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Clinical features, diagnosis and differential diagnosis of spontaneous bacterial peritonitis

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Клинична картина, диагноза и диференциална диагноза на спонтанния бактериален перитонит

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РЕЗЮМЕ

Спонтанният бактериален перитонит (СБП) е главна причина за смърт при болните с чернодробна цироза. Коремната болка (чувствителност), температурата, промените в броя на левкоцитите не са чести при случаите със СБП. Той може да протече и напълно безсимптомно (~10%). Кръвоизливът от варици на хранопровода в днешно време не е основен рисков фактор за СБП. Чернодробната енцефалопатия е честа. Бъбречната дисфункция е независим фактор, предсказващ смъртността при пациентите със СБП. Концентрацията

ABSTRACT

Spontaneous bacterial peritonitis (SBP) is the main cause of death in patients with liver cirrhosis. Abdominal pain (tenderness), fever, altered white blood cell (WBC) count are not frequent in cases with SBP. SBP may be completely asymptomatic (~10%). Variceal bleeding is not the main risk factor for SBP nowadays. Hepatic encephalopathy is frequent. Renal dysfunction has been show to be an independent predictor of mortality in patients with SBP. Protein concentration and WBC increase in ascitic fluid (AF) during diuretic therapy. The serum

на белтък и левкоцити се увеличава в асцитната течност (АТ) по време на диуретична терапия. Серумно-асцитният албуминен градиент превъзхожда опредеилянето на трансудат/ексудат в диференциалната диагноза на асцита. Полиморфонуклеарни левкоцити в АТ ≥250/mm^3 (определени микроскопски), независимо от резултата на бактериалните култури от асцита, са универсално възприети като най-добър маркер за диагноза на СБП. Симптомите и смъртността на болните с културело-негативен неутрофилен асцит (КННА) са подобни с тези при СБП. Бактериалните култури от асцитната течност често са негативни, дори ако посявките се вземат в бульон за хемокултури. Тест лентите (за урина) отчитат естеразната активност на левкоцититите, но имат ниска чувствителност и използването им в диагнозата на СБП не се препоръчва. Те могат да са полезни обаче за диагнозата на вторичния бактериален перитонит (ВБП), поради моного високото съдържание на неутрофили в асцита. ВБП се подозира (въз основа на АТ), когато се установят два или повече от следните критерии: белтък >10 g/l, глюкоза <2,7 mmol/l и лактатдехидрогеназа в по-високи стойности от плазмените. Мономикробният бактерасцит (БА) без повишени неутрофили в АТ е рядък (~5%). БА протича обикновено (но не винаги) клинично безсимптомно и е спонтанно реверзибилен. СБП и ВБП са животозастрашаващи заболявания (усложнения). Оперативното лечение или антибактериалната терапия могат да са еднакво опасни при грешна диагноза. Литературният обзор е посветен на клиничната картина, диагноза и диференциална диагноза на СБП.

Ключови думи: спонтанен бактериален перитонит, вторичен бактериален перитонит, асцитна течност, културело негативен неутрофилен асцит, бактерасцит

ascites albumin gradient in the different diagnosis of ascites is superior to the exudate/transudate concept. An AF polymorphonuclear leucocyte count ≥250/mm^3 (determined by microscopy) – irrespective of the AF culture result – is universally accepted as the best surrogate marker for diagnosis of SBP. Symptoms and mortality in patients with culture-negative neutrophylic ascites (CNNA) are similar to the course of desease in patients with diagnosed SBP. Ascitic fluid culture is frequently negative even if performed in blood culture bottles. Leucocyte esterase reagent strips (RS) have low sensitivity and the use of RS (Multistix) cannot recommend for the rapid diagnosis of SBP, but RS may be useful for diagnosis of secondary bacterial peritonitis (SecBP) – very high ascitic neutrophyl count. SecBP may be suspected (based on AF) when two or more of following are identiefed: proteins >10 g/l, glucose <2,7 mmol/l and lactate dehydrogenase higher than in plasma. Monomicrobial non-neutrocytic bacterascites (BA) has rarely been described (~5%). BA is usually (but not always) clinically asymptomatic and spontaneously reversible. SBP and SecBP are lifethreaten diseases (complications). Surgical treatment or antibacterial therapy may be equal dangerous in cases with wrong diagnosis. The review focuses upon clinical features, diagnosis and differentiale diagnosisof SBP.

Key words: spontaneous bacterial peritonitis, secondary bacterial peritonitis, ascitic fluid, culture-negative neutrophylic ascites, bacterascites

Infections in patients with cirrhosis increase mortality rate four times (11), and the risk of death within one year but is 30% (15). Spontaneous bacterial peritonitis (SBS) is the most common infection in cirrhosis and it is currently the main cause of death in these patients (20).

CLINICAL FEATURES.

There are three clinical forms of SBP – latent, classical (with little expression) and fulminant (46). Third (31.7%) of clinical suspected cases for SBP are confirmed (33).

Half of the patients with SBP have one during hospitalization (21), but almost third of cas-

es with SBP (29%) are due to nosocomial infection (9). Resistance to treatment in nosocomial infection is high (64%) and lethality – also (28).

The classic symptoms of peritonitis are often missing in SBP and is usually found only slightly increased sensitivity of the abdominal wall (30–47%) (48). Physical examination is not sensitive method, since 85% of cases with cirrhosis and ascites without SBP have pain or tenderness in the abdomen (17). In cases with ascites over 10 l, there is no peritoneal symptoms even in perforative peritonitis (45). Half of the patients with SBP have not pain, fever and leukocytosis (44). Completely asymptomatic are 10% of the patients with SBP (49). Hepatic encephalopathy and fever are the only symptoms in 10–50% of patients (19).

Bacterial infection deteriorate liver function, and may cause hepatic encephalopathy (15). Aspiration is common in encephalopathy and mortality in intubated patients is 33–60%. SIRS (systemic inflamatory response syndrome) can cause respiratory distress syndrome (ARDS) (15). Mortality from sepsis in cirrhosis is 26–44% (11.15).

Acute renal disease may also be manifestation of SBP (36). Renal function is impaired in third of the patients with SBP (31). Acute renal dysfunction in patients with advanced cirrhosis occurs in 27–34% with 40–50% mortality (15). Renal failure (RF) caused by a bacterial infection that is not being controlled by an infusion of albumin, today belongs to the Hepatorenal syndrome(HRS) (15). RF may progress despite infection have been controled (15). SBP combines with HRS type 1 (10), which have a poor prognosis than type 2 HRS.

Proinflammatory cytokines and sepsis worsen impaired coagulation in cirrhosis and enhance fibrinolysis, and prolong and aggravate bleeding from oesophageal varices and tendency to relapse (15).

According to the consensus of the European Association for the Study of the Liver (EASL) since 2010. patients with SBP have at least one of the following symptoms: 1. Abdominal pain or tenderness in the abdomen, vomiting, diarrhea or ileus, 2. Signs of systemic infection: hypothermia, fever, tachycardia, tachypnea, leukocytosis, 3. Worsening of liver function 4. Hepatic encephalopathy 5. Shock 6. Renal failure 7. Gastrointestinal bleeding. It is accentuate that the SBP can occur asymptomatic (21).

Relapse of SBP is suspected in the values of total protein in the ascites <10 g/l, bilirubin> 55 μmol/l and platelets <98x10^9 (8). In these cases, prophylactic treatment is indicated (8).

