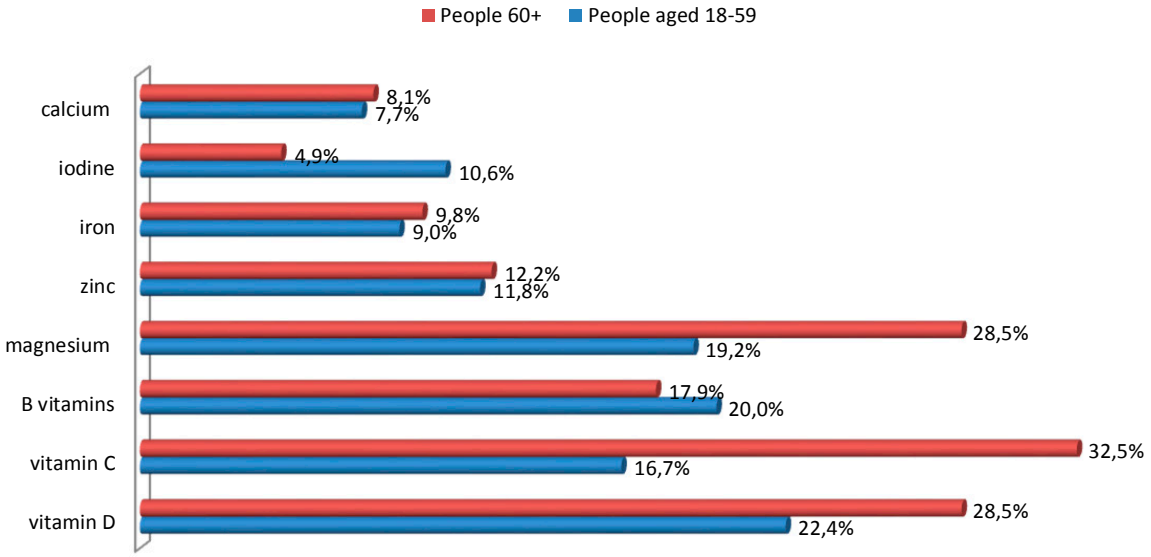


**Figure 2.** The percentage of people using vitamins and minerals

## Discussion

Increasing number of people over 60 years old has been observed in Poland and other developed countries. Drop in birth rates and longer life expectancy of society were the main reasons for this phenomenon. The ageing process is associated with numerous physiological and biochemical changes, leading to difficulties in health maintenance. This is a period, while the border between physiological and pathological processes often becomes invisible. The structural and functional changes following in the ageing organism increase the vulnerability to various diseases. This is particularly visible in a digestive system, which improper functioning, affects to other systems of the body [5, 9]. The adherence to a balanced diet, covering the demand for all nutrients, including vitamins, minerals and trace elements, as well as reducing the risk of diseases is very important in this period of life. Scientific studies indicate that the elderly people do not always follow to nutritional recommendations [5, 10].

Between the years 1994 to 1996 in the United States, Sebastian et al. reported that 42% of people aged 51–70 and 43% of respondents over 70 years old consumed dietary supplements [11]. Similar results were obtained in a study conducted in 1999 among 206 75–80 years old people by Kaluza et al. This research determined that dietary supplements were used by 42% of people [6]. In the last 10–20 years this proportion increased by nearly 20 percentage points. The studies conducted by Saran and Duda between the years 2005 to 2006, concerning using dietary supplements among older adults, have shown that consumption of these preparations declare 64.8% of respondents. In these analyses the similar percentage of population 60+ (64.2%) assured of the dietary supplements intake (more or less regularly) [2].

Studies conducted by Saran and Duda indicated the relationship between using supplementation and the level of education in the group of elderly people. Dietary preparations were more often used by respondents with higher education than the other [2]. In population 60+ over discussed correlation did not occur in these analyses. Only in the group below 59 years old the highest percentage of representatives using dietary supplementation (independently of age) constituted the people with at least secondary education. During the similar research, conducted in 2006 by He et al. among people over 45 years old, indicated no relationship between the education and using dietary supplements. However, with increasing economic status, the frequency of this preparations intake also upswing [12].

As like in the current study, Saran and Duda has not determined a significantly relationship between using the dietary supplements and financial situation or health in the group of surveyed people [2]. Conducted analyses did not confirm the relationship between dietary supplements intake and place of residence, whereas Kaluza et al. and He et al. determined that city inhabitants more often than rural residents absorbed these preparations [6, 12]. The differences

between presented study and analyses other authors may resulted from more interest dietary supplements, accessibility and universality an advertisements of the dietary supplements, also addressed to the consumers from little town and villages.

The discussed study showed that the most common cause of dietary supplements intake in the group of elderly people was a need to boost the immune system. Valid reason for taking supplements was also recommendation delivered by a doctor. A need to improve health was also a main reason for the implementation of supplements by the elderly people in a research executed by Saran and Duda [2]. It is worth noting that in the analyses respondents could choose one of many answers to define causes of dietary supplementation (**Figure 1**), which in most cases were associated with the general health improvement, also considering the need to improve the health condition of skin, hair, and nails (the main reason of supplementation in younger group of respondents). At the same time, it was found that after using dietary supplements, health in the elderly people was insignificantly improved, while the substantial benefits were observed in the second group. The coexistence of many diseases and polypharmacy in the elderly population, as opposed to younger may be a cause of this phenomenon [6].

Vitamin mostly consumed as a dietary supplement was ascorbic acid, as evidenced own study and analysis conducted in the US by Sebastian et al. The American team of researchers determined that vitamin C was used by 33% of respondents between 51–70 years old and by 30% of those over 70 years old [10]. In the analyses conducted by Kaluza et al. ascorbic acid was used by 38% of women and 20% of men. Considering the fact, that the intake of vitamin C in the diet does not cover the demand for this nutrient, ascorbic acid supplementation in the elderly can be justified [6]. Vitamin C is an important antioxidant, reducing the risk of cardiovascular diseases, which are a frequent cause of death, especially in the elderly. Moreover, due to a dental problems occurring in this population, consuming the main sources of vitamin C (mainly raw fruits and vegetables) is significantly reduced [6, 13].

The presented analyses, as well as the work of Kaluza et al. showed a similar percentage of the older people supplementing with magnesium, although the average consumption of this macronutrient with a diet in the elderly was at a sufficient level [6]. Comparable results received Markiewicz et al. who analysed the magnesium intake with food in the elderly people living the Social Care Home in Bialystok, both in the summer how winter. Magnesium is an important component of diet in a prevention and treatment of hypertension and also in reduction the risk of coronary heart disease. Magnesium deficiencies predispose to development of type 2 diabetes and postmenopausal osteoporosis in women, due to the impact on calcium homeostasis [10]. Daily intake of magnesium with diet in the elderly population is usually at the adequate level, so the potential introduction of supplementation should be consulted with specialist, in order to avoid side effects of hypermagnesaemia, especially in patients with renal failure.

The particular attention was given to supplementation of vitamin D, because most of the data in over discussed study were obtained in months with a low sun exposure in Poland. Cholecalciferol deficiencies, resulting from reduced synthesis in older adults, are very widespread among the Polish population. Low blood levels of vitamin D may affect 80% of the elderly, especially living in the nursing homes. In presented analyses only about 30% of older adults declared consuming of this vitamin. It is worth noting that this was a bigger percentage of respondents than in the younger population. The former works showed a disturbing fact of the insufficient knowledge and awareness of people over 60 years old, associated with a necessity of cholecalciferol supplementation during periods, in which endogens synthesis in the cells of skin is quite reduced. A number of scientific reports indicate that the deficit of vitamin D in the elderly people, increase the risk of developing osteoporosis, bone fractures, falls, muscle weakness, cardiovascular diseases, cognitive disorders, and cancers [14, 15].

The popularity of calcium supplementation in presented study in both groups turned out to be very low. According to the studies of various authors examining the intake of this component from food, the elderly people are often exposed on calcium deficiency. The causes of this phenomenon are low dairy products intake (mainly because of lactose intolerance), reducing the vitamin D synthesis in the skin cells or decreasing the calcium binding proteins and protein transport. The studies of Markiewicz et al. and Kaluza et al. confirmed the fact that calcium is a macronutrient, which supplementation in the elderly seems to be justified or even indicated, but only in population without contraindications (e.g. without diagnosed nephrolithiasis). Tufts University's USDA Human Nutrition Research Center on Aging suggested a need of calcium and vitamin D supplementation as part of the basic nutritional recommendations in the elderly [1, 6, 10, 14].

Presented research provided alarming results regarding folic acid supplementation in the elderly. The study conducted by Stawarska et al., evaluating the individual vitamins and minerals intake by elderly people, determined a significant shortage of folic acid. Deficits of folate are very common in the elderly, and also dangerous due to the potential development of cardiovascular diseases, cancers, megaloblastic anaemia, rheumatoid arthritis and because of the predisposition toward development of dementia and deepening depression. Supplementation of folic acid should be used in older adults with confirmed deficiencies of folate, simultaneously with vitamin $B_{12}$ (wherein the absorption is reduced in the elderly). Presented study demonstrated a small percentage of people supplementing B vitamins, including cobalamin. The long-term deficits of vitamin $B_{12}$ in the elderly contribute to the development of hematopoietic disorders manifesting by macrocytic anaemia, increase of homocysteine levels, and neurological symptoms e.g. peripheral neuropathy, demyelination of the white matter of the brain, paresthesias, lethary and apathy. The inadequate blood level of vitamin $B_{12}$ in older adults should be eliminated through the oral supplementation [1, 9, 16–18].

The current study reported that the iron and zinc supplementation is rare. The analyses carried out by Madej et al. among 102 people over 65 years old showed that traditional diet covered the demand for iron of almost all studied people. A different result was obtained in the case of zinc, which turned out to be insufficiency micronutrient in the elderly. This element is important in maintaining the integrity of a number of homeostatic mechanisms, including the immune function. Zinc is also the component allowing sense of taste and smell, proper functioning of the thyroid gland and pancreas, blood clotting, wound healing, cognitive function or appropriate function of the heart. Inasmuch as many studies confirm that older adults are exposed to zinc deficiency, therefore the use of dietary supplements containing this micronutrient in the elderly should be considered [5, 19].

The conducted analyses indicate that every fifth person over 60 years old consume polyunsaturated fatty acids omega-3 (n-3) as a supplement. Jablonowska et al. confirmed the low intake of these nutrients by the Polish population. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) supplementation is particularly important for people, who in daily diet rarely include foods rich in over discussed components (e.g. marine fish, fish products, margarine). Before prescribing supplementation should be encourage to frequent consumption of food containing natural forms of these acids. The numerous scientific reports indicated that n-3 fatty acids minimalize the risk of coronary heart disease, type 2 diabetes, cancers, depression and inflammation. In the study conducted in the US by Farzaneh-Far et al. suggested that the omega-3 fatty acids contribute to slowing down the aging process (by influencing the length of telomeres) [20, 21].

## Conclusion

1) Vitamins and minerals as dietary supplements were common used by elderly people, regardless of education, place of residence, health and financial situation with a minor influence on health benefits. This phenomenon may be a result of co-existence of many diseases in the elderly population, compared to younger, in which the effect of supplementation were more visible.
2) Decisions about supplements intake by the elderly untaken with a physician may protect against health effects caused the abuse of these preparations. Health security is difficult to ensure in young adults and middle-aged people, suggesting the unverified data from the Internet.
3) The low intake of vitamin D, which deficiencies in the elderly are common, as well as the overuse of some dietary supplements without medical consultation, may be a reason of the high risk of hypo- or hypervitaminosis. There is a need to expand education among the elderly in the field of nutritional deficiencies, products rich in over discussed nutrients and potential dietary supplementation.


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

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**Correspondence address:**
Division of Biology of Civilization-Linked Diseases,
Department of Chemistry and Clinical Biochemistry
Poznan University of Medical Sciences
6 Swiecickiego Street, 60-781 Poznan, Poland
phone: +48 61 8546476
fax: +48 618546476
email: katarzynasuliga@poczta.onet.pl

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# Comparison of the quality of life of women with breast cancer after mastectomy and after breast-conserving therapy: prospective observational study

Iwona Głowacka-Mrotek[2], Magdalena Sowa[1], Krystyna Nowacka[2], Tomasz Nowikiewicz[1], Wojciech Hagner[2], Wojciech Zegarski[1]

[1] Department of Surgical Oncology, Collegium Medicum of the Nicolaus Copernicus University in Torun, Oncology Center in Bydgoszcz, Poland
[2] Department of Rehabilitation, Collegium Medicum of the Nicolaus Copernicus University in Torun, Poland

ABSTRACT

**Introduction.** Breast cancer is the most common malignancy in women in developed countries. Treatment of this disease affects the quality of life of patients. Quality of life is an ambiguous concept, which refers to the state of health, severity of symptoms, and implemented treatment. It is also linked to meeting individual needs of each person.
**Aim.** The aim of the study was to assess the quality of life of breast cancer patients according to the type of previous surgery.
**Material and Methods.** The study was conducted prospectively. The study population included 101 women with breast cancer after surgical treatment in the period from October 2012 to October 2014 (51 cases after mastectomy, the remaining ones after breast-conserving therapy). Standard questionnaires EORTC QLQ-C30 and QLQ-BR23 were used to assess the quality of life (assessment on the day of admission to the department, two months and one year after surgery).
**Results.** The analysis of QLQ-C30 revealed no statistically significant differences between the compared groups of patients. Regarding the analysis of QLQ-BR23, statistically significant differences related to the assessment of the patient's own body and life perspectives, evaluation of sexual feelings and social roles (they were not found in the evaluation of sexual functioning, undesirable effects of treatment or symptoms associated with the affected breast).
**Conclusions.** Regardless of the type of surgery performed, breast cancer patients require similar psychological actions supporting their possibility of adapting to the new situation and dealing with negative effects of surgical treatment.

**Keywords:** breast cancer, quality of life, mastectomy, breast-conserving therapy, QLQ-C30 questionnaire, QLQ-BR23 questionnaire.

## Introduction

Quality of life is associated with physical, mental, and social well-being. It is also related to satisfaction with daily functioning [1].

A neoplastic disease may cause numerous unfavorable changes in the daily functioning of patients, thus decreasing their quality of life [2]. The diagnosis of cancer also interferes with patient's social function-

ing; withdrawal mechanisms are activated, followed by limited interpersonal contacts, reduced willingness to take social activity and impaired functioning in social groups [3, 4].

One of the oldest and the largest research groups involved in the standardization of questionnaires to assess the quality of life is the European Organization for Research and Treatment of Cancer (EORTC). The EORTC QLQ-C30 questionnaire is a basic tool for measuring the quality of life of cancer patients regardless of the type, form and location of the primary tumor [5]. The questionnaire includes modules for various diseases. The module for breast cancer consists of 23 questions (QLQ-BR23).

## Aim

The aim of the study was to assess the quality of life of breast cancer patients according to the type of prior surgery.

## Material and Methods

The study was conducted prospectively, on the basis of the consent of the Bioethics Committee of CM UMK KB 226/2011, in the period from October 2012 to October 2014.

The study population included 101 consecutive women with breast cancer scheduled for surgery at the Clinical Department of Breast Cancer and Reconstructive Surgery in Bydgoszcz; 51 patients underwent mastectomy (AMP group), whereas 50 women received breast-conserving therapy (BCT group).

Standard questionnaires EORTC QLQ-C30 and QLQ-BR23 were used to assess the quality of life in both study groups (the version translated by the Institute of Oncology in Warsaw). Assessment of the quality of life using QLQ-C30 and QLQ-BR23 was performed three times (on admission of patients to the Department, two months after surgery, and one year after surgery).

## Procedures

Assessment of the quality of life using QLQ-C30 and QLQ-BR23 was performed three times.

- Exam I – on the day before the surgical procedure, the point of this exam was to show QLQ before surgery,
- Exam II – two months after the surgical procedure; adjuvant treatment had been initiated by that time, the point of this exam was to show QLQ during adjuvant treatment,
- Exam III – one year after the surgical procedure, all patient had finished adjuvant treatment, excluding those with hormone therapy, point of this exam was to show QLQ after adjuvant.

Patient inclusion criteria were as follows:

- provision of an informed consent to participate in a research study,
- patient age between 25 and 70 years.

Patient exclusion criteria were as follows:

- the necessity to radicalize the treatment while in the study (i.e. to perform mastectomy in a patient following previous breast conservation therapy),
- breast reconstruction surgery while in the study.

The statistic package PQStat ver. 1.4.2.324 was used for a detailed analysis of the results. Results of the analyzed parameters of the quality of life were compared between the two study groups (AMP and BCT) using the Mann-Whitney U test. The Friedman test and the post-hoc Dunn's test were used for the evaluation of the results obtained at the subsequent time points (I, II, III) in each study group. The choice of nonparametric methods of data analysis was preceded by checking the normality of distribution using the Shapiro-Wilk test and the Levene's test for homogeneity of variances.

Test probability was statistically significant at $P < 0.05$, and highly significant at $P < 0.01$.

## Results

A total of 101 women were included in the analysis: 50 (49,9%) underwent BCT, 51 underwent MAS (50,1%).

The sociodemographic and clinical characteristics of the operated groups are summarized in **Table 1**. Patients who underwent BCT were older than those who underwent MAS, but there were no statistical differences ($p = 0,8913$). There were no statistical differences with regard to menopausal status and BMI (body mass index). More patients in MAS group had an axillary dissection, than those who underwent BCT. More patients in the MAS group had an advanced stage of cancer than those in BCT ($p < 0,001$). Neoadjuvant chemotherapy was preformed in 23,5% patient in MAS group. All study patients were subjected to post-operative treatment. The most common type of adjuvant treatment in both the MAS and the BCT group included combination of CHTH and RTH. More patient in MAS group had an advanced stage of cancer ($p < 0,001$).

**Table 2** shows the scales of functioning in MAS and BCT groups and relationship between the groups at the subsequent time points on the basis of the EORTC

**Table 1.** Description of study population by age, menopause status, type of axillary operation, neoadjuvant and adjuvant therapy, stage of disease

| Characteristics | MAS (n = 51) | BCT (n = 50) | P value |
|---|---|---|---|
| Age, mean year (SD) | 54.0 (7.91) | 55.2 (9.3) | 0.405 |
| BMI (SD) | 27.26 (5.5) | 26.92 (4.9) | 0.8785 |
| Menopause, n(%) | | | |
| – Yes | 33 (64.7) | 33 (66.0) | |
| – No | 18 (35.3) | 17 (34.0) | 0.8913 |
| Type of axillary operation, n(%) | | | |
| – No operation | 0 (0) | 0 (0) | |
| – Sentinel lymph node biopsy | 32 (62.0) | 18 (32.3) | |
| – Axillary dissection | 32 (67.7) | 19 (38.0) | < 0.001 |
| Neoadjuvant therapy, n(%) | | | |
| – CHTH | 12 (23,5) | 0 (0) | |
| – RTH | (0) | 0 (0) | |
| – HTH | (0) | 0 (0) | < 0.001 |
| Adjuvant therapy, n(%) | | | |
| – CHTH. RTH | 27 (52.9) | 28 (56.0) | |
| – CHTH | 14 (27.4) | 0 (0) | |
| – RTH | 3 (5.9) | 21 (42.0) | |
| – HTH | 7 (13.7) | 1 (2.0) | < 0.001 |
| Stage of disease, n(%) | | | |
| – I A | 14 (27) | 38 (76) | |
| – II A | 17 (33) | 12 (24) | |
| – II B | 11 (21.5) | 0 (0) | |
| – III A | 5 (9.8) | 0 (0) | |
| – III B | 4 (7.8) | 0 (0) | < 0.001 |

BCT – group treated by breast conserving therapy, MAS – group treated by mastectomy, BMI – body mass index, CHTH – chemotherapy, RTH – radiotherapy, HTH – hormone therapy.

**Table 2.** Scales of functioning in MAS and BCT groups and relationship between the groups at the subsequent time points on the basis of the EORTC QLQ-C30

| QLQ-C30 scales of functioning | MAS (n = 51) | | | | BCT (n = 50) | | | | Mann--Whitney U test |
|---|---|---|---|---|---|---|---|---|---|
| | Arithmetic mean | | | | Arithmetic mean | | | | |
| | I | II | III | Test F | I | II | III | Test F | |
| Physical functioning | 82.48 | 81.05 | 83.79 | p > 0.05 | 82.53 | 84.80 | 80.13 | p > 0.05 | I 0.8812<br>II 0.1074<br>III 0.1038 |
| Role functioning | 89.00 | 84.67 | 80.33 | p < 0.001 | 92.16 | 82.35 | 90.20 | p < 0.05 | I 0.8147<br>II 0.6176<br>III 0.0016 |
| Cognitive functioning | 84.31 | 80.72 | 80.72 | p > 0.05 | 84.00 | 83.67 | 81.33 | p > 0.05 | I 0.7937<br>II 0.5144<br>III 0.7316 |
| Emotional functioning | 68.30 | 67.81 | 61.60 | p > 0.05 | 65.17 | 63.78 | 77.67 | p < 0.05 | I 0.6200<br>II 0.3503<br>III 0.0001 |
| Social functioning | 89.87 | 79.74 | 81.05 | p > 0.05 | 85.00 | 78.67 | 76.33 | p > 0.05 | I 0.0241<br>II 0.6224<br>III 0.8068 |
| Overall quality of life | 59.31 | 52.61 | 59.31 | p > 0.05 | 60.17 | 58.00 | 59.33 | p > 0.05 | I 0.1638<br>II 0.6369<br>III 0.3331 |

EORTC QLQ C-30 – European Organization for Research and Treatment of Cancer Quality of Live Questionnaire Core 30, BCT – group treated by breast conserving therapy, MAS – group treated by mastectomy, Test F – the Friedman Test.

QLQ-C30. The assessment included results obtained for MAS and BCT groups at each measurement time point. In the scale of the overall quality of life as well as physical and social functioning, comparison of the results showed no statistically significant differences ($p > 0.05$). The assessment of roles functioning showed statistically significant differences. Patients in the MAS group had highly significant worse scores than patients in the BCT group in III examination ($p = 0.0016$). There was also statistically significant

difference in MAS group in functioning roles between each examination (p = 0,0016). In BCT group QLQ in functioning roles decreased between I and II examination p < 0,05) but one year after surgery increased (BCT, III = 90.20). With regard to emotional functioning patient in MAS group had worse scores than patient in the BCT in III examinations (p = 0.0001). There was also statistically significant difference in BCT group in emotional functioning between II and III examination (p < 0,05) it means that QLQ in emotional functioning increased in that time.

**Table 3** shows evaluation of the symptoms related to the treatment of breast cancer in MAS and BCT groups. and the relationship between the groups at the subsequent time points on the basis of the EORTC QLQ-C30. Evaluation of nausea and vomiting at I study time points revealed statistically significant differences between the results in both study groups. The MAS group has more symptoms nausea and vomiting than BCT (p = 0.038). The results of subjective assessment of insomnia in the period before surgery were significantly different, in the BCT group, sleep disorders were more severe (p = 0.0241). Evaluation of pain showed significantly difference in III examination, in the BCT group pain was more common (p = 0,0120). In the scale of fatique, dyspnoea, loss of appetite, constipation, diarrhea and financial difficulty, comparison of the results showed no statistically significant differences (p > 0.05).

Results obtained using EORTC BR-23 scale in both groups are shown in **Table 4**. When evaluating the quality of life in terms of sexual functioning, undesirable effects of treatment, and symptoms related to the affected breast, there were no statistically significant differences between the compared groups of patients in each examinations. Statistically significant differences were demonstrated for the body image patient in MAS group had significantly worse results than those who underwent BCT (p = 0.0352). Sexual enjoyment was higher in III examination in MAS group was higher than in BCT group p = 0.0104). Complaining of arm symptoms was in I examination higher in the MAS group than in BCT group (p = 0.0045).

**Table 3.** Evaluation of the symptoms related to the treatment of breast cancer in MAS and BCT groups and the relationship between the groups at the subsequent time points on the basis of the EORTC QLQ-C30

| QLQ-C30 scales of symptoms | MAS (n = 51) | | | | BCT (n = 50) | | | | Mann--Whitney U test |
|---|---|---|---|---|---|---|---|---|---|
| | Arithmetic mean | | | | Arithmetic mean | | | | |
| | I | II | III | Test F | I | II | III | Test F | |
| Fatigue | 27.02 | 27.67 | 27.02 | p > 0.05 | 26.44 | 24.67 | 26.89 | p > 0.05 | I 0.8893<br>II 0.2306<br>III 0.9162 |
| Nausea and vomiting | 6.86 | 10.78 | 2.61 | p < 0.05 | 0.67 | 2.67 | 1.56 | p > 0.05 | I 0.0380<br>II 0.0692<br>III 0.8126 |
| Pain | 17.97 | 18.63 | 14.71 | p > 0.05 | 18.00 | 17.00 | 22.67 | p > 0.05 | I 0.9404<br>II 0.3263<br>III 0.0120 |
| Dyspnoea | 9.80 | 12.42 | 10.46 | p > 0.05 | 12.00 | 9.33 | 15.33 | p > 0.05 | I 0.5799<br>II 0.7062<br>III 0.3791 |
| Insomnia | 25.49 | 32.68 | 35.95 | p > 0.05 | 40.00 | 32.00 | 32.67 | p > 0.05 | I 0.0241<br>II 0.6224<br>III 0.8068 |
| Loss of appetite | 8.50 | 11.11 | 9.15 | p > 0.05 | 10.67 | 10.00 | 12.00 | p > 0.05 | I 0.8016<br>II 0.7806<br>III 0.5799 |
| Constipation | 17.65 | 16.99 | 20.26 | p > 0.05 | 12.67 | 14.00 | 18.00 | p > 0.05 | I 0.2771<br>II 0.2817<br>III 0.4715 |
| Diarrhea | 5.88 | 6.54 | 4.58 | p > 0.05 | 4.00 | 6.67 | 6.67 | p > 0.05 | I 0.4407<br>II 0.9081<br>III 0.5892 |
| Financial difficulty | 21.57 | 23.53 | 22.88 | p > 0.05 | 26.67 | 26.67 | 31.33 | p > 0.05 | I 0.8919<br>II 0.5254<br>III 0.0961 |

EORTC QLQ C-30 – European Organization for Research and Treatment of Cancer Quality of Live Questionnaire Core 30, BCT – group treated by breast conserving therapy, MAS – group treated by mastectomy, Test F – the Friedman Test.