The most common cause of death in the SBP are shock, septicaemia (25–30%), gastrointestinal bleeding (10–18%), other infections (10–20%), multiorgan failure (20–30%), progressive cardiovascular, liver (30–44%) and renal failure (6, 12). Currently mortality in SBP is reduced from 90% to 20% (21, 46). In Bulgaria HRS had 52% of patients with SBP, upper gastrointestinal bleeding – 21% and hepatic encephalopathy – 10.5% (3).

EXAMINATION OF ASCITES – DIAGNOSTIC APPROACH AND CLINICAL INTERPRETATION.

In ascites changes establish faster and more accurate than blood (4).

Criteria for diagnosis of SBP are polymorphonuclear leucocytes (PMN)≥ 250 mm^3 or positive bacterial cultures (21). For every hospitalization of a patient with cirrhosis and ascites is recommended to perform diagnostic paracentesis (21, 39), as half of patients have SBP during hospitalization (21). Control Testing of ascites (biochemical and microbiological) is not always necessary after treatment of SBP, although PMN decreased by 25% in the 48th hour (exudative phase continuing from hours to days) (4, 21) from the beginning of therapy in almost all patients (90–94%).

BIOCHEMICAL EXAMINATION OF ASCITES.

Since 1992. is advised to use serum-ascites albumin gradient (SAAG), instead determining transudate/exudate (38, 40). Ascites is almost „pure“ filtrate and his protein composition re-

semble a plasma but in smaller quantities (4). In 80–90% of cases, the etiology of transudate is non-inflammatory (4). SAAG sensitivity is 97% with ascites caused by portal hypertension, whereas examination transudate/exudates it is 56% (38).

Exudate is observed predominantly in the peritoneum diseases – inflammatory, neoplastic and others (carcinomatosis, tuberculosis, mesothelioma, bacterial, mycoses or parazitic peritonitis, but in myxoedema, chilous ascites, etc.) (2). Specific weight of ascites depends on the content of protein in it (1, 4). The percentage of albumin is between 65 and 85% for transudate and 50–60% for exudate. Protein values in ascites > 30 g/l in combination with elevated lactate dehydrogenase (LDH)> 200 U/l in ascites have high sensitivity and specificity for exudate – respectively 93% and 85%. High protein ascites explains the absence of SBP in malignant ascites and congestive heart failure (satisfactory opsonization of microorganisms in ascites) (6).

LDH in ascites exceed serum LDH in 60% of cases with malignant ascites (1). LDH can be increased in secondary (including non-perforative) peritonitis (> 225 U/l) (5, 39). Ratio LDH in ascites to LDH in serum <0.8 with high sensitivity and specificity for transudate (1). Examination of LDH is not recommended for SBP because of low sensitivity and specificity (21).

PH <7.3 correlates with the increase in LDH, as usually is concern about exudate in malignant ascites fluid. PH <7.26 was observed in tuberculous peritonitis (12). Lactate and pH in ascites have little diagnostic value because of their low sensitivity (respectively 54% and 58%).

Alkaline phosphatase (ALP) in the ascites increase in hemorrhagic malignant ascites (usually of ovarian origin) values corresponding to serum or above (1), and in secondary bacterial peritonitis [non-perforative or perforative peritonitis (> 240 U/l)] (5,39,50).

Total fat in chilous ascites reach 4–50 g / l (4). Triglycerides in serum ascites exceed chilous ascites and lower in pseudochilous (4). Lipid content of the ascites is similar as plasma lipids in pseudohylouse ascites (43). The concentration of cholesterol in ascites is higher than in serum in pseudohylouse ascites (4). It is observed predominantly in malignant diseases (1), but also as a complication of cirrhosis (43), and tuberculosis (4).

When bilirubin levels in ascites> 102 μmol/l or a ratio of bilirubin in ascites to serum bilirubin> 1, the most likely case of leakage of bile (7) or bile peritonitis.

Ascites glucose in perforative peritonitis is <2,78 mmol/l, because glycolysis by bacteria, macrophages and other cells (4, 39), and a disturbance in its transport (4). Study of ascites glucose is not recommended by EASL at SBP (21).

Values of ammonia in ascites > 300 μg/dl are observed in perforative peritonitis (1).

During perforation in ascites is increased carcinoembriotic antigen (CEA)> 5 ng/ml, not only ALP (50). CEA (> 2 ng/ml) and CA 125 (> 35 U/l) increase in malignant ascites.

Testing for lactoferrin (reference values up to 242 ng/ml) in the ascites (ELISA) with sensitivity 95.5% and specificity 97% for SBP (32).

β-microglobulin is increased in ascites in lymphoma, myeloma and less frequently in collagenosis (1).

The study of hyaluronic acid in ascites is important in mesothelioma (1).

FEATURES OF CHANGES IN ASCITES IN SOME DISEASES.

Liver cirrhosis. Ascites total protein is <25 g/l (2,5 g/dl) (transudate), and often <20 g/l, but after diuretic treatment the total protein ascites in two thirds of patients is > 25 g/l (23). Total protein in ascites <10 g/l increases risk of SBP tenfold due to reduced opsonic activity of ascites (6, 27), but in values> 10 g/l is unlikely to have a patient with SBP (negative predictive value 95%)

Heart failure. In the so-called „cardiac ascites" body fluid should be transudate (2), but almost in all patients is> 25 g/l, whereas patients with

tuberculous peritonitis in half the cases the total protein in the ascites is <25 g/l (37).

Nephrotic syndrome. Protein in the ascites was reduced (2).

Pancreatogenic ascites. The average value of total protein in ascites in these patients is 32 g/l, but SAAG is increased in half of the cases due to concomitant alcoholic liver disease with portal hypertension (41). Amylase in ascites is greatly increased and considerably exceed serum. About 10% of pancreatogenic ascites are mallignant (1, 2). Hyperamilasemia can occur in any states occurring with the picture of an acute abdomen. PMN can also elevated in ascites in pancreatitis (like SBP) (41).

Tuberculous peritonitis (TBC peritonei). Total protein and lymphocytes in ascites are increased. Studied adenosine desaminase (ADA), Quantiferon test, possibly and PCR (polymerase chain reaction). Values of ADA > 33 U/l are with sensitivity, specificity, positive and negative predictive value respectively 89%, 100%, 100% and 94% (12). ADA is an enzyme that is produced by lymphocytes and macrophages as a result of T-cell stimulation by peritoneal tuberculosis (33). ADA is with reduced vaues in SBP (12). Not only lymphocytes but also PMN may be elevated in ascites in tuberculous peritonitis (41). Quantiferon test (ELISA) established interferon-gamma (IFN-γ), produced by T-lymphocytes due to two antigens originating only from Mycobacterium tuberculosis. The best method to prove tuberculous peritonitis has remaine laparoscopy and biopsy for histological and microbiological examination (40).

Secondary bacterial peritonitis (SecBP). SecBP represents 10–15% of infected ascites (6) and is due to perforation or inflammation of intra-abdominal organ (21). Operative death in SBP is 80%, while treatment of peritonitis in perforative ascites is surgical (14).