**Table 4.** Evaluation of the quality of life based on the EORTC QLQ-BR23 in MAS and BCT groups at the subsequent study time points

| Assessment with QLQ-BR23 scale | MAS (n = 51) | | | | BCT (n = 50) | | | | Mann--Whitney U test |
|---|---|---|---|---|---|---|---|---|---|
| | Arithmetic mean | | | Test F | Arithmetic mean | | | Test F | |
| | I | II | III | | I | II | III | | |
| Body image | 1.53 | 1.57 | 1.61 | p > 0.05 | 1.44 | 1.59 | 1.40 | p > 0.05 | I 0.2468<br>II 0.9756<br>III 0.0352 |
| Sexual functioning | 1.52 | 1.51 | 1.62 | p > 0.05 | 1.61 | 1.50 | 1.58 | p > 0.05 | I 0.3556<br>II 0.8678<br>III 0.9108 |
| Sexual enjoyment | 2.75 | 2.35 | 2.63 | p > 0.05 | 2.28 | 2.45 | 2.04 | p > 0.05 | I 0.1407<br>II 0.7313<br>III 0.0104 |
| Future perspectives | 2.84 | 2.67 | 3.00 | p > 0.05 | 2.86 | 3.04 | 2.42 | p < 0.05 | I 0.9810<br>II 0.1024<br>III 0.0072 |
| Therapy side effects | 1.47 | 1.50 | 1.56 | p > 0.05 | 1.44 | 1.42 | 1.55 | p > 0.05 | I 0.7885<br>II 0.1414<br>III 0.6564 |
| Breast symptoms | 1.47 | 1.60 | 1.46 | p > 0.05 | 1.34 | 1.45 | 1.53 | p > 0.05 | I 0.2151<br>II 0.2189<br>III 0.2567 |
| Arm symptoms | 1.46 | 1.49 | 1.61 | p > 0.05 | 1.15 | 1.35 | 1.39 | p > 0.05 | I 0.0045<br>II 0.2127<br>III 0.0625 |

EORTC QLQ-BR23 – European Organization for Research and Treatment of Cancer Quality of Live Questionnaire breast cancer-specific modules, BCT – group treated by breast conserving therapy, MAS – group treated by mastectomy, Test F – the Friedman Test.

## Discussion

In this study standardize questionnaire (QLQ-C30 and QLQ-BR23) were used to compare the quality of life of breast cancer patients according to the type of previous surgery (total mastectomy vs breast conserving-therapy). The same patient was examined three times (before surgery, 2 month after surgery an one year after surgery). In our study, the overall assessment of the quality of life of patients did not change during the whole study period. Results obtained in our study in this scale ranged from 52.61 to 60.17 points (out of 100 points maximum), and were consistent with the values reported by Ganz et al. [6]. Studies carried out by other authors have demonstrated that the stress related to the treatment process can last up to two years after surgery [7, 8].

Our results showed that some very specific benefits of BCT, such a better body image, better roles functioning, and emotional functioning are visible during one year after surgery. There is a lot of studies focused on the quality of life of women with breast cancer [9]

In our study, there were no statistically significant changes in the overall quality of life as well as physical, cognitive and social functioning of the analyzed subjects, which could indicate an improvement or deterioration of the quality of life of patients throughout the study period, and this has been shown in a number of previous studies [11, 12]. In our study showed that women who underwent MAS had worse scores in social roles and emotional functioning one year after surgery. Similar results for this assessment were obtained in previous study [11]. Symptoms scale in our study showed that patient who underwent MAS had more symptoms nausea and vomiting in first examination that BCT patient. It is strictly connected with neoadjuvant hemotherapy. In other symptoms (fatigue, dyspnea, loss of appetite, constipation, diarrhea or financial problems) no statistically difference were found. However, a common upward trend for the worsening of symptoms was observed in both groups, especially between II and III examination. Similar results, suggesting an increase in the severity of symptoms assessed using the QLQ-C30 were obtained by Arora et al. [12] Evaluation of nausea and vomiting at I study time points revealed statistically significant differences between the results in both study groups. The MAS group had more symptoms nausea and vomiting than BCT. It had connection with adjuvant chemotherapy. Nausea and vomiting are basics side effect of chemotherapy [13].

Analysis of EORTC QLQ-BR23 questionnaire showed that women who underwent MAS had worse

body image than women with underwent BCT. Similar conclusions can be drawn from the other studies [14, 15]. The studies by Ganz et al. showed no differences in the mental functioning of women treated with mastectomy or BCT during the first year after surgery [10].

Statistically significant differences in the assessment of sexual pleasure (a year after surgery) were observed in the analyzed group of patients. More favorable study results concerning sexual satisfaction were obtained in patients after mastectomy. It is worth noting that only sexually active women answered the questions about sexual pleasure. It was observed that questions about this particular sphere of life, especially in women over 60 years old, were frequently omitted in the study. Similar observations were presented in other studies [16].

The treatment of neoplastic disease may also result in financial problems, as well as the feeling of being an incompetent family member. Severity of these changes is related to the age and social function performed by the patient [17].

As it has been shown in studies by Wrońska et al., support provided by the members of the immediate family results in the fact that a significant number of patients requiring mastectomy do not experience the feeling of rejection or isolation [18]. Similar results were presented by Trojanowski et al. [18] and Zanapalıoğlu Y et al. [19], who demonstrated that mastectomy did not result in the deterioration of family relations.

This study is prospective study and has a limitation in QLQ evaluation, there was statistically significant difference between adjuvant therapy in study group. In our study we didn't compare difference between adjuvant therapy and degree of lymph node dissection and the results of QLQ. Other authors noted that both chemotherapy and other methods of antitumor therapy reduced the assessed quality of life of patients [20]. The observation term was one year after surgery. It was stressful time for examined patient. Studies carried out by other authors have demonstrated that the stress related to the treatment process can last up to two years after surgery [21]. Other study demonstrated that higher scores of the overall quality of life in women after BCT were statistically significant five years after surgery [22]. The use of quality of life assessments in breast cancer patients has an significant role as a risk for treatment outcome and prognosis [23–25].

Our study suggest that breast cancer patients who underwent BCT experienced more positive outcomes in roles functioning, emotional functioning, body image. Patient who underwent mastectomy had better results with sexual functioning. Our study findings also that intense physical therapy and psychological intervention is required both in patients who underwent breast conserving therapy and mastectomy. Diagnosed breast cancer, regardless of the type of surgery, resulted in similar changes regarding the evaluation of the quality of life by patients. Perhaps, the phenomenon of "a half-woman complex", attributed to mastectomy as a kind of mental disability, also applies to women receiving breast-conserving therapy.

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

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**Correspondence address:**
Iwona Głowacka-Mrotek
9 Maria Curie-Skłodowskiej Street
85-094 Bydgoszcz, Poland
phone: +48 791225318
email: iwona.glowacka@cm.umk.pl

REVIEW PAPER

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# Glucocorticoids and regulation of brown adipose tissue in humans – physiological and pathophysiological considerations

Aleksander Rajczewski, Magdalena Gibas-Dorna

Department of Physiology, Poznan University of Medical Sciences, Poland

ABSTRACT

This review discusses the effects of glucocorticoids (GCs) on brown adipose tissue (BAT) in the context of obesity prevention and therapy. Due to the unique expression of the uncoupling protein 1 (UCP1), BAT is capable of non-shivering thermogenesis, also defined as a metabolic heat production, related to increased metabolic rate. All processes that contribute to an increase in activity and/or quantity of BAT are able to upturn metabolism, and thus enable the above therapeutic goals to be achieved. GCs may stimulate BAT differentiation and proliferation. In the case of differentiation, the opposite effect of GCs has been also described. Within white adipose tissue (WAT) GCs inhibit the formation of so called beige adipocytes that are functionally and morphologically similar to the adipocytes from BAT. The activity of GCs with concomitant inhibition of WAT browning is mediated by the induction of microRNA-27b (MIR27B) expression. GCs are responsible for the decline in BAT activity as the body ages. Depriving the body of an enzyme responsible for local reduction of cortisone into an active GC-cortisol in BAT (11β-hydroxysteroid dehydrogenase type 1; 11β-HSD1) prevents the reduction of BAT activity. The effects of high doses of GCs on BAT generally depend on the exposure time. Prolonged elevation in GCs level decreases BAT activity. During adrenergic stimulation the effect of GCs on BAT is ambiguous, because both decrease and increase in activity has been described. A full understanding of the GCs impact on brown remodeling in WAT may reveal a discovery of a novel preventive and therapeutic strategies for obesity and possibly other metabolic disorders.

**Keywords:** brown adipose tissue, glucocorticoids, obesity therapy, obesity, browning.

## Introduction

In human body, there are two types of adipose tissue with different locations, structures, colors, and pathologic characteristics. WAT's content is far greater and its main function is the accumulation of triglycerides (TG) and endocrine activity. While WAT builds up energy in the form of TG, BAT being more metabolically active, works for energy expenditure and heat dissipation. A high amount of BAT was primarily found in newborn humans and hibernating mammals. In recent years, using functional imaging techniques, studies have demonstrated presence and physiological significance of BAT in adults [1]. Mature BAT contains higher number of mitochondria than WAT and possess unique ability of non-shivering thermogenesis, possibly due to the presence of mitochondrial uncoupling protein 1 (UCP1) [2]. Through the mechanisms of non-shivering/metabolic thermogenesis BAT accelerates weight loss increasing the basal metabolic rate (BMR) and reduces risk for obesity. Processes associated with BAT activity may be of particular importance for a new therapeutic strategies to treat obesity and obesity-related disorders. Additionally, endo- and exogenous factors (eg. GCs)

that impact beneficial effects related with BAT-stimulated energy expenditure require detailed investigation.

The aim of this review was to present the current state of knowledge about the influence of GCs on brown fat cells and ways through which GCs affect functioning of BAT.

## Effect of GCs on BAT's development and proliferation

### Differentiation

GCs significantly influence BAT differentiation process. Most of the reports confirm their stimulating effect, however, the mechanisms still remain not fully explained. GCs stimulate gene expression of proteins that are crucial for non-shivering thermogenesis in humans [3]. GCs deficiency inhibits the expression of genes responsible for storage of triglycerides (TG) in the mice's differentiated brown adipocytes [4]. They also affect factors inhibiting thermogenesis and differentiation, e.g. PREF-1 [5]. Armengol et al. [6] have demonstrated that mice treated with dexamethasone (DEXA) exhibit suppression of PREF-1 down-regulation observed during differentiation of brown preadipocytes, and increase in transcription factor C/EBPδ (CCAAT-enhancer binding protein delta). They suggested that induction of C/EBPδ by GCs may provide an indirect mechanism for stimulation of PREF-1 gene by GCs in brown preadipocytes. Complete understanding of the GC's influence on BAT differentiation may play role in the future procedures that therapeutically target this process. However, given that the current results are still incomplete and often conflicting, more studies are required.

### Browning of WAT

In the WAT, GCs affect formation of so called beige adipocytes (browning of WAT), which are functionally and anatomically related to brown fat cells by the ability to induce thermogenesis due to their high mitochondrial UCP1 content [7]. It has been demonstrated that corticosterone inhibits formation of beige adipocytes in the mice's inguinal WAT [8]. Studies investigating the effect of dexamethasone on the WAT browning have revealed the role of MIR27B in this process [9]. DEXA induced MIR27B expression, which in turn, by affecting the three major untranslated regions of PRDM16 mRNA, led to inhibited transformation of WAT into BAT [10]. GCs stimulated expression of MIR27B gene through GC-receptor dependent mechanism at post-transcriptional level. Similar results were observed when MIR27B function was antagonized and mice treated with DEXA *in vivo* exhibited an efficient induction of WAT browning [9]. Therefore, it has been suggested that antagonizing MIR27B may serve as a new target for the obesity prevention and/or treatment. Additionally and perhaps more importantly, this process can obstruct GC's unfavorable influence on the WAT in the context of the development of central obesity [11]. Thus, patients with chronically elevated levels of GCs (eg. with Cushing syndrome, or being treated with high doses of GCs) may benefit from these new discoveries.

### Proliferation

An in vitro study by Barclay et al. [3] has shown that DEXA markedly stimulates the proliferation and differentiation of brown preadipocytes, while lowering the proliferation of white preadipocytes. On the other hand, DEXA inhibited adrenergic stimulation of the cultured brown adipocytes indicating a complexity of GC action within fat cells.

### BAT ageing

Animal model studies have shown that as the body ages, expression and activity of an enzyme capable of converting inactive cortisone into active cortisol (11β-HSD1) increase [12]. Cortisol is likely to impair BAT's thermogenic activity. It has been observed that aged 11β-HSD1 deficient mice exhibit a higher level of UCP1, and hence the desirable for non-shivering thermogenesis characteristic of brown adipose tissue, as well as greater number of mitochondria in the brown fat cells [12]. Further research on the 11β-HSD1 may provide a background for the genotherapy of obesity, because reducing local cortisol level within BAT can ensure significant thermogenesis and increase BMR.

## Comparison of the effects of high concentrations of GCs on metabolic effect in BAT according to exposure time

### Chronic effect

GCs in high concentrations cause various effects depending on BAT exposure time to their elevated level. Several authors have shown that in response to the chronic GCs' influence, thermogenesis rate is decreased [12, 13] and oxygen consumption in brown adipocytes is reduced [9]. It has been postulated that these processes may also occur in the human body [12]. In another study, ablation of LSD1 (lysine-specific demethylase 1 responsible for the repression of 11β-HSD1), in mice showed enhanced 11β-HSD1 activity accompanied by

elevated GCs level and disturbances in BAT metabolism [14]. Chronic exposure to GCs accompanies, among others, abdominal obesity and hepatic steatosis [13], and DEXA in a long term interaction with BAT may work as one of the major causative factors for "buffalo hump" in Cushing's syndrome [3]. It was also discovered that cold-induced increase in adrenergic activity partially reverses hypertriglyceridemia triggered by GCs via stimulation of BAT metabolism [8]. Given the negative effect of chronically elevated GCs on BAT thermogenic activity, it is clinically important to understand and evaluate all the processes and factors that may participate in inhibition of GCs' influence on BAT.

### Short-term effect

Nonshivering thermogenesis as a defense against cold and obesity/obesity related disorders occurs in response to cold-induced β-adrenergic stimulation. As shown by authors, intracellular conversion of thyroxine (T4) to triiodothyronine (T3) is essential for the optimal thermogenic function of BAT [15, 16]. T3 is required for and potentiates the adrenergic stimulation of deiodinase 2 (DIO2) activity in BAT, which participates in the formation of T3. Depending on the time of exposure and concentration of GCs, DEXA and hydrocortisone regulate adrenergic stimulation and DIO2 activity in different ways. In a short time high doses of hydrocortisone (1–10 μM) inhibit DIO2, whereas low doses (1–100 nM) of hydrocortisone and longer time of exposure to DEXA results in an increase of DIO2 activity [17]. This observation allows researchers to consider GCs as a potential factors stabilizing activity of DIO2 and mRNA of DIO2.

Although a large number of studies confirm negative acute effect of high dose GCs on metabolic thermogenesis in BAT [3, 18], Ramage et al., in their recent work, reported that prednisolone significantly increases the uptake of 18-fluorodeoxyglucose by brown adipose tissue in lean healthy men during exposure to mild cold (16–17°C). This was followed by an increase in energy expenditure, likely by increasing BAT activity. The same study provided significant observation regarding different effects of isoprenaline on intracellular respiration and UCP1 stimulation in human versus mice BAT. In human primary brown fat cells GCs increased stimulated by isoprenaline intracellular respiration and UCP1 but significantly reduced both parameters in primary murine beige and BAT adipocytes [19]. Taken together, the existence of non-consistent findings about how GCs influence BAT metabolism in humans suggests that any interpretation should be made with caution and further replicate experiments are required. In this connection, it should be borne in mind that there are anatomical and functional differences regarding brown adipocytes in mice as compared with humans [7]. In mice BAT is more important for the survival in a dynamic temperature environment generating heat and to maintaining constant body temperature in response to cold acclimation (up to 60% of entire animal's energy expenditure [20]), whereas human BAT plays similar role only in neonates. In adult humans brown fat cells are more significant for the metabolic processes and their depots significantly decline with ageing [21].

Full understanding of the short-term effects of GC on BAT under adrenergic stimulation is clinically relevant. For example, short-term BAT exposure to GCs administered by local injections might increase BMR and, therefore, could possibly serve as a helpful additional procedure controlling body weight for patients who cannot be physically active for medical reasons.

## Summary

GCs may influence beneficial effects related to BAT-stimulated energy expenditure. Based on the previous studies, one may conclude that the effect of GCs on brown adipocyte metabolism is rather complex. Recently, particular attention has been paid to the interaction between GCs and PRDM16, 11β-HSD1 proteins/microRNA (MIR27B), which are all recognized as key regulators in the BAT energy management. Understanding the impact of GCs on human BAT can be an attractive medical background for medical purposes in order to develop a novel strategies to combat obesity. Thus, the main targets of future therapeutic procedures may include induction of the brown fat formation, browning of WAT, as well as stimulation and maintaining of brown and beige fat cells activity.


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**Correspondence address:**
Magdalena Gibas-Dorna
Department of Physiology
Poznan University of Medical Sciences
6 Swiecickiego Street, 60-781 Poznan, Poland
phone: +48 618546540
email: physioplus@wp.pl

REVIEW PAPER

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# From the carrier of active substance to drug delivery systems

Barbara Jadach

Department of Pharmaceutical Technology, Faculty of Pharmacy, Poznan University of Medical Sciences

ABSTRACT

Development and innovation all the time are in interests of pharmaceutical science and evaluation of different dosage forms. They are concerned with the aim of compliance of patients. All the time different research groups try to develop and improve form of drugs to receive better bioavailability or strict control of dose, place and time of action of active substances. This is possible by using different excipients, biodegradable, biocompatible polymers that work like carriers, developing simple drug delivery systems, which in time became more and more complicated, nanotechnology that control size, shape and multi-functionality of particulate drug delivery systems. This review shows the main directions in the evaluation of pharmaceutical researches from simple carriers of active substances to drug delivery systems.

**Keywords:** carrier, drug delivery system, sustained release, zero-order kinetic, nanoparticle.

## Introduction

Modern pharmaceutical technology is focused on reaching the target therapeutic effect while minimizing adverse side effects of medicinal substances called API (Active Pharmaceutical Ingredient). Hence, for many years, research centers and research and development departments deal with the development of new APIs, but also the search for novel carriers that could improve bioavailability of APIs. Research and development on the use of different carriers are closely related to the development of drug delivery systems (DDSs) and controlling the active substance release from such systems (controlled drug delivery systems CDDs) [1–4]. They provide drug delivery in the right dose, at a specific location of the body, and its release for a defined period of time.

The dosage form is a mixture of the active substance with excipients which has been given for the required form of drug. After administration of the dosage form the drug release appears at the place of administration, as for example, oral administration of a tablet involves the dissolution of the active substance in the stomach, and thus the passage of substances from the solid form to the solution. The next steps are the absorption, distribution, biotransformation, elimination. During the absorption of the drug substance passes (penetrates) into the blood, crossing biological membranes. Prolonged drug delivery is obtained by special processes, which provides a single dose to quickly reach the minimum concentration of acting substances in the body and maintaining it for a longer time than with a conventional form with immediate release of API (unmodified) [4]. Most of the new APIs are poorly soluble in water and thus have a low bioavailability. That is why, researchers work not only with sustained release but also with increasing the solubility of APIs.

## Main directions of research and development in pharmaceutical technology

Reviewing trends in the development of drug delivery systems can be noticed few periods [1–4] that are described in **Table 1**.

All activities in the development of pharmaceutical dosage forms are associated with the need to search for excipients which allow to control the rate of release of active substance. They will provide an improvement in bioavailability or localize action of the active substance in a specific place [3].

**Table 1.** Trends in the development of pharmaceutical technology

| Period of time | Area of research | References |
|---|---|---|
| 40 and 50-ies of 20$^{th}$ century | – the study on the sustained release of the drug substance, the aim to extend the duration of action of drugs | 5–9 |
| 60 and 70-ies of 20$^{th}$ century | – first reports of the use of implant with zero-order kinetics of the drug release in vivo;<br>– intensive development of delivery systems with zero-order kinetics and controlled release;<br>– therapeutic systems Ocusert® and Progestesert® were introduced into the treatment | 10–23 |
| 80-ies of 20$^{th}$ century | – theoretical analysis of the kinetics of release of drugs;<br>– nasal, oral and mucoadhesive adhesion systems for the application 1 or 2 times per day, based on the release of the drug substance by dissolution, diffusion, osmosis or ion exchange;<br>– OROS (Osmotic Release Oral System) was introduced into the treatment | 24–25 |
| 90-ies of 20$^{th}$ century | – intelligent polymers and hydrogels activated by changes in pH or temperature;<br>– development of nanotechnology: nanoparticles obtained using biodegradable polymers, micelles, dendrimers | 27–29 |
| since 2000 | – modular systems for the targeted activity, a long-term action with little initial burst effect;<br>– developing a correlation of in vitro – in vivo (prediction of drug release in the body on the basis of the dissolution studies in vitro);<br>– intensive development of nanotechnology | 30–38 |

## Dosage forms with prolonged release

The first attempts to use the form of drug with slow release were conducted in the years 1932 to 1937, when the implantation of sterile pellet containing hormones were used. These pellets were invented in 1861 by Lafage [4].

Introduction to the treatment the first antibiotic penicillin resulted in the need to control its concentration in the blood. Penicillin has a short half-life ($t_{0.5}$ = 0.5 h), and therefore for effective therapy it should be administered by injection every 3 h. Romansky and Rittmann [5] used in 1944 the suspension of sodium salt of penicillin in oil with some wax instead of the aqueous solution. The dose of penicillin 200 000 IU in the form of suspension provided suitable concentration in the blood for 12 h after administration [5]. The next step was to use in 1945 calcium salt of penicillin (sparingly soluble salt), with increasing amount of wax in the formulation. After administration of 300,000 IU in such dosage form the concentration of penicillin was maintained in the blood for 12–24h [6]. In 1948 E. Lilly Laboratory introduced the preparation Duracilin, including the composition of the suspension of procaine penicillin in oil, which provided further extension of the action.

Another way of achieving a depot effect was the investigation prepared by H. and A. Choay in 1947 who increased the size of the molecule of insulin by forming an adduct with polyvinylpyrrolidone. Thus prolonged the duration of action of insulin [8].

In the same time in United States Smith, Kline and French Laboratories (SK&F) ran research concerned with enteric coatings. The results were the starting point for Blythe's concept, based on involving the use of many small coated beads to release the drug substance independently of environmental pH. The technique of their manufacture was developed by MacDonnell [4]. On sugar pellets various lipid coating was applied to give different release. The first oral formulation of prolonged action were Dexedrine® Spansule introduced into medical practice in the United States in 1952. This product was based on gelatin capsules filled with pellets coated with various waxes.

In 1950s in Europe Saunders and Srivastava put forward the concept of placement of the therapeutic substances to ion exchange resins in order to obtain a prolonged action [8] and this idea was patented by Keating or Hays at the beginning of 1960s [10–12]. Ion exchange resins become "carriers" for acting substances and started to be used for the formulation of the dosage form.

Ion exchange resins produced from polymers containing functional groups capable of ion exchange

have been known since 1938. The cation exchange resins contain acid groups and form the connection with basic drug substances, while anion exchangers contain basic groups and combine with acidic substances to form salts. The resulting connection drug-ion exchange resin is insoluble in water but after oral administration in the digestive tract they release the active drug, thereby providing sustained release [4, 29, 40]. Initially the use of ion exchange resins emphasized their advantages for extending the release of acting substances, but over time it was observed, that long-term use may cause disturbances in electrolyte in the body due to the reverse ion exchange [4].

The 1950s were a period of intensive research on the development of solid dosage forms of prolonged action. Two-layer tablets occurred during that time, for example SK&F Company introduced a tablet with theophylline consisting of two layers, from one layer acting substance is released immediately and the other layer release theophylline slower. Ciba firm introduced to the market product Lontab® containing a prolonged core surrounded on all sides by the layer immediately release the drug substance [4]. The same ideas are used by other pharmaceutical companies all the time.

The next stage of the development of oral sustained-release tablets was the preparation of tablets that contain insoluble in gastric juice coat between the tablet core and the immediate release coat (Duplex). Also compressing the mixture of granulates with different release rates or the introduction of matrix tablets of plastic or polymers developed modified release products [4]. Such formulation was patented in 1959 by Fryklöf, Sandwell and Ostholm (Duretter®). In the same year the first oral liquid dosage form of the sustained release was also developed [9], using for the first time hydrogenated castor oil and ethylcellulose as excipients.

## Dosage forms that release drug with zero-order kinetic

Further development of oral forms of drugs was based primarily on the search of formulations that release the acting substance according to zero-order kinetics, that is, the speed of release is independent of the amount of the substance remaining in the form. The consequence of this was the development of technologies of therapeutic systems. Between 1964 and 1966 Folkman and co-workers [13, 15] proposed a drug delivery system in the form of implant of Silastic® material (rubber silica). They used the term "carrier" as first with respect to the excipient regulating the release rate of the drug.

Chemist A. Zaffaroni, who in the late 60s of 20$^{th}$ century founded the company ALZA, was inspired with Folkman's work. ALZA intensively took up the idea of controlled drug delivery systems. Zaffaroni cooperated with J. Folkman and also with T. Higuchi, who was a pioneer in the study of mechanisms of release of therapeutic substances with controlled systems [1]. Higuchi gave the basis of studies of the kinetics of release of acting substances from dosage forms that are used to this day.

Folkman's suggestions caused also that Zaffaroni introduce in 1971 the term "Therapeutic system". It was identified as a device or dosage form comprising the drug substance (or mixture of substances) that is released continuously at a predetermined rate for a predetermined time and at a particular site of administration [4, 17]. The company ALZA introduced therapeutic systems: Ocusert® (eye system) and Progestesert® (intraurethral), developed therapeutic systems in the form of skin patches [1, 18], and in 1974 patented oral therapeutic system OROS®.