The guideline of the 2010 EASL not discussed the ascites total protein as a diagnostic criterion for the SBP, but only indicates that the protein is elevated in SecBP, without specifying values (21). However, total protein in ascites below or above 15 g/l (1,0 g/dl) remains one of the major differential diagnostic criteria between SBS and SeBP. Ascites protein> 15 g/l is one of the three criteria for SecBP, but only in combination with at least one of the following items – ascites glucose <50 mg/dl (2,7 mmol/l) and LDH values in ascites exceed those in plasma (13). Due to the low specificity of these tests is recommended to also include CEA (carcinoembriotic antigen) > 5 ng/ml and ALP > 240 U/l (only in perforative peritonitis) (13). Without defined as criteria for the diagnosis of SecBP, in support of disease are polymicrobial flore in ascites (8% in SBP) isolation of anaerobes (4% in SBP), enterococcus, fung (13), and the very high count of PMN (21) and non affective from treatment (13). In these cases (eventually with possible non-perforative secondary bacterial peritonitis) antibacterial therapy is effective against anaerobic infections and enterococcus (13). Surgery or antibacterial therapy can be equally harmful if misdiagnosed.

Other conditions occurring with increased polymorphonuclear leucocytes in ascites. These are peritoneal tuberculosis and carcinomatosis, pancreatitis, hemorrhagic ascites (41).

Chlamydial infection. Ascites content manly lymphocytes, like tuberculous peritonitis (47). This infection usually affects young, sexually active women (the female peritoneal cavity is not closed).

First stage in the study of ascites. In anticipation uncomplicated ascites due to cirrhosis is advised to initially examine the ascites total protein, albumin, the quantity of cells and differential count (40). Tracking the dynamics of the inflammatory process is essential (4). Exudative (neutrophilic phase) lasts from hours to days (4). Body fluids are unfavorable location for surviving of blood cells (4). The more pronounced pleocytosis is, the more pronounced is the neutrophilosis, with rare exceptions (4). Diuretic therapy may cause an increase in leucocytes (pleocytosis) in ascites, like the total protein in it, but not and PMN (23, 37). PMN are increased in ascites only during infection (37).

PMN in ascites increased rapidly after infection (4 hours in rats) (42).

It is possible to make quick examination of neutrophils in ascites with test strips for urine (Multistix 8 SG RS) (21). The principle of this study is to establish colorimetric esterase activity of neutrophils (21). The negative predictive value of the test is 95% (24). However, the method has low diagnostic accuracy and the possibility of false negative results in SBP (21) and is not recommended by EASL (21). Although these tests have high specificity (92%) (30), all of which have low sensitivity to PMN <1000/mm^3 (16), which occurs quite often in the SBP. Test is fast (from 30' to 2 min), indicative and used for screening of SBP (24), but „manually" count it exceeds with 21% (35). Some authors use the combined use of Multistix and examining ascites pH (24), and others – Multistix and study of lactoferrin (34). In view of the increased sensitivity Multistix in PMN> 1000/mm^3, the use of the test is warranted in suspected secondary bacterial peritonitis.

The so-called „manual" counting PMN is carried out with an optical microscope after centrifugation and stain Giemsa (21) Sometimes, however, PMN lysed during transportation (32).

Automatic counting of neutrophils is fast, affordable, easy and reliable (34). It can permanently replace the „manually" counting (9, 34). In PMN> 250/mm^3 automatic counting sensitivity is 86–100% (29). There is good correlation between „manual" and automatic counting (21). However, the automatic counting is not recommended by EASL (21).

In lymphocytic ascites is suspected tuberculosis, lymphoma, myeloma, fungal or chlamydial infection (1, 7).

Eosinophils in ascites can be increased not only in parasitic diseases, but also in subserous eosinophilic gasstroenteritis (2).

SECOND STAGE IN THE STUDY OF ASCITES.

Microbiological testing of ascites. Held in pain (or tenderness in the abdomen), fever or encephalopathy (40) and others. (renal dysfunction, bleeding, etc.).. The material is taken in a blood culture bottle (21) at the bedside (21). The sensitivity of the examination taken material increases to 70% (50–93%) (7, 21, 37). The sensitivity of routine microbiological testing is at least 10% (51), and in our country – 14.8% (3). The reason for the rare positive of bacterial cultures is very small concentration of microorganisms in ascites – less than 1 bacteria in 1 ml (37). Cultures are positive after 12 to 72 hours, but using a colorimetric method (BacT/LERT) they have bacterial growth after 13 hours, a period equal for growth of blood cultures (9).

In quarter to third (34%) of patients with cirrhosis and ascites (without SBP) is identifies bacterial DNA in ascites (26), but this is not demonstrated in any disease on continuous prophylaxis with norfloxacin. Cytokines in serum and ascites (IFN-α, IL-12, IFN-γ) correspond to the identified bacterial DNA in effusion. Antibiotics do not eliminate bacterial DNA from body fluid in the short term (before 72 hours) (22).

More recent studies have found that 30% of the gram-negative flora is resistant to quinolones, especially if carried prophylaxis with norfloxacin, and another 30% were resistant to trimethoprim/sulfamethoxazole (17% – simultaneously to both) (21). Resistance to third generation cephalosporins is lower in cases after quinolone prophylaxis. The patients treated with norfloxacin, may develop SBP caused by gram-positive cocci (21).

Blood cultures. Bacteraemia in SBP is found in 55–60% (58.2%), and mortality is high – 37.3% (18). In 30–40% of patients with SBP in blood cultures and ascites is isolate the same organism, and where cultivation of ascites are sterile, bacteria isolated in blood cultures is a primer cause of SBP (36).

Urine culture. Bacteriuria is more common in class B – Child-Pugh, unlike SBP, and often significant in patients with SBP compared with patients with sterile ascites (7) (50% vs. 10%). Urinary tract infection is usually caused by E. coli and Klebsiella pneumoniae, as in SBP (18).

Third stage in the study of ascites. It includes cytology (39). This stage is the most expensive (39). According to other authors, however, in any patient with newly ascites should be performed and cytological examination (first phase) (27). The sensitivity of cytology for blastoma cells in the first examination is 82.8%, in the second – 93.3%, while the third – 96.7% (37). Adenocarcinoma is the most common tumor that metastasizing in peritoneum (1). PMN can be elevated in ascites in carcinomatosis peritonei (41).

FORMS OF SBP.

1. **Spontaneous bacterial peritonitis.** The establishment of PMN> 250 cells/mm^3 in ascites (sensitivity 98.1%) and positive bacterial cultures it (the second stage) diagnosed SBP. The absence of these two criteria, safe exclude SBP (17). Specificity of the assay is increased PMN> 500 cells/mm3 but the limit of PMN> 250/mm^3 is considered sufficient (21).
2. **Culture negative neutrophilic ascites (CNNA).** Where are PMN> 250/mm^3, but cultures are negative, it is case of CNNA. It is completely equivalent (variant) of SBS, as there is no significant difference in survival compared to SBP (25) and needs treatment (21).
3. **Bakterascites (BA).** In its positive bacterial cultures, but no increase in PMN (> 250/mm^3). BA is very rare (5%) and a phase (stage) of SBP (36). Bakterascites (BA) occurs usually asymptomatic, often resolve spontaneously (36), but can pass in SBP, especially if there is clinical expression. When BA is due to secondary colonization of ascites from extraperitoneal infection (including pulmonary), there are usually common symptoms of infection (21).

Spontaneous bacterial empyema. About 5% of patients with cirrhosis and ascites have hydrothorax (4). An infected hepatic hydrothorax is called „spontaneous bacterial empyema" (21). Only half of the cases with spontaneous empyema have SBP (21). Therefore, pleural effusion may be infected directly from ascites or from bacteremia circulation (such as some cases of SBP). The criteria for the diagnosis of spontaneous bacterial empyema are the same as SBP (PMN> 250/mm^3 and positive bacterial cultures), but when culture are negative (unlike CNNA) is need to pleural punctate PMN to be> 500/mm^3 (21).