## Micro- and nanoparticles as carriers and drug delivery systems

In the 60s of the 20$^{th}$ century, the research on drug delivery systems, are beginning to include not only systems in the "macro", there is also interest in scale "micro" and "nano" [1]. For the first time at the University of Cambridge A.D. Bangham discovered liposomes [14] and Schmitt and Polistina from Davis & Geck Company, Cyanamid Co. synthesized and patented polymer of glycolic acid (PGA), which has been used as biodegradable carrier [16].

Liposomes are small structure in which is possible to place both hydrophilic and lipophilic drugs. They are constructed with one or more phospholipid bilayers closing the interior aqueous phase. The interest in liposomes as carriers of therapeutic substances developed primarily G. Gregoriadis [20, 21]. Number of publications have appeared describing possible use of liposomes as carriers for anticancer drugs [39,41].

Polymers are macromolecular structure capable of forming a micro-/nanocapsules or micro-/nanospheres. Polymer of glycolic acid (PGA), polymer of lactic acid (PLA) and copolymer of lactic acid and glycolic acid (PLGA) were the first to be used. In the late 60s in the Du Pont company Boswel and Scriber used PLA to connect it with protein drugs. They produced microparticles that worked like depot drug delivery system. Technology of preparation of microparticle

has been patented by Boswel and Scribner in 1973 [19]. Boswel in U.S. patent used the term "carrier or matrix" to the PLGA used in order to obtain sustained release of the drug [1, 19]. At the same time Speiser and Kreuter [22] also use methacrylic acid polymer (polymethacrylate methyl) to obtain polymer nanoparticles.

The 70s of the 20th century was a period when polymeric nanoparticles with a diameter of 100 nm were used for the first time and polymers become the basis for the "carriers" of acting substances [2]. Polymers have been used in the preparation of sustained release drugs, and their main task is to ensure a therapeutic level of the acting substance in the body of the patient for a defined period of time without taking next dose during the day [25, 33]. The idea of using micro or nanoparticles in drug delivery began over five decades ago, and the unique skills of small particle size in drug delivery have been appreciated by scientists. From now term "carriers" refers not only to the additives, but also for systems with small size like: liposomes, polymeric micro- and nanoparticles. The terms "carrier" and "drug delivery system" are combined or used interchangeably. In the following years, new reports on the use of biodegradable or non-biodegradable polymers and the development of new micro and nanoparticle methods are emerging.

The most popular nanosystems include hydrogels, cyclodextrins, liquid crystalline phase and nanoparticles: liposomes, polymeric nanoparticles, polymeric micelles, nanoparticles of silica, gold, silver or other metals, carbon nanotubes, solid lipid particles, niosomes, dendrimers and hybrid particles with a porous core [32,34–39].

In the 80s, it is launched a new line of research. In 1984, Hiroshi Maeda of the University of Kumamoto discovered enhanced penetration and retention of nanoparticles (EPR). At this point, the idea of developing a drug form with targeted action. Maeda used in studies styrene-maleic anhydride (SMA) conjugated to the anti-cancer peptide drug, neocarcinostatin (NCS), which he called "SMANCS" and he had labeled the conjugate with a dye [26]. He noted that the dye accumulated in tumor tissue, on this basis concluded that the vascular system created around the growing tumor is leaky, so that allows to collect the drug in the tissue [1, 26].

In the 1980–90s, from drug delivery systems in scale "nano" were developed especially PEGylated polymeric micelles and liposomes. In Japan, K. Kataoka, T. Okano, and M. Yokoyama synthesized poly(ethylene glycol)-poly(aspartic acid) a block copolymer [27].

The new compound showed the ability to form the PEGylated micelles. It was possible to load small drug molecules in micelles on the basis of the physical load or connection with free amino or carboxyl groups. The free hydroxyl groups of the polyethylene glycol were ligands that allowed the micelles reach the tumor cells [1, 27]. At the same time, in the US, A. Kabanov worked out PEGylated micelles produced with Pluronic, nonionic triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) (PEO-PPO-PEO) [1].

Since the 90s there is a lot of reports about the use of polymeric micelles or liposomes as carriers of acting substances that are able to achieve the targeted action [1]. Gregoriadis and Torchilini developed many liposome formulations, both for imaging and drug delivery systems where the hydrophilic medicinal substances may be placed inside the liposomes and hydrophobic materials in the lipid bilayer. PEGylated liposomes containing doxorubicin have been introduced into medical practice as Doxil ® in 1995 [1].

The "nano" DDS systems are constantly being developed, including dendrimers or other branched polymer systems [1]. Dendrimers were synthesized for the first time in the period 1970–1990 by two different groups: Buhleier and co-workers and Tomali and co-workers [23, 24]. In contrast to the polymers with linear structure dendrimers developed by these two groups have precisely controlled spatial structure [38, 42].

These materials have a uniform and well-defined size and shape, and therefore are of great interest in the biomedical sciences. They have the ability to penetrate cell membranes, and are not rapidly eliminated. The high degree of order of the spatial structure causes that seem to be the ideal carriers [38, 42, 43]. They may be used in controlled release systems applied intravenously or orally, directly into the lungs, as a system on the eye or on the skin. After joining the respective ligand they can also be used for targeted therapy [31, 42, 40].

As already mentioned, most of the emerging medical substance is poorly soluble in water and therefore has low bioavailability. Hence, there is a desire to improve the solubility of such APIs thus improving the availability of drug and later bioavailability.

There are many methods to improve the availability of pharmaceutical drugs sparingly soluble in water. The appropriate "carriers" that influence the improvement of API solubility can be found here. Among the methods which used carriers can be distinguished: the formation of complexes, eg. with cyclodextrins, modification of the crystalline form by loading to mesoporous silica materials or the use of lipid carriers.

The first cyclodextrins were discovered in 1891 by Villiers [44]. The following years were the precise characteristics of the structure and the development of the theory of their use. The formation of complexes of cyclodextrins and drugs was first used in the 70s of 20$^{th}$ century [45]. Cyclodextrins are characterized by the presence of hydroxyl groups at the surface, making them soluble in water. Its interior forms a hydrophobic microenvironment suitable for encapsulating drugs that dissolve better in lipids [46].

Mesoporous silica materials were synthesized in the late 90s of the last century. Due to its characteristics: high surface area and the pore volume became of interest as carriers of drugs and such a use for the first time proposed Valet-Regi and co-workers [47]. From that time these materials are used for loading drug substances which can improve the solubility of the API by changing and/or prevent the formation of crystalline form of API.

In recent years, there is a great interest in self-emulsifying drug delivery systems (SEDDS) forming spontaneously microemulsions (SMEDDS) or nanoemulsions (SNEDDS).

SEDDS formulations are simple binary systems: lipophilic phase and drug, or lipophilic phase, surfactant and drug. A lipid component prevents sudden precipitation of API and surfactant present in the system improve the wetting and the penetration of the dissolution fluid [36]. In such a type of dosage form micro-/nanoemulsion place role of "carrier" and "delivery system" at the same type. Lipid carriers are used since 1996 when Müller and Lucks introduced solid lipid particles (SLN) and patented the method of their production towards the high-pressure homogenization [28, 37].

## Conclusion

All the time continuous improvement of controlled drug delivery systems is observed. The development of knowledge of molecular biology and medicine enable the manufacture and use of carriers apply to the "nano", which will be able to be taken up by specific cells/receptors so that activity of the drug will be directed at specific places and routes inside cells. Increased ability to control the efficiency and specificity of the delivery process will minimize side effects. Knowledge of the processes and DNA sequence encoding the disease could be used to create personalized medicines. The development of controlled drug delivery systems has evolved from macro-, micro- and nano- by using polymers at each stage.


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**Correspondence address:**
Barbara Jadach
Department of Pharmaceutical Technology
Poznan University of Medical Sciences
6 Grunwaldzka Street, 60-780 Poznań, Poland
email: bajadach@ump.edu.pl

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# The effect of endometrioma on ovarian reserve

Małgorzata Szczepańska[1], Paweł P. Jagodziński[2], Ewa Wender-Ożegowska[1]

[1] Department of Obstetrics, Gynecology and Gynecological Oncology, Division of Reproduction, Poznan University of Medical Sciences, Poland
[2] Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland

ABSTRACT

An ovarian endometrioma is a very common form of endometriosis in women of reproductive age. This review presents the current state of research on ovarian reserve in women with ovarian endometriomas. Endometrioma can negatively affect ovarian markers: the anti-Müllerian hormone (AMH), antral follicle count (AFC) and *in vitro* fertilisation (IVF) results. Decisions on the surgical treatment of endometrial cysts should be carefully thought through, especially in women who have not given birth.

**Keywords:** endometriosis, AMH, ovarian reserve.

Most ovarian cysts which are recognized in women of reproductive age are related to ovulation and are resolved spontaneously. Ovarian endometrioma is the most common appearance of endometriosis and accounts for 17–44% of women with this enigmatic disorder [1, 2]. The natural course of an endometrioma is unknown, because of the lack of follow-up studies involving untreated women with endometrial cyst [3]. As with dermoid cysts and cystadenomas, endometriomas require surgical treatment. In contrast to other non-functional, benign ovarian tumors, endometrioma *per se* and cystectomy of endometriomas negatively affect ovarian reserve. This article will review the literature on the impact of endometriomas on ovarian reserve. The pathogenesis of infertility in women with endometriosis is not fully understood, except in the case of distorted pelvic anatomy. Among the reasons mentioned as the cause of the inability to become pregnant, impaired folliculogenesis, poor quality of oocytes and embryos and implantation defects are most common [4, 5]. The effect of endometriomas on fertility has not been fully established, although there is some evidence of abnormal physiological mechanisms of ovulation in ovaries with endometriomas, the exact causes are unknown. The inflammatory reaction––typically associated with the presence of endometriosis––may play a role [6]. Another possible mechanism could be mechanical damage to the ovarian tissue or disturbance of the vascularization of the organ by the presence of an expanding ovarian cyst [7].

Currently, there are insufficient data to clarify whether endometrioma- related damage to the ovarian reserve precedes or follows surgery.

An endometrioma is best defined as an ovarian pseudocyst developing from metaplasia of the coelomic epithelium [8] or originating from ectopic endometrial tissue, which constantly invaginates the ovarian cortex [9]. There are several prospective studies, which indicate that patients with endometriomas have reduced ovarian reserve [10, 11]. Ovarian reserve is defined as the functional potential of the ovary that reflects the number and quality of the remaining primordial follicles left in the ovary at any given time [12]. In clinical practice, it is possible to measure only the functional ovarian reserve (maturing, growing follicles), which represents a small percentage of the total ovarian reserve (including non-growing follicles). AFC is the number of small (2–10 mm in diameter) antral follicles. It can be decremented by transvaginal ultrasonography between days 2 and 4 of the menstrual cycle [13].

The most common laboratory tests which are used to measure ovarian reserve are the AMH and follicle stimulating hormone (FSH) tests. AMH belongs to the transforming growth factor-beta family, and is produced by the granulose cells of primary, pre-antral and small antral follicles. Serum AMH levels represent the most reliable marker of ovarian reserve, because they are menstrual cycle independent and are not subject to alteration under the influence of contraceptive pills or gonadotrophin releasing hormone agonists [14].

In a prospective cohort study by Hwu et al. (2011), the impact of unilateral and bilateral endometriomas on serum AMH levels was compared both before and after surgery. The mean baseline serum AMH level was significantly lower in patients with bilateral endometriomas (n = 32) compared to that of patients with unilateral endometriomas (n = 109) (1.56 ± 0.24 (SEM) vs. 2.45 ± 0.17 ng/ml, P < 0.05). The mean serum AMH level was significantly lower in patients treated with bilateral cystectomy than in patients treated with unilateral cystectomy (1.01 ± 0.11 vs. 1.48 ± 0.14 ng/ml, P < 0.05) [10].

In another prospective study, Goodman et al. (2016) evaluated patients with endometriomas (n = 58), pelvic endometriosis (n = 29) and volunteers with no endometriosis (n = 29) to assess AMH levels before and after surgery. This study also demonstrated that baseline AMH values were significantly lower (45%) in the endometrioma vs negative laparoscopy group (1.8 ng/mL [95% confidence interval, 1.2–2.4 ng/mL] vs. 3.2 ng/mL [95% confidence interval, 2.0–4.4 ng/mL]; *P* < 0.02). Only patients with endometriomas had a significant decline in ovarian reserve at 1 month after surgery (-48%; 95% confidence interval, -54 to -18%; *P* < 0.01; mean AMH baseline value, 1.77–1.12 ng/mL at 1 month after surgery). Six months after surgery, AMH values continued to be depressed from the baseline, but these were no longer significantly different [11]. The impact of endometriomas on ovulation and hyperstymulation have been intensively studied [7, 15–18].