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Clinical peculiarities of Wilson's disease

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Клинични особености на болестта на Wilson

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РЕЗЮМЕ

Болестта на Wilson, наричана още хепатолентикуларна дегенерация, е наследствено автозомно рецесивно заболяване. Развива се в резултат на нарушен метаболизъм на медта, нейното натрупване и токсично действие главно в черния дроб, мозъка, корнеята и бъбреците. Протича с широк спектър чернодробни нарушения – от безсимптомна хепатоспленомегалия или повишени аминотрансферази, до разгърнатата клинична картина на чернодробна цироза или фулминантен хепатит. Неврологичните симптоми са свързани с нарушения в двигателната функция. При част от пациентите са налице психични отклонения. Хемолизата в резултат на токсичното въздействие на медта върху еритроцитите и хемолитичната анемия може да са първа изява на болестта. Важни извънчернодробни прояви са бъбречното и мускулоскелетното засягане, увреждането на сърцето и ендокринните жлези, както и кожните промени. Представена е фенотипна класификация на заболяването. Познаването на многообразните клинични изяви на болестта позволя-

ABSTRACT

Wilson's disease also known as hepatolenticular degeneration is a hereditary autosome recessive disease. It is caused by a disturbed copper metabolism and the accumulation and toxic action of copper mainly in liver, brain, cornea and kidneys. It presents with a broad scope of hepatic disturbances ranging from asymptomatic hepatosplenomegaly or elevated aminotransferases to well-manifested clinical symptoms of liver cirrhosis or even fulminant hepatitis. Neurological signs are related to movement disorders. Some patients present with psychiatric abnormalities. Haemolysis results from copper toxic action on erythrocytes as haemolytic anemia can represent the first manifestation of Wilson's disease. The affections of the kidneys, musculoskeletal system, heart and endocrine glands as well as skin lesions belong to the important extrahepatic manifestations of the disease. The phenotypic classification of Wilson's disease has been demonstrated. Knowledge of the variety of clinical manifestations of this disease enables its timely diagnosis and adequate treatment.

ва нейното навременно диагностициране и адекватно лечение.

Ключови думи: болест на Wilson, клинични форми, извънчернодробни прояви, фенотипна класификация

Key words: Wilson's disease, clinical forms, extrahepatic manifestations, phenotypic classification

INTRODUCTION

Wilson's disease is a hereditary autosomal recessive disorder of copper transport. It is due to the deficiency or dysfunction of copper-transporting P-type ATP-ase which plays a crucial role in copper biliary excretion and incorporation into ceruloplasmin. Consequently, copper accumulates in liver, and later on in other tissues and organs, predominantly in brain, eyes, and kidneys.

In 1912, British neurologist Samuel Alexander Kinnier Wilson described the disease as a 'progressive lenticular degeneration', a familial, lethal neurological disease accompanied by a chronic liver disease leading to cirrhosis (2,13,20,26). In 1993, the gene responsible for Wilson's disease was cloned (5,8,13).

CLINICAL FORMS

There exist various classifications of the course and fo4rms of Wilson's disease. Based on his great experience and observations of patients with this disease, Kolarski (1) systematizes the following two stages in the course of the disease:

1. Stage of asymptomatic course – it lasts differently, usually between 5 and 7 years. During this period, there are no manifested clinical signs yet. In most cases, liver is within normal size or slightly enlarged, with the characteristics of hepatic steatosis that can be echographically and morphologically confirmed. This stage is considered a latent period of the disease.
2. Stage of clinically manifested disease. It presents with:
   - an acute form also called abdominal form, i. e. fulminant hepatitis, and
   - chronic forms consisting of:
     i) classic or mixed form,
     ii) hepatic form such as chronic active hepatitis and liver cirrhosis, and
     iii) predominantly neurological form.

Most commonly, the first manifestation of the disease is between the first and fourth decade of life. A two-year old infant and a 72-year old patient presenting with a first manifestation of the diseases have been reported (4,18). The main clinical forms include a hepatic, haemolytic, neurological and psychiatric disease. Both genders are equally affected as the fulminant form occurs more often in females than in males as this ratio varies between 2:1 (22) and 4:1 (1,23).

LIVER DAMAGE

The disturbances of liver function are the most common initial clinical symptom occurring at the age of about 15 years. It represents the most common initial manifestation in childhood (7,17).

Liver damage varies in a broad scope – from asymptomatic hepatomegaly and/or splenomegaly, hepatic steatosis and elevated aminotransferases to acute hepatitis with an outlined cytolysis, liver failure and jaundice along with signs of chronic liver disease and developed liver cirrhosis

(18,20). Most patients with neurological and psychiatric manifestations present with a unrecognized liver disease, usually with cirrhosis, however, commonly, without any symptoms on the part of the hepatic disease at all (3,20,23).

Wilson's disease can present with features mimicking other etiological forms of the active hepatitis such as viral hepatitis, chronic autoimmune hepatitis or drug-induced hepatitis (20,26). In such cases, nothing could distinguish Wilson's disease from viral hepatitis but the negative virus markers. In a patient who has contacted the hepatitis virus in the past and, at

presence, he is positive for one of the viral tests, there is a high probability to omit the diagnosis of Wilson's disease (9). That is why the patients with proved autoimmune hepatitis in childhood or adult patients with suspected autoimmune hepatitis that does not respond to therapy should be carefully examined for Wilson's disease because the increased immunoglobulin levels and the detectable non-specific autoantibodies can be established in both pathological conditions (20).

Liver cirrhosis in Wilson's disease is the most common initial manifestation of liver damage and presents with classical symptoms and signs of portal hypertension as well. If the patient presents with a history of alcohol consumption then the diagnosis of Wilson's disease can be omitted and not suspected further.

While most patients with liver cirrhosis present with an elevated risk of development of hepatocellular carcinoma (HCC), this risk is relatively much lower in Wilson's disease as compared to that in the patients with haemochromatosis (12). It is, probably, related to the significantly shorter time of observation of the patients who have not been treated in the past and there was no time enough for them to develop HCC. The rising number of reported cases demonstrates that this incidence rate will increase along with survival improvement (20,23). In this respect, the protective role of copper as well as the contrary opinion about its procancerogenic effect are discussed (14,15,25).

Wilson's disease should be taken into consideration in any unclear liver disease in the individuals aged between 3 and 55 years. One should think about it in case of virus-negative hepatitis, elevated aminotransferases, steatosis, and signs of a chronic liver disease as well. One should bear it in mind also in the patients aged below 40 years with hepatitis and positive viral markers, with cirrhosis even with evidence of alcohol misuse, or with liver insufficiency (20,23).

Kayser-Fleischer's rings representing copper accumulations in the cornea occur in 50–60 per cent of the patients with a liver form of the disease only (18,20,21).

About 5 per cent of the patients with Wilson's disease are diagnosed with the symptoms of a fulminant hepatitis, i. e., with an acute form of the disease. It occurs mainly in early childhood. Its course is rapid and unfavourably and it advances to an acute hepatic failure with lethal outcome. It manifests itself by jaundice, haemorrhagic diathesis, haemolytic anemia, oedema-ascites syndrome, renal failure, hepatic encephalopathy, and coma. Most patients already present with cirrhosis. The acute intravasal haemolysis results from erythrocyte destruction caused by copper from the destroyed hepatocytes that suddenly enters blood circulation. Both urinary and serum copper levels are very high. Usually, ceruloplasmin is low; it can, however, be normal or elevated as it represents an acute-phase reactant and increases due to the active liver disease. The serum levels of transaminases and alkaline phosphatase are discrepantly low for a fulminant hepatitis which, on its part, can develop after the long-lasting cessation of treatment, too (10).