In 2008 Horikawa et al. investigated the rate of ovulation in 28 infertile patients with unilateral endometriomas and found a 34% ovulation rate in affected gonads [15]. In a study published in 2009, Benaglia et al. examined ovulation rates in 70 women, based on the assumption that the expected ratio of ovulation in healthy and affected ovaries is about 1:1. Ovulation occurred in affected ovaries in only 22 cases (31%; 95% CI: 22–43%), this difference was statistically significant (*P* = 0.002) [7]. In a prospective study, Somigliana et al. (2006) evaluated responsiveness to ovarian hyperstymulation during IVF-ET cycles in 36 women with unilateral endometriomas who had not undergone previous ovarian surgery. In this study, the number of codominant follicles developing in affected gonads was reduced when compared with the contralateral intact ovaries of the same patients [16]. In a multicenter retrospective cohort study, Benaglia et al. (2013) explored ovarian responsiveness and oocyte quality in 39 unoperated women with bilateral endometriomas and 78 control women. The number of developing follicles and the number of oocytes retrieved was significantly lower in women with bilateral endometriomas. The total numbers of developing follicles in case and control subjects were 9.6 ± 3.3 and 14.1 ± 6.8, respectively. The numbers of oocytes retrieved were 7.1 ± 3.2 and 9.8 ± 5.5, respectively. However, this did not lead to significant differences in the quality of oocytes obtained or the chances of pregnancy [17]. Similar findings were demonstrated by Hamdan et al. (2015) in a meta–analysis, which included 33 studies. Compared with healthy women, women with endometriomas had a lower mean number of oocytes retrieved, required a higher FSH dosage for ovarian stimulation and had a lower AFC, suggesting that their ovarian reserve was diminished prior to IVF/ICSI [18].

Based on this literature review, it can concluded that the presence of endometriomas reduces ovarian reserve. Decisions on the surgical treatment of endometrial cysts should be carefully thought through, especially in women who have not given birth. According to Carvahlo et al., the assessment of AMH levels prior to surgery should be measured in order to discuss the risks and benefits of surgery with patients [19].

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**Correspondence address:**
Małgorzata Szczepańska
Department of Obstetrics,
Gynecology and Gynecological Oncology
Division of Reproduction
Poznan University of Medical Sciences
33 Polna Street, 60-535 Poznan, Poland
phone: +48618419302
fax: +48618419625
email: mal.gin@poczta.fm

CASE STUDY

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# Surgical treatment of combined thoraco--abdominal injury of liver with the mechanism of the hydrodynamic shock

Igor Khomenko[1], Vitalii Shapovalov[2], Ievgen Tsema[1, 3], Georgii Makarov[1], Roman Palytsia[1], Andrii Dinets[3], Ievgen Zavodovskyi[3], Ivan Ishchenko[1]

[1] National Military Medical Clinical Center of Ministry of Defense of Ukraine, Ukraine, Kyiv
[2] Military Medical Clinical Center of South Region of Ministry of Defense of Ukraine, Ukraine, Odesa
[3] Bogomolets National Medical University of Ministry of Health of Ukraine, Ukraine, Kyiv

ABSTRACT

This article presents a clinical case of successful multilevel treatment the combined gunshot wound of liver with the mechanism of hydrodynamic shock using «damage control» tactic. The patient received a combined thoraco-abdominal injured as a result of warfare on the east of Ukraine. The blind fragmental penetrating trauma of the chest cavity led to the rupture of the right dome of diaphragm and liver ($S_3$–$S_6$) by the mechanism of hydrodynamic kick (fragments the wreckage and input/output openings in the abdominal cavity were not found). The «damage control» tactic was implemented as follows. On the second level of medical care the rupture of the liver parenchyma eliminated by repeated firmwares with omentopexy. The post-traumatic period proceeded with the several consecutive complications: cutting the liver sutures, the arrosive bleeding from the right hepatic artery and the irreversible ischemia of the liver $S_5$-$S_6$. These complications were liquidated gradually on the IV level of medical care by the transpupillary choledochal stenting, vascular suture and the imposition of a typical anatomical resection of liver $S_{5-6}$. Successful treatment of patients with the severe fragmental gunshot thoraco-abdominal injuries should be based on a clear understanding of the stages of traumatic disease and expected complications of parallel conduct medical evacuation, using «damage control» tactic on the all levels of medical care.

**Keywords:** a combined thoraco-abdominal injured, levels of the medical care, warfare on the East of Ukraine.

## Introduction

Due to the warfare on the East of Ukraine in recent years, importance of problem in treatment of patients with gunshot wounds is increasing, as a result of mortar attacks and artillery shelling reactive. The most serious injuries include multiple thoraco-abdominal fragmental wounds of gunshot injuries of internal organs [1, 2, 5–7]. The important elements in achieving a successful result of treatment of such injuries are: coordinated work of sorting injured, understanding the stage of traumatic disease process and the possibility of using modern surgical technologies on the all levels of medical care [3, 4, 8]. Patients who achieved serious injuries as a result of warfare on the East of Ukraine provided a multilevel medical care, which includes pre-hospital and hospital stage of treatment. On the pre-hospital stage wounded provided first medical and pre-medical care (basic level) and first medical care (I-level). First medical care is provided on the place of damaged by the self- and mutual aid, when the wounded are being evacuated by the sanitary transport to the nearest place of qualified medical care. If evacuation is

impossible to implement a stage of qualified care for 1 hour (the principle of the «golden hour») the wounded is provided first medical care on the stabilization post where emergency care doctors are available. First aid is provided by nurses during the evacuation [3–5, 8, 9].

Hospital stage of medical care to the wounded in the combat area includes II, III, IV and V levels of medical care. The qualified surgical care (second level) is provided in city and district hospitals, which are groups enhancement of military doctors and deployed military mobile hospitals. These hospitals deployed to the minimum allowable proximity to the contact line, contributing to the implementation of the principle of the «golden hour» in 80% of the wounded. Recently at this level has implemented some elements of specialized surgical care through completing these medical units with laparoscopic racks. In step skilled care injured gets, usually within 60 minutes after the injury.

Specialized surgical care (level III) is provided in one of the three frontline multi-disciplinary hospitals: Military Medical Clinical Center of the North Region (Kharkov), Dnipropetrovsk military hospital (m. Dnipro), Mechnikov Regional Clinical Hospital (m. Dnipro). Term evacuation on the third level of care should not exceed 6–8 hours with injuries of the head and eyes, all other are evacuated after stabilization of the overall situation. The evacuation of this level carried by road (reanimobile), rail (train «InterCity») and air transport (helicopter). The IV level of medical care provides an exhaustive list of the wounded specialized care using high-tech equipment. This level of care is provided in the National (Kyiv) or regional Military Medical Clinic Centers (Vinnytsya, Odesa, Lviv) where wounded evacuated by air.

The V level of care provides a rehabilitation in specialized military and civilian medical facilities.

The course of traumatic disease characterized by a certain phasing course which determines the choice of treatment strategy and forecasting the possible occurrence of vital dangerous complications of traumatic disease and previously performed surgery. An important role in achieving positive outcomes for severe gunshot wounds played availability and accessibility during specialized surgical help of modern high-tech equipment: video endoscopic rack (laparoscopy, thoracoscopy, arthroscopy), systems for VAC therapy, equipment for endoscopic transpapillary surgery etc. [1, 4, 6].

In the present clinical case has demonstrated coordinated stages providing appropriate levels of surgical care and medical evacuation using «damage control» tactic.

## Case report

### Patient

The patient was a 22-years-old soldier (male), who during the fighting in the area of locality Krasnogorivka (Donetsk region) 16.12.2016 around 23:45 received gunshot multifragmental combined thoraco-abdominal injuries as a result of shelling. The nature of the wounds: gunshot blind debris penetrating chest injury case; right-handed haemopneumothorax; firearm fracture 5, 6, 7 ribs on the right; break right dome diaphragm and parenchyma right and left liver fate of the «hydrodynamic shock».

**First aid (basic level)** was given in place of injured in the order of mutual assistance: had superimposed aseptic dressings, intramuscularly had administered analgesic. From the get injured patient immediately was evacuated sanitary transport to the stage of qualified surgical assistance. Since the evacuation lasted less than 60 minutes (the principle of the «golden hour») wounded were evacuated immediately to the second level of care.

**Qualified surgical care (second level)** wounded was provided in the 66th Military Medical Hospital, where the wounded with vital indications were implemented immediate measures of surgical intervention: thoracentesis of pleural cavity drainage right on the right haemopneumothorax; primary surgical treatment of gunshot wounds. During laparocentesis of the abdominal cavity revealed hemorrhagic content – laparotomy performed. During the revision of the abdominal organs revealed the rapture of the right dome of the diaphragm and the ruptures of 3, 4, 5 and 6 segments of the liver such as «hydrodynamic shock» (input / output holes and fragments on the CT abdominal organs didn't found). According to the severity of the patient, due to the course of traumatic disease and massive blood loss minimum volume measures of skilled care was made – suturing of gunshot wounds of the liver, abdominal drainage, primary surgical treatment of gunshot wounds of the extremities. During subsequent audit revealed additional strain in the area of liver gallbladder fossa – performed cholecystectomy, closure strain, drainage of the abdominal cavity. Given the questionable viability of liver $S_{5-6}$, the question arose about the feasibility of resection of liver ischemic segments. Based on the principles of «damage control» due to the critical condition of the wounded (hemodynamics maintained large doses sympathomimetics) decided to limit the minimum amount of surgery – stop bleeding by taking in lots of gaps omentopexy liver, abdominal drainage.

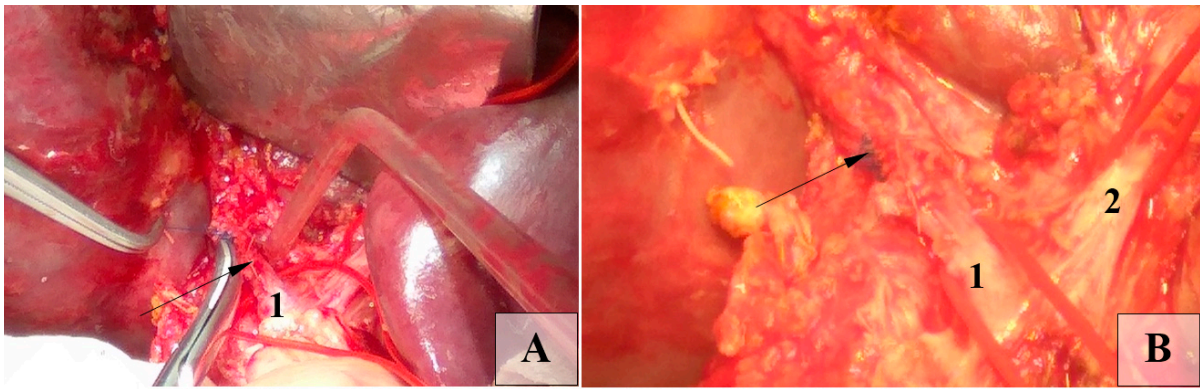


**Figure 1.** Suturing of arrosive defect of right hepatic artery. A. The area of arrosive defect (pointed by the arrow) of right hepatic artery; the turnstile is placed under the vessel (1). B. The hemostasis has achieved by suturing on the right hepatic artery (suture line pointed by the arrow): 1 – right hepatic artery; 2 – left hepatic artery on the turnstile

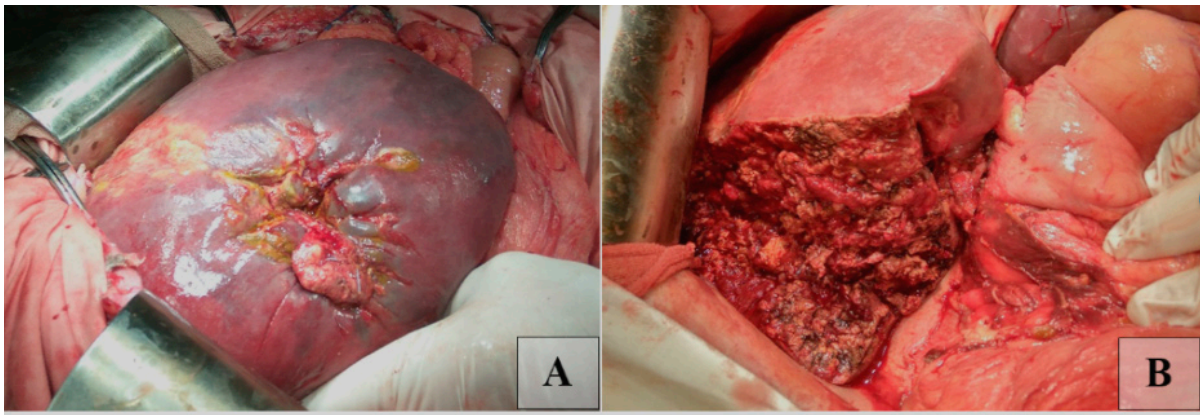


**Figure 2.** Patient's liver on 16th day after gunshot wound. A. The condition after suturing of liver's gunshot wounds (17.12.2016) and repeated suturing of liver's injury with omentopexy because of failure of the stitches (12.18.2016). B. Liver's diaphragmatic surface after the 5th and 6th segments' resection within healthy tissues (01.01.2017 p.)

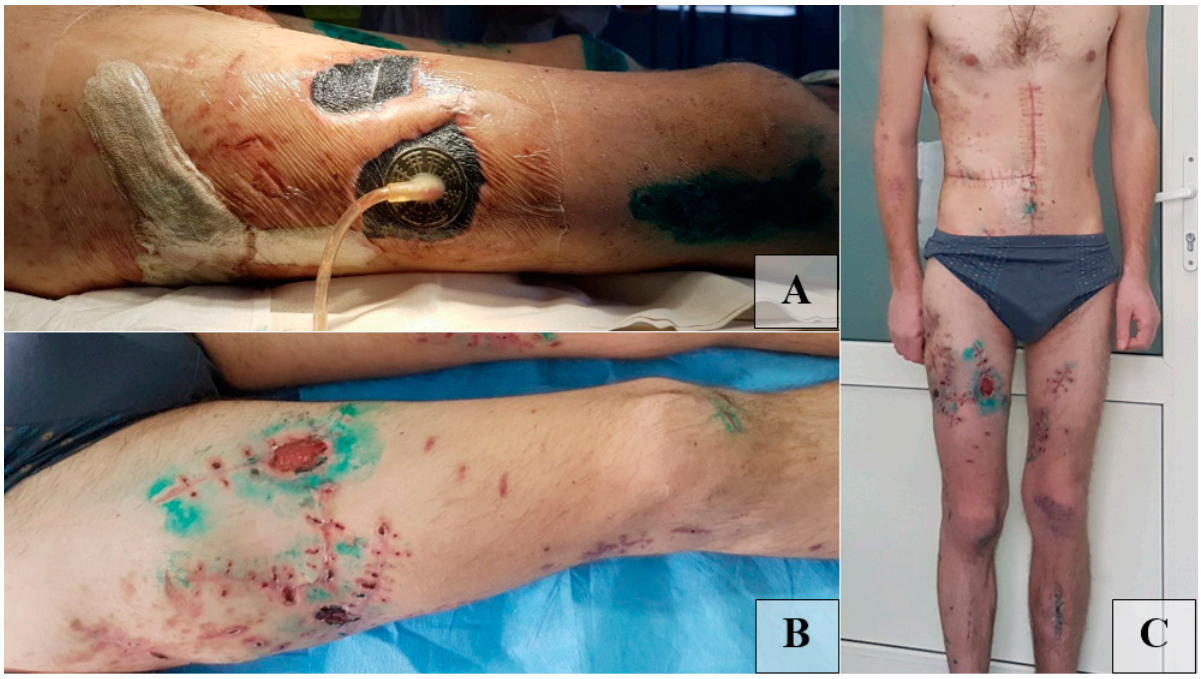


**Figure 3.** The dynamics of wounds' healing of the soft tissues of the right extremity. A. The view of the shrapnel wounds of the right thigh with installing VAC-system on the 21th day after the injury. B. The view of patient's right thigh on the 49th day after the injury. C. Patient Zh., 1995 year of birth, before discharge from the hospital on the 49th day after wounded

**Specialized surgical care (level III).** 18.12.2016 for further treatment the wounded was transferred to the Mechnikov Regional Clinical Hospital (Dnipro) by sanitary transport, where he received the infusion, antibacterial (aurotaz, dorybaks, amitsyl), symptomatic therapy, was conducted prevention of thrombo-embolic complications daily dressings.

**Specialized surgical care (IV level).** 21.12.2016 the patient had been brought by the air to the National Military Medical Clinical Center of Ukraine (Kyiv), where he was placed in the department of reanimation and intensive care because his serious condition. The general condition of the patient remained difficult due to course the third period of traumatic disease. For drainage of subhepatic space recorded daily flow rate of bile in volume to 300 ml. It was decided that one of the reasons the continuation of bile from the place of suturing a gunshot wound of the liver may be the intrahepatic biliary hypertension as a result of post-traumatic edema of liver parenchyma. In order to decompress biliary hypertension 12.22.2016 (6th day after the injury) the transpapillar intervention had been performed: endoscopic papillosphincterotomy, endoscopic retrograde cholecystopancreatography, stenting of the common bile duct; for enteral nutrition the probe had been set behind Treitz ligament. Implementation of endoscopic decompression of the bile duct had a positive effect in the form of a gradual reduction of bile from subhepatic space from 300 ml to 50 ml per day.

Given the earlier signs of liver ischemia injured areas of patient test performed CT scans of the liver, which resulted in the patient showed a trend to increase in areas of ischemic lesions of the liver parenchyma. According to «damage control» tactic the patient was preparing for liver resection of the affected segment, to prevent necrosis and abscess formation. However, on 16th days (01.01.2017) after the injury the patient had occurred an intra-abdominal bleeding. In an emergency laparotomy revealed arrosive wall defect right hepatic artery (**Figure 1A**), due to the vessel wall arrosive bile and chronic inflammation. Imposed on vascular suture site arrosive defect – achieved stable hemostasis (**Figure 1B**). With further revision including early sutured the liver gunshot wounds, surgical wounds were found covered with layers of fibrin, has been a partial failure of previously imposed joints, signs of bile, the liver tissue $S_{5-6}$ had expressed ischemic changes with a corresponding change in the consistency of focal liver parenchyma softening type (**Figure 2A**). In order to prevent further secretion of bile and failure formed vascular suture decided to perform anatomic resection of segments 5 and 6 of the liver (**Figure 2B**). Due to the critical condition of the patient and guided by «damage control» tactic the surgery had stopped for 2 hours, then held blood transfusion, stabilized hemodynamic, operating crew completed by surgeon-hepathologist (surgery was performing at New Year's Eve). Due to the irreversible changes in liver sections after repeated suturing of gunshot wounds, reaching hemodynamic stabilization, the patient had been performed anatomical resection of liver $S_{5-6}$ within the viable tissue. In general, due to intraperitoneal bleeding episode 11 doses of one-group packed red blood cells had been transfused to the patient. The postoperative period was uneventful, on the 9th day after the last operation patient was transferred to the specialized department.

Along with the performance of thoracic and abdominal surgery patient was held repeated surgical treatments of multiple fragmental injuries of soft tissues of the head, chest, abdominal, upper and lower extremities. The most traumatic injury suffered soft tissue arrays right lower limb, so for their treatment used VAC-treatment system rewiring them every 3–4 days (**Figure 3A, 3B**).

Further post-traumatic and postoperative periods proceeded smoothly, postoperative wounds had healed without complications, sutures had been removed. The patient was discharged in satisfactory condition from the hospital on 49th day after receiving a gunshot multifragmental wound (**Figure 3C**). Decision military medical commission wounded granted 45 days for rehabilitation (V level) before continuing performance of military duties.

## Conclusions

1. Treatment of patients with serious gunshot penetrating wounds should be based on a clear understanding of the stages of traumatic disease course and expected complications with parallel conduct medical evacuation, using «damage control» tactic at all levels of care and attachment of all possibilities of modern surgical technology.
2. In terms of the use of high-energy rocket launchers gunshot liver damage can occur not only by clastic injured, but the mechanism of hydrodynamic impact.
3. The third period of traumatic disease with severe gunshot wounds the linked liver may be accompanied by a number of life-deferred dangerous complications that are caused by the partial failure of seams after a gunshot wound suturing the liver, bile seepage and hepatic bile arrosive vessels.

4. Bile from wounds after a gunshot injury of the liver can be effectively eliminated by endoscopic transpapillary decompression biliary tract.
5. An effective method of finally stopping arrosive delayed bleeding from the hepatic artery after gunshot injury can be suturing arrosive defect of vessel with resectional removal source of arrosion (liver resection).

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**Correspondence address:**
Ievgen Tsema
National Military Medical Clinical Center
of Ministry of Defense of Ukraine,
Abdominal Surgery Department
18 Hospitalna Street, 01133 Ukraine, Kyiv
email: hemorrhoid@ukr.net
phone: +38 (063) 7315995

CASE REPORT

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# A giant rapidly progressive breast phyllodes tumour causing a skin rupture. A case report and literature review

Abdalla Saad Abdalla Al-Zawi[1], Ali Salih[1], Wayne Chicken[1], Turhan Comez[1], Kingsley Osayi[1], Marina Barron[1], Beat Adamczyk[2]

[1] Basildon University Hospital, Essex, United Kingdom
[2] Greater Poland Cancer Centre, Poznan, Poland

ABSTRACT

Large phyllodes tumours may behave in a rare way, in this paper we present a case of large phyllodes tumour with a female lady aged 42. The tumour caused a skin rupture due to rapid growth.

**Keywords:** breast phyllodes, mammogram, mastectomy.

## Introduction

1% of all known breast neoplasms are phyllodes. In 1838, Johannes Muller of Germany suggested the term cystosarcoma phyllodes to describe them. Breast phyllodes tumour (BPT) is a tumour of fibro-epithelial cells with classically deep "leaf-like" projections into cystic spaces and sarcomatous stroma[1]. As 70% of theses lesions are benign, and only rarely demonstrate cystic features. World Health Organization (WHO) currently recognised the term Phyllodes tumour as the most appropriate nomenclature. The tumour size is variable, ranging from 1 cm to 50 cm [2]. The histological classification is determined by different elements including stromal atypia, stromal overgrowth, mitotic count and tumour margins. After the analysis of these criteria the tumour will be given a grade from a spectrum of benign, borderline, borderline/malignant and malignant features [3]. This is a paper reporting a very large phyllodes tumour with unusual skin manifestations, due to large tumour size, rapid growth, high body mass index (BMI) and large breast size the patient developed skin rupture.

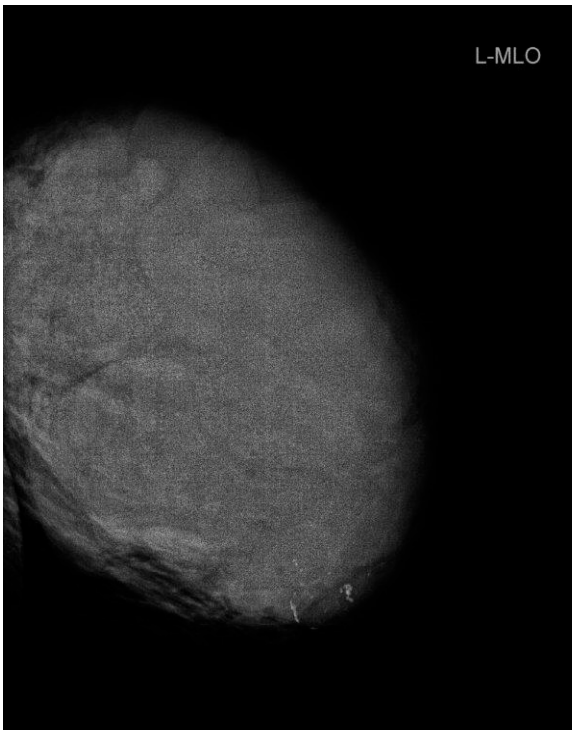


**Figure 1.** Mammogram revealed diffuse density of the whole breast

## Case report

A 42-year-old previous fit female patient presented a two weeks history of palpable mass on the left breast, with accelerated growth pattern. There was no personal or family history of breast or ovarian cancer. Clinically in spite the patient has high BMI, there was obvious breast asymmetry the whole left breast was occupied by large lobulated firm mass and stretching the skin over. There were no signs of axillary lymphadenopathy.

Breast imaging with mammogram (**Figure 1**), breast ultrasound and CT scan (**Figure 2**) reported, a large lesion with internal vascularity and a heterogeneous echo texture is seen encompassing the entire left breast. In the axillary tail adjacent to the mass there are two cystic areas. Core biopsy was taken, the histology showed borderline phyllodes pathology. The patient in