HAEMOLYSIS

Haemolysis can be observed in the cases of acute liver insufficiency and thus it represents one of the signs most often omitted during the diagnosis of Wilson's disease (18). Coombs-negative haemolytic anemia can be the first symptom in 10–15 per cent of the patients. It is due to the destruction of the erythrocytes by copper from the destroyed hepatocytes that has suddenly entered blood circulation. Wilson's disease-related light-degree haemolysis can be established prior to the clinical proof of the liver disease. The haemolysis can either be chronic, isolated, or combined with portal hypertension and splenomegaly. Cholelithiasis can secondarily develop as a result from haemolysis (20,26).

NEUROLOGICAL MANIFESTATIONS

Neurological symptoms represent the first clinical manifestation in about 40–60 per cent of the patients (3,6,18). Usually, they develop later than the liver disease, most commonly, during the second and third decade of life; they can, however, occur already in childhood, too (9,20). Brain areas affected in Wilson's disease coordinate movement. A slow (hypokinetic) or disturbed speech, tremor, dystonia, discoordination and dysphagia are typical symptoms. The patients can present with symptoms similar to Parkinson disease as copper, typically, accumulates in the areas of the basal ganglia affected by Parkinson disease (3,18,23). Epileptic state (24) and polyneuropathy of the extremities (16) were described as initial clinical manifestations of the disease. Kayser-Fleischer's rings are established in almost all the patients with neurological or psychotic manifestations (18,21).

PSYCHIATRIC MANIFESTATIONS

Psychiatric symptoms can occur prior to the hepatic or neurological manifestation, most commonly, between 2 and 5 years before it. They are established in 20–50 per cent of the patients being the first clinical symptom in 10–20 per cent of the cases. They can be classified into four basic categories (3,23):

- behavioural and personality disorders;
- affective disorders;
- schizophrenia-like disturbances (psychosis), and
- cognitive disorders.

The patients can develop depression leading sometimes to suicide attempts, paranoea, hallucinations, excitability, loss of sexual inhibitions or reduced concentration at school or at work. The behavioural and cognitive symptoms can be reversible during one-two years since treatment onwards (18).

OTHER MANIFESTATIONS

Wilson's disease patients can present with important extrahepatic manifestations independently of the neurological or psychotic symptoms.

Renal manifestations in Wilson's disease involve glomerules and tubules as well. Azotemia of different degree can be observed in more that 20 per cent of the patients. Glomerular filtration reduction varies between 10 and 14 per cent. Urinary copper causes tubular dysfunction. These alterations are similar to Fanconi's syndrome, i. e. to proximal tubular acidosis. A considerable loss of amino acids, i. e. aminoaciduria, of glucose, uric acids, calcium and phosphates can be established. These abnormalities lead to nephrocalcinosis and nephrolithiasis (17,20,21). Haematuria and nephrocalcinosis along with proteinuria can be observed prior to treatment as a component of the pathological process and as a side effect of D-penicillamine.

Musculoskeletal damage includes preterm osteopenia and osteoporosis in about 50 per cent of the patients as well as arthropathy, rhabdomyolysis, hydrocalcinosis, patellar chondromalacia and *osteochondrosis dissecans* (18,20,23,26).

Heart involvement presents with cardiomyopathy that can lead to congestive heart failure and rhythmic disturbances.

The anomalies in the functions of the endocrine glands include pancreatitis, hypoparathyroidism, menstrual disorders, secondary amenorrhea, infertility and habitual abortions (18,20,23,26).

Skin changes are expressed by hyperpigmentation of the lower limbs, bluish staining of nail lunula and *acantosis nigricans* (9).

Up to 25 per cent of the patients can present with the affection of more than one organ or system (18).

PHENOTYPIC CLASSIFICATION

At the 8th international meeting on Wilson's disease and Menkes' disease held on April 16–18, 2001 in Leipzig, Germany, Wilson's disease has been classified according to its type of presentation into three categories (11): hepatic (H) presentation, neurological (N) presentation and a category of others (O). The disease is classified as hepatic after exclusion of any

neurological symptoms. H1 refers to the acute hepatic form of the disease presenting with jaundice in a previously healthy individual because of hepatitis, Coombs-negative haemolytic anemia, or both of them. H2 describes any kind of chronic liver disease that can lead to or present with a decompensated hepatic cirrhosis. The neurological form includes clear neuropsychotic symptoms when diagnosed. N1 is related to a clinically manifested liver disease N2 is, however, not associated with a symptomatic liver disease. Documentation of an absence of a significant liver disease requires liver biopsy; fibrosis/steatosis can exist at any time. NX means that the patient has not been examined concerning the presence of a liver disease at all.

CONCLUSION

Wilson's disease is a rare inherited, however, a curable disturbance of copper metabolism that involves numerous organs and systems. It is an important reason for a chronic hepatic disease. There is a great variety of clinical manifestations of the disease. The diagnosis is easy in the patients who present with the classical manifestations of liver damage, Kayser-Fleischer's ring and neurological symptoms as well. However, it is difficult in the patients with fulminant hepatitis. The patients with a liver disease only represent a diagnostic challenge as the typical Kayser-Fleischer's rings are often absent. One should never forget the rare clinical manifestations, uncommon age, or the presence of an accompanying other liver disease. Knowledge of the clinical variety of Wilson's disease is of importance because early diagnosis and adequate treatment can warrant a good individual quality of life and survival of the patients.

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Anti-Müllerian hormone serum levels in men and women with overweight and obesity

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Серумните нива на Антимюлеровия хормон при мъже и жени с наднормено тегло и затлъстяване

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РЕЗЮМЕ

Целта на проучването е да се търсят отклонения в нивата на антимюлеровия хормон (AMH) и се установи връзката им с наднорменото тегло при жени със синдрома на поликистичните яйчници (PCOS) и мъже с метаболитен синдром. Ние изследвахме 22 жени с това заболяване и сравнихме техните резултати с тези на 20 здрави жени на същата възраст, а също така и 20 мъже с метаболитен синдром и 20 мъже с нормално тегло като контролна група. Серумните нива на AMH, тестостерона, гонадотропините, свързващия половите хормони глобулин, естрадиола, инсулина, кръвната захар и липидите бяха определени в кръвни проби, получени сутрин на гладно. Базалните нива на AMH при жените с PCOS (42.34 ± 6.42 pmol/L) бяха сигнификантно повишени в сравнение с контролите (21.58 ± 3.41 pmol/L), p=0.008, но при мъжете с метаболитен синдром нивата на AMH (30.84 ± 13.14 pmol/L) се оказаха сигнификантно по-ниски в сравнение с тези на здравите участници (43.14 ± 9.66 pmol/L), (p=0.002).

В заключение, серумните нива на AMH са повишени при жени с PCOS, но при мъже с метаболитен синдром концентрациите му са сиг-

ABSTRACT

The aim of the present study was to investigate the alterations of anti-müllerian hormone (AMH) in women with polycystic ovary syndrome (PCOS) and in men with metabolic syndrome. We have studied 22 females with this disorder and compared their results to those of 20 healthy age-matched women as well as 20 men with metabolic syndrome and 20 non obese men as control group. Serum levels of AMH, testosterone, gonadotropins, sex hormone binding globulin, estradiol, insulin, blood glucose and lipids were measured in blood samples taken in the morning after overnight fast. The basal AMH levels in women with PCOS (42.34 ± 6.42 pmol/L) were significantly higher in comparison with those of the controls (21.58 ± 3.41 pmol/L), p=0.008, but in men with metabolic syndrome AMH levels (30.84 ± 13.14 pmol/L) were significantly lower in comparison to the other healthy participants (43.14 ± 9.66 pmol/L), (p=0.002).

In conclusion, serum AMH levels are elevated in women with PCOS, but in men with metabolic syndrome its concentrations are significantly lower. The disturbances in androgen levels and metabolic state in both sexes are probably the cause for alterations of AMH levels than body weight per se.

нификатно по-ниски. Промените в нивата на андрогените и метаболитните нарушения са водещата причина и при двата пола за отклоненията в нивата на АМН, а не повишеното тегло per se.

Ключови думи: Антимюлеров хормон, синдром на поликистичните яйчници, затлъстяване, тестостерон, метаболитен синдром

Key words: Anti-Müllerian hormone, polycystic ovary syndrome, obesity, testosterone, metabolic syndrome

INTRODUCTION

The Anti-Müllerian hormone (AMH) was first mentioned in 1940, when A. Jost pointed to a protein substance, different from testosterone, formed in testes of mammals including man, which seemed to be responsible for regression of Müllerian ductus. It was named initially „Müllerian Inhibiting Substance" (9). This hormone is a dimeric glycoprotein, a member of the transforming growth factor-β (TGF-β) superfamily (2), which seems to act only in the reproductive organs (23).

In males, AMH causes regression of the Müllerian ducts during the fetal sex differentiation (2). It is synthesized in Sertoli cells of the testes since the 5th week of the embryonal development and then during the whole life (9). Serum AMH is an excellent marker of follicle stimulating hormone (FSH) and androgen action in the testis (8).

In females, AMH is involved in the regulation of follicular growth and development. It is produced postnatally by granulosa cells from preantral and small antral follicles (6,27). As AMH is largely expressed throughout folliculogenesis, from the primary follicular stage towards the antral stage, serum levels of AMH may represent both the quantity and quality of the ovarian follicle pool (15). It is formed in ovaries from 36th week gestation (9). Undetectable concentrations after spontaneous menopause have been reported (25).

In the past decade, measurements of serum AMH have provided useful tools for clinical investigation in several gonadal disorders (14). Some authors reported that serum AMH levels in women with polycystic ovary syndrome (PCOS) were higher than in controls (1,4,16) and were significantly related to the follicle number (20,25). In addition, its diagnostic application as a sensitive characteristic sign for primary and recurrent granulosa cell tumors has been proposed (7, 15). Different studies find reduced testosterone levels in obese men and in patients with metabolic syndrome (24), but scarce information is available about the effects of increased body weight on the AMH levels in men as well in women.

In view of these data, the purpose of our study was to assess the changes of AMH level and its relationship with some hormonal indices in overweight and obese men with metabolic syndrome and women with PCOS.

MATERIALS AND METHODS

Twenty two women of mean age 26.59 ± 1.10 years, x ± SEM (range 18 – 35) and body mass index (BMI) 30.73 ± 1.29 кg/m^2 were diagnosed with PCOS according to the Rotterdam consensus (22). They had oligo- or amenorrhoea, clinical hyperandrogenism and polycystic ovaries. Twenty healthy, age – matched women with regular menstrual cycles served as controls.

Forty men in reproductive age 18–56 years (mean 33.22 ± 2.68) were included in the study. Twenty of them fulfilled the criteria for metabolic syndrome, according to the International Diabetes Federation (10). The patients were compared to 20 non obese age-matched volunteers without gonadal disturbances.

Prior to enrolment in the study, all subjects underwent a complete general assessment, in-

cluding height, weight, waist circumference, blood sugar, lipid profile and blood pressure. Additionally in females ultrasonographic ovarian examination and Ferriman-Gallwey hirsutism scoring were done. Nine of the males had carbohydrate disturbances. BMI was calculated according to the formula: weight (kg) divided by the square of height (m^2). The quantity and percentage of fat tissue were determined by professional analyzer of body composition – Tanita TBF-300M.

Serum levels of AMH were measured in blood samples taken in the morning between 8:00 and 10:00 a.m. after an overnight fast (in women on day 3–4 of spontaneous or induced menses). After centrifugation sera were frozen at -20 ^{O}C. In the same blood samples serum levels of testosterone, luteinizing hormone (LH), FSH, sex hormone binding globulin (SHBG), estradiol, insulin, blood glucose and lipids were determined. The metabolic profile was investigated by measurements of oral glucose tolerance test (OGTT) and serum lipid levels. The homeostasis model assessment of insulin resistance (HOMA-IR) is used to quantify insulin resistance and beta-cell (17). In addition, using total testosterone and SHBG, we calculated the free androgen index (FAI) (FAI = total testosterone/SHBG) and the free testosterone using the method of Vermeulen et al (26).

Serum levels of AMH levels were measured using a commercially available ultrasensitive two-side enzyme immunoassay (ELISA), (Immunotech, Marseille, France). The assay sensitivity was 0.7 pmol/L. Inter- and intra-assay coefficients of variation were below or equal of 14.2 % and 12.3 %, respectively. The serum levels of LH, FSH, testosterone and SHBG were determened by time resolved fluoroimmunoassay (DELFIA) methods with commercial kits (Perkin Elmer, Wallac Oy, Turku, Finland). Serum HDL-ch and triglycerides were analyzed on an autoanalyzer Cobas Mira Plus (Hoffman La Roche) using enzymatic colorimetric method.

Statistical evaluation of the data was carried out using SPSS v.11 for Windows (SPSS Inc, Chicago, IL, USA). The Kolmogorov-Smirnov test for normality of the distribution was performed and accordingly Student's t-test or Mann-Whitney test were used to established the differences between the groups. Two-tailed Pearson or Spearman correlation analyses were used where appropriate. The results are expressed as the mean ± SEM. A p value of less than 0.05 was considered statistically significant.

RESULTS

The clinical, biochemical and hormonal parameters of the women with PCOS and healthy persons are given in Table 1.

Table 1. Clinical and hormonal data of women with PCOS and healthy controls (mean ± SEM)

Parameters	Patients with PCOS n=22	Controls n=20	P value
Age (yr)	26.59 ± 1.10	28.85 ± 0.80	>0.05
BMI (kg/m^2)	30.73 ± 1.90	22.11 ± 0.95	0.001
Waist circumference (cm)	89.64 ± 4.63	73.35 ± 2.56	0.005
Ferriman-Gallwey score	8.20 ± 1.02	4.30 ± 0.98	0.009
LH IU/L	7.07 ± 1.31	5.11 ± 0.55	>0.05
FSH IU/L	5.75 ± 0.86	5.44 ± 0.30	>0.05
Testosterone (nmol/L)	3.81 ± 0.29	2.45 ± 0.17	0.001
Free testosterone (nmol/L)	0.070 ± 0.009	0.026 ± 0.003	0.001
Free androgen index	12.17 ± 2.25	3.23 ± 0.31	0.001
SHBG (nmol/L)	46.17 ± 5.36	77.78 ± 4.97	0.001
Estradiol (pmol/L)	322.22 ± 39.16	257.75 ± 25.59	>0.05
Insulin (μU/mL)	15.97 ± 2.06	8.69 ± 1.25	0.005
AMH (pmol/L)	42.34 ± 6.42	21.58 ± 3.41	0.008
Body fat (%)	37.16 ± 2.25	26.99 ± 2.31	0.003
Body fat (kg)	32.47 ± 3.92	18.29 ± 2.70	0.006
HDL cholesterol (mmol/L)	1.39 ± 0.07	1.55 ± 0.10	> 0.05
Triglycerides (mmol/L)	1.20 ± 0.16	0.61 ± 0.04	0.001
HOMA	3.31 ± 0.47	1.39 ± 0.12	0.001

<0.05 considered statistically significant by t-test

As expected, Ferriman-Gallwey score, testosterone levels, free testosterone and FAI were significantly higher in the patients than in the control group (Table 1). The basal levels of serum AMH in patients with PCOS (42.34 ± 6.42 pmol/L) were significantly higher in comparison with those in the controls (21.58 ± 3.41 pmol/L), p=0.008. Among the patients with PCOS, overweight and obese patients (n=14) had 32.16% higher AMH levels (47.01 ± 8.34 pmol/L) than those with normal weight (35.57± 11.56 pmol/L), but difference was not statistically significant. However among the controls, AMH levels in normally weight healthy subjects (23.44 ± 3.99 pmol/L) were very close to those who were overweight (20.09 ± 6.78 pmol/L).