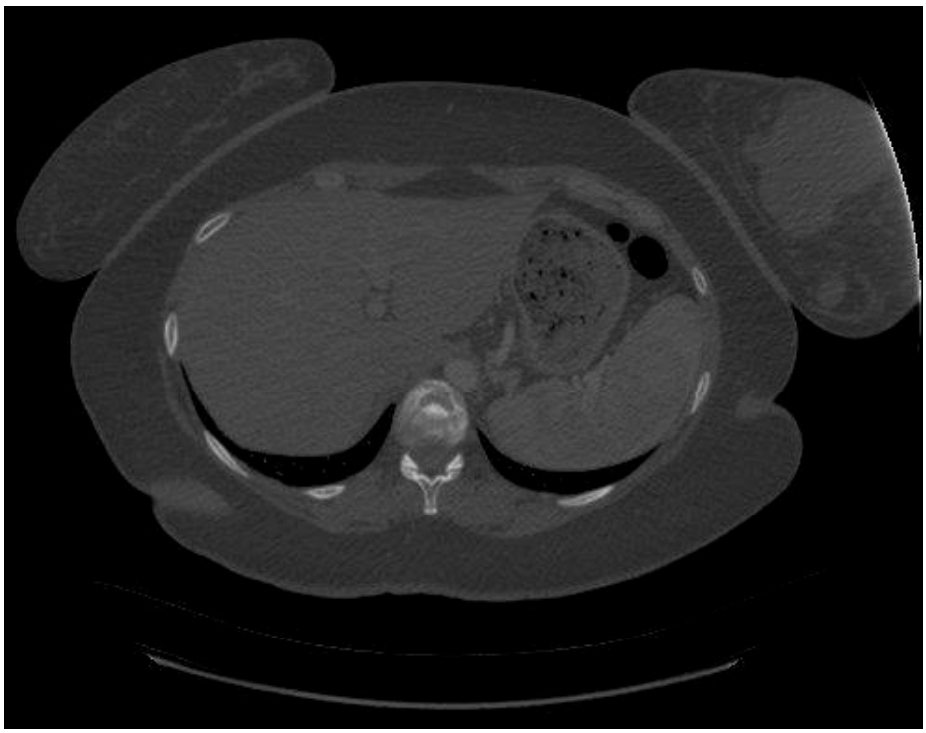

**Figure 2.** CT scan showing a large lobulated soft tissue mass in the left breast

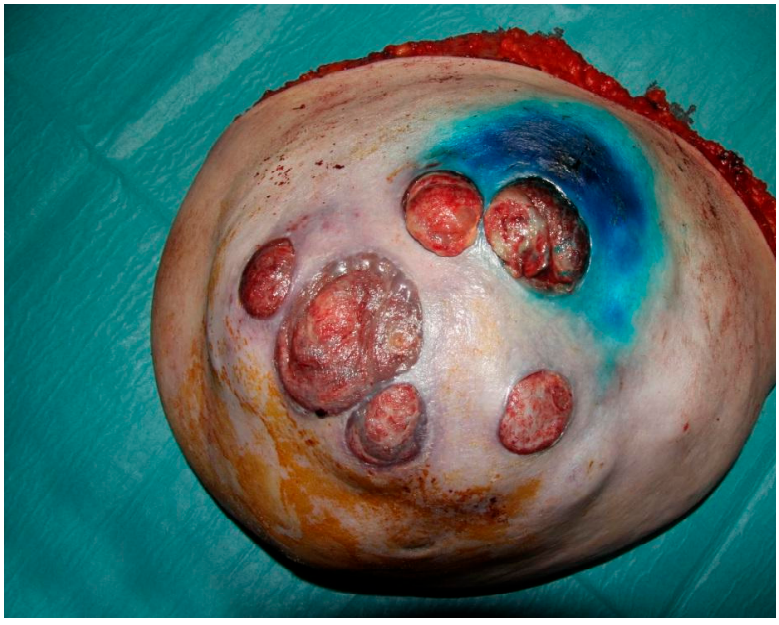

**Figure 3.** Mastectomy specimen with tumour protrusion through skin rupture

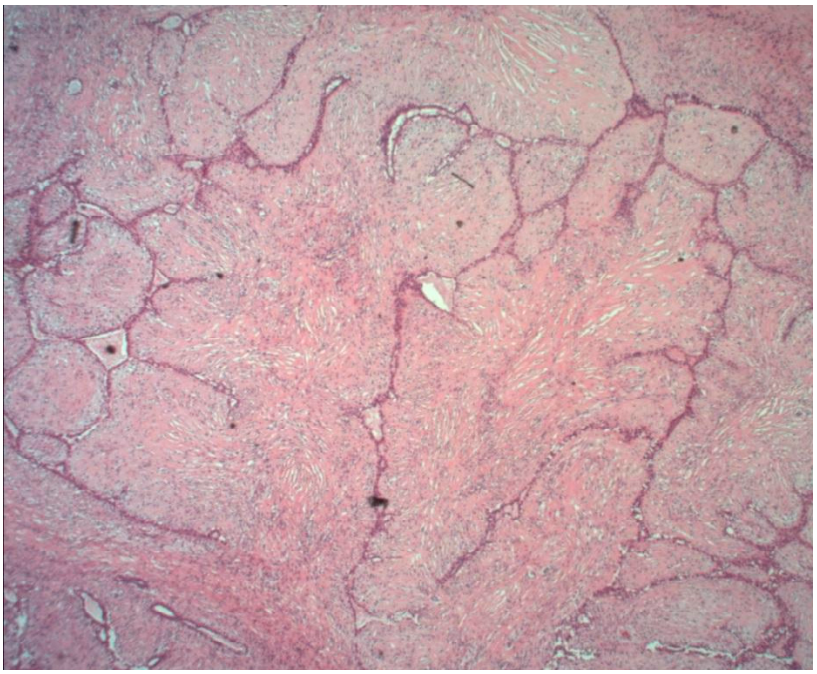

**Figure 4.** Low grade phyllodes displaying complex epithelial arrangement, cleft-like spaces and concentration of tumour cells beneath the epithelium

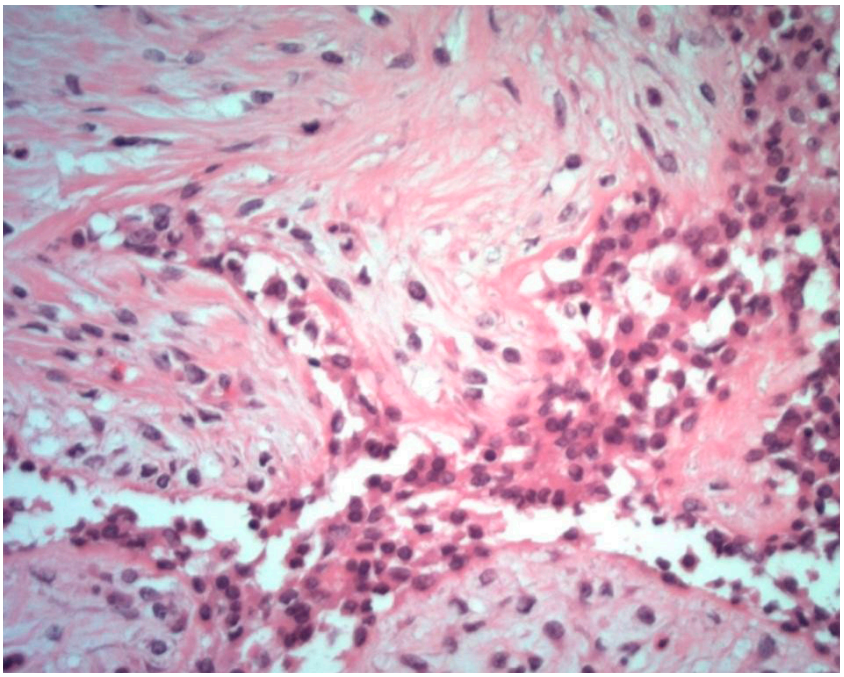

**Figure 5.** Low grade phyllodes with single mitotic figure in tumour cells beneath the epithelium

few days developed skin rupture ulcerations and the tumour protruded through the skin defect. An expeditious surgical management with mastectomy and sentinel lymph node biopsy performed after discussion in multidisciplinary team meeting. The postoperative histology showed fibro-adenomatous growth pattern with a cellular stroma component. There is a surface ulceration and areas exhibiting a leaf like growth pattern. This confirmed the pre-operative diagnosis of border line phyllodes pathology (**Figures 4, 5**).

## Discussion

The phyllodes tumor was first described in 1827 by Chelius [4], however in 1838, Johannes Muller was the first one who applied the name cystosarcoma phyl-

lodes, and described it as a huge neoplasia with a cystic lobulated section and rapid growth [5].Initially it has been attracted as totally benign, but Cooper and Ackerman, in the year 1943 reported on the potential malignant biological pattern of this tumour [6]. Breast phyllodes peak incidence between 35–55 years of age in white females [7, 8], also prevalent in Latin American, and Asian populations [6]. Usually are unilateral, single, nodular, painless masses with an insidious onset and slow progression [9] with size may grow up to 50 cm in neglected cases [6, 10]. A rare features as skin rupture, may be related to rapid growth of the lesion. Wijeyaratne in 2010 and Nabi et al, in 2013 reported a cases of breast phyllodes causing breast skin rupture [11, 12]. As as in our case, probably the obesity and high volume of breast masked the breast tumor growth and contributed to skin rupture.

Phyllodes tumours of the breast arises from the fibroepithelial component of the breast and are rare in general and their incidence ranges from 0.3–1% of all breast tuomors [13], also they form a rate of 2% to 3% in all breast fibrous epithelial tumors. Rowell in 1993, reported the incidence is about 2.1 in one million [14]. They are characterised by local recurrence which the most important prognostic feature of this condition where the nodal or distant metastases are rare. The local recurrence incidence rate is about 15%, it is has been reported that, the incomplete excision is a contributary factor to the recurrence [13]. Borderline and malignant phyllodes tumor metastasis rate is about 25% to 31%, however The overall rate of all phyllodes tumor metastasis being 4% [15, 16]. The modes of tumor metastasis are primarily via blood, rarely lymph nodes. Chest wall soft tissue was the most frequently affected site of distant metastases, followed by the lungs, thoracic cavity, bones, and pleura. A rarer sites for metastatic lesions as vulva also has reported [17]. Chest wall soft tissue lesions may be related to incomplete local tumour excision and most patients with distant metastasis had progressed from local recurrence. The predictive factors for local recurrence were age, tumor size, histotype, and margin status. It is generally advised that the resection margin distance should be at least 1 cm for adequate surgical resection [18]. In addition to local recurrence, other risk factor for distant metastases are stromal overgrowth [19] and the tumour histological subtype. Kim et al. reported that the expression of stromal matrix metalloprotein-14 (MMP-14) was associated with a higher rate of recurrence [20]. There is a clinical diagnostic dilemma, as the history and clinical findings often mimic that of the common benign presentation of fibroadenoma or even cancerous lesions in rare occasions. The importance of phyllodes tumours today lies in the need to differentiate them from other benign breast lesions. The most common presentation is a solitary and unilateral breast lump, as do fibroadenomas. There are no pathognomonic radiological features could be detected in the mammogram or breast ultrasound for phyllodes tumour [21].Features that may indicate phyllodes diagnosis are: a large mass, 3rd and 5th decade of life, an increase in size of a previously stable mass and a rapid growth pattern. Other non-specific features as dilated skin veins, blue skin discolouration and nipple retraction [8]. This rare presentation should raise strong suspicion for Phyllodes. This is essential to differentiate it from fibroadenomas to avoid incomplete excision and local recurrence, also to differentiate it from breast cancer to avoid over treatment. Malignant transformation of a phyllodes tumour is a rare form of breast cancer, accounting for just 0.1–3% of all breast cancer cases [3, 8].

The tumour rarely shows cutaneous manifestations which may be confused with a cancer fungation. In this case the fast growth of the tumour exceeds the skin ability to stretch. This results in skin rupture and tumour protrusion through the skin defects. Also the tumour had expanded so rapidly that bleeding, infarction, necrosis and degenerative changes result in cystic lesions [22].

The histopathological morphology of the disease presents a continuum from benign to malignant nature. In 1981, the World Health Organisation (WHO), adopted the term phyllodes tumour. WHO classification of the phyllodes tumour into benign, borderline and malignant phyllodes, is based on amalgamation of various histopathological characteristics, including stromal cellularity, nuclear atypia, mitotic activity per high power field (HPF), stromal overgrowth, and tumor margin appearance [23, 24], see **Table 1**.

Surgery has been agreed the gold standard in the management of the phyllodes tumours ever since they were first described. Breast conservation surgery for small tumours and reasonable breast size. A clear margin of 1 cm is required to improve the outcome and reduce the risk of disease recurrence. Mastectomy is advisable in large tumours or small breast size. Immediate or delayed reconstruction still can be offered for mastectomy Adequate surgical excision with clear margins is the most essential preventive measures to minimize the disease recurrence. Using adjuvant treatment as radiotherapy or chemotherapy is debetable,

**Table 1.** Main histological features of the 3 grading subgroups for phyllodes tumours. Tavassoli FA, Devilee P. World Health Organization Classification of Tumours. 2003. HPF: High Power Field

| | Benign | Borderline | Malignant |
|---|---|---|---|
| Stromal hypercellularity | Modest | Modest | Marked |
| Cellular pleomorphism | Little | Moderate | Marked |
| Mitosis | Few if any | Intermediate | Numerous (more than 10per 10 HPF |
| Margins | Well circumscribed pushing | Intermediate | Invasive |
| Stromal pattern | Uniform stromal distribution | Heterogeneous stromal expansion | Marked stromal overgrowth |
| Heterologous stromal differentiation | Rare | Rare | Not uncommon |
| Overall average distribution | 60% | 20% | 20% |

however some authors advise to use them in cases of malignant phyllodes [25, 26].

## Conclusion

The patterns of phyllodes tumour are widely varied, making the clinical diagnosis. There should be a high index of suspicion for this diagnosis if there is a rapid progression of the tumour. It may causes rupture of the breast skin and presents as an external fungating breast mass, a presentation which is exceedingly rare. High body mass index or large breast size may mask the large tumour size and skin rupture may happens also. In spite it is a benign entity in most of the cases, an adequate clear surgical margins are very essential to minimise the recurrence and radical tumour removal remains the most crucial preventive measure against disease recurrence. Simple mastectomy is advisable if breast conservation will not secure a clear margin. Nodal metastases are rare and routine auxiliary dissection is not recommended.


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The authors declare no conflict of interest.

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**Correspondence address:**
Abdalla Saad Abdalla Al-Zawi
Breast Unit, Basildon University Hospoital
Essex, United Kingdom
email: abdalasaad@gmail.com

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**Standard journal articles**

1. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.

2. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013 Mar;45(3):279–284.

**Books**
Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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