In the female controls we established a significant correlation of the basal levels of AMH with basal insulin concentration (r=-0.569, p=0.009), as well as with HOMA-IR (r=-0.613, p=0.026), with testosterone (r=+0.505, p=0.028), with FAI (r=+0.486, p=0.035), with LH (r=+0.480, p=0.044) and with LH/FSH ratio (r=+0.486, p=0.035). However, in the patients with PCOS no correlation was observed between AMH levels on the one hand and investigated metabolic and hormonal data on the other hand.

The main characteristics of the two groups of men as well as the statistically significant differences are shown on Table 2.

The levels of total testosterone, SHBG and free testosterone were significantly lower in men with metabolic syndrome. The concentrations of LH were similar in both groups while the FSH values were slightly, but significantly elevated in the patients (Table 2). In obese men with metabolic disturbances AMH levels were significantly lower than in the controls. The difference was significant between patients with carbohydrate disturbances and the other obese patients (24.06 ± 3.16 pmol/L) versus (36.38 ± 4.05 pmol/L, p= 0.033).

In all 40 men, AMH was negatively related to age (r=-0.544, p<0.001), BMI (r=-0.368, p=0.020), waist circumference (r=-0.435, p=0.005), and FSH (r=-0.365, p=0.020) and positively to free testosterone (r=+0.332, p=0.036).

DISCUSSION

We found that serum AMH levels are elevated in women with PCOS, but no correlation with body weight was noted in patients as well in the healthy women.

Some recent studies show that in the ovary AMH is produced by the granulosa cells and correlates with the count of small antral follicles (13). AMH basically has three main effects in the ovaries. One is that it does inhibit recruitment of follicles out of the primordial follicle pool (6,27). Secondly it seems to determine the sensitivity of ovarian follicles to FSH (6). Thirdly, it does inhibit aromatize activity which is responsible for the aromatization of androgens into estrogens (5). This later effect is largely responsible for the observation that there is a good correlation between AMH levels and androgen levels. Moreover, if androgen levels are high they might induce arrest in growing follicles, which increases the cohort of small antral follicles, and they in turn produce

Table 2. Anthropometric and hormonal data of men with metabolic syndrome and healthy controls (mean ± SEM)

Parameters	Metabolic syndrome n=20	Controls n=20	P value
Age (yr)	36.35 ± 2.78	30.10 ± 2.58	>0.05
BMI (kg/m^2)	35.59 ± 2.15	23.43 ± 0.50	0.001
Waist circumference (cm)	116.95 ± 4.43	81.60 ± 1.79	0.001
LH IU/L	4.05 ± 0.65	4.12 ± 0.48	>0.05
FSH IU/L	4.39 ± 0.55	2.85 ± 0.24	0.015
Testosterone (nmol/L)	15.74 ± 1.55	27.84 ± 2.86	0.001
Free testosterone (pmol/L)	430.35 ± 53.08	613.85 ± 67.90	0.040
SHBG (nmol/L)	21.71 ± 2.48	38.80 ± 3.91	0.001
Estradiol (pmol/L)	158.23 ± 21.04	118.37 ± 10.65	>0.05
AMH (pmol/L)	30.84 ± 2.94	43.14 ± 2.16	0.002

<0.05 considered statistically significant by t-test

more and more AMH. The significant increase of AMH is not surprising in this situation of follicle excess, which is a typical feature of the PCOS. It has been assumed that the intraovarian hyperandrogenism can cause an increase of the number of early stages follicles, and as a consequence the serum AMH concentrations are enhanced (13,20). Therefore, AMH might even constitute a more sensitive marker of ovarian dysfunction in women with PCOS than the ultrasound image! Moreover, AMH serum levels correlate well with other parameters indicative for the ovarian dysfunction in PCOS such as elevated LH and testosterone concentrations (1).

Patients with PCOS have a number of metabolic and endocrine disturbances, which interfere active in the pathogenesis of the syndrome. The studies on the relationship between AMH and metabolic disturbances in patients with PCOS are few (18). The results of our study demonstrate significant differences in the hormonal profile of the patients with PCOS compared to the healthy subjects. Hyperinsulinemia augments androgen production in PCOS. It has been postulated that insulin, acting through the IGF-1 and insulin receptors in the ovary, may stimulate androgen secretion (21). Also, insulin merely acts as a co-gonadotrophin, which is an additional cause for even more increased androgen levels. We found a positive correlation of insulin levels with androgens and body weight in our patients with PCOS, which is in agreement with these studies. Obesity and insulin resistance may enhance the follicular excess through the dysregulation of AMH or through the pathway of hyperandrogenemia. These findings might partly explain why adequate body weight correction and decrease in insulin resistance can improve the ovulatory function in women with PCOS (3). Because the levels of AMH in healthy normal and overweight healthy women are nearly the same we suppose that metabolic alterations in patients with PCOS are more important for the changes in AMH than body weight per se.

In males with metabolic syndrome we found significantly lower levels of AMH, which correlated negatively with obesity indices. These results are unexpected in vew of the known mechanisms of the hormonal regulation. AMH is a marker of the immature Sertoli cells and its concentrations remain high until the onset of puberty. Thereafter, the levels of AMH decrease in coincidence with the increase of intratesticular testosterone concentrations. Androgens are responsible for AMH down-regulation independently of age and gonadotropin levels. Their inhibitory effect prevails over the positive effect of FSH on AMH secretion in the pubertal testis (8).

Increasing abdominal obesity leads to increased activity of the enzyme aromatase, present in adipose tissue, which converts testosterone to estrogen. The resulting low testosterone leads to insulin resistance which in turn causes further androgen deficiency and visceral fat deposition, that suppress hypothalamic-pituitary axis (11). If metabolic syndrome is a state of an isolated secondary hypogonadotropic hypogonadism, raised AMH concentrations might be expected because of the lower testosterone levels. The results of our study for lower AMH in patients with metabolic syndrome could not be explained by the action of gonadotropins since the FSH levels were found in the normal ranges and even slightly higher in patients than in controls. Consequently, Sertoli cell impairment in obese men with metabolic disturbances might be assumed.

We hypothesized that the prolonged synergic influence of several risk factors as insulin resistance and higher levels of different adipocytokines related to increased visceral obesity could suppress more deeply Sertoli cell function. The difference in AMH levels between obese men and women with PCOS could be due to the different androgen concentrations in both sexes. Additionally, adipocitokines could play an important role.

The hypotheses of primary testicular dysfunction in men with metabolic syndrome was

supported by several findings in the literature. In healthy women without PCOS serum AMH levels correlated negatively with insulin, fasting glucose and HOMA index (19). Likewise, a significant positive correlation between the AMH levels and adiponectin was found (19). The influence of adipocitokines on testicular function was already demonstrated in humans (12).

In conclusion, AMH levels are vastly elevated in women with PCOS but lower in men with metabolic syndrome, which may be related to the alterations in the Sertoli cells function. The changes in androgen levels and metabolic state (especially hyperinsulinemia) in both sexes seem to the main cause for the disturbed AMH secretion.

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Specific aspects of the quality of life of patients with refractory epilepsy

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Специфични аспекти на качеството на живот на пациентите с резистентна епилепсия

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РЕЗЮМЕ

Цел: Да оценим качеството на живот (КЖ) на пациенти с резистентна и фармакочувствителна епилепсия (ФЧЕ) и да определим специфичните аспекти на качеството на живот на пациентите с резистентна епилепсия (РЕ).

Методи: В проучването участваха 70 възрастни пациента с РЕ без когнитивни нарушения, прогресивни соматични, неврологични заболявания, и пристъпи в последните 24 часа, и 70 пациенти с ФЧЕ. Всички участници попълниха въпросника за КЖ QOLIE-89.

Резултати: Общата оценка от QOLIE-89 (Т-стойност) на пациентите с РЕ е малко под средната за епилептичната популация (47.80). Ниски средни оценки (Т-стойности) са получени за подскалите „перцепции за здраве“ (39.43), „сексуални взаимоотношения“ (42.50) и „цялостно КЖ“ (42.79). Средните оценки на всички останали подскали (Т-стойности) са близки до средните за епилептичната популация (46–55). Установяваме статистически значима разлика между оценките на пациентите с РЕ и ФЧЕ за всички подскали с изключение на подскалата „промяна в здравето“ – оценките на пациентите с РЕ са по-ниски.

ABSTRACT

Purpose: To assess the quality of life (QOL) of patients with refractory and pharmacosensitive epilepsy and to determine the specific aspects of the quality of life of the patients with refractory epilepsy.

Methods: We studied 70 adult patients with refractory epilepsy (RE), without cognitive impairment, progressive somatic, neurological disease or recent seizures, and 70 patients with pharmacosensitive epilepsy. All participants completed QOLIE-89.

Results: The overall T-score of QOLIE-89 calculated for the patients with RE was slightly below the mean for the epileptic population (47.80). Low mean T-scores were calculated for the subscales „health perceptions“ (39.43), „sexual relations“ (42.50), and „overall QOL“ (42.79). The mean T-scores of all other subscales were close to the mean for the epileptic population (46–55). A significant difference between the scores of the patients with RE and PSE for all subscales with the exception of the subscale „change in health“ was found – the scores given by the patients with RE were lower.

Conclusion: RE has a negative impact on all aspects of QOL (with the exception of the change in health in the last year) and mostly on health perceptions, sexual relations, and the overall QOL.

Заключение: РЕ оказва негативно влияние върху всички аспекти на КЖ (с изключение на промяна в здравето през последната 1 година) и в най-голяма степен върху перцепциите за здраве, сексуалните взаимоотношения и цялостното КЖ.

Ключови думи: резистентна, фармакочувствителна, епилепсия, качество на живот

Key words refractory, pharmacosensitive, epilepsy, quality of life

INTRODUCTION

Epilepsy has a great influence on the three levels of quality of life (physical, mental and social health), an influence that is exercised directly by affecting physical and mental health and indirectly by introducing limitations and decreasing the opportunities for taking part in quality of life improving activities. It is associated mostly with the uncertainty about the disease development. The quality of life (QOL) of the patients with refractory epilepsy (RE) is even lower than that of the general epileptic population and than the QOL of the patients having other chronic diseases. The influence of the various psychological, social, clinical and demographic factors on the QOL correlates with: activity and mobility limitations, sleep disorders, fear of seizures and death, depression, anxiety, cognitive impairment, education difficulties, stigma, lower self-esteem and worse social adaptation, lower life satisfaction, family dynamics and/or parental anxiety, employment difficulties, worries about independence, knowledge about epilepsy, self-management, reproductive functions, ability of decisions taking, seizure frequency and severity, duration of epilepsy, number of antiepileptic drugs, and adverse drug events (1, 2, 3).

AIM

Our purpose was to assess the quality of life of patients with refractory and pharmacosensitive epilepsy and to determine the specific aspects of the quality of life of the patients with refractory epilepsy.

PATIENTS AND METHODS

The study was performed with the participation of 176 consecutive patients with RE and 70 consecutive patients with pharmacosensitive epilepsy (PSE) who attended the Clinic of Neurology at the University Hospital in Plovdiv, Bulgaria for a regular examination or in cases of unsatisfactory seizure control or adverse events from treatment.

All study procedures were performed after the approval of the Local Ethics Commission at the University of Medicine, Plovdiv. Every patient was introduced to the study design and signed an informed consent form before participating in the study procedures.

The following inclusion criteria were used: a signed informed consent form; age between 18 and 65 years; a diagnosis of RE or PSE; lack of cognitive impairment based on Evaluation Rapide des Fonctions Cognitives (ERFC; Gil and Toullat, 1986) with a score < 47 in patients up to 60 years of age and primary education or < 46 in patients between 60 and 65 years of age and less than a primary education or illiterate; lack of progressive somatic or neurological disease; lack of a simple or complex partial seizure in the last 4 hours; and lack of generalised tonic-clonic seizure in the last 24 hours. Epilepsy was accepted as refractory in cases in which adequate seizure control had not been achieved with at least two potentially effective anti-epileptic drugs prescribed as mono- or poly-therapy at maximally tolerated doses. After excluding 39 patients with pseudo-refractory epilepsy (in cases with diagnostic, therapeutic errors or poor compliance), 2 patients older

than 65 years, 2 patients with progressive neurological disease, 5 patients with a simple or complex partial seizure in the last 4 hours or a generalised tonic-clonic seizure in the last 24 hours, and 58 patients with cognitive impairment, 70 patients with RE and 70 patients with pharmacosensitive (PSE) were included in the study. Both groups were similar with respect to age and gender.

The data were collected by a trained health professional using a purposeful interview on the patients' disease history, as well as by examining the patients' medical documentation. All participants completed QOLIE-89.

Twenty one (30.0% ± 5.5) of the participants with refractory epilepsy were men; the remaining 49 (70.0% ± 5.5) were women. The mean patient age was 41.7 ± 1.1 years. Most participants (76.6%) were between 30 and 60 years of age. The mean disease duration was 25.1 ± 1.3 years. Of the patients with PSE, 34 (48.6%) were men, and 36 (51.4%) were women; their mean age was 36.7 ± 1.5 years. There was no significant difference between both groups regarding their gender ($P > 0.05$, $\chi^2 = 2.4$) and age ($P > 0.05$, $u = 0.6$).

QOLIE-89 is the most understandable and most widely used instrument for quality of life assessment in patients with epilepsy. It is the scale that includes the greatest number of epilepsy-associated factors. QOLIE-89 has been approved for research, and it is completed in 45 minutes. This scale enables the discrimination of minimally expressed but significant life quality changes in these patients. QOLIE-89 contains 89 items that are distributed in 17 subscales, characterizing 4 basic factors directed towards epilepsy, physical, mental, and social health. Except for the standard scores, the so-called „T-scores" for each of the 17 scale final scores and the overall score are calculated. The T-scores represent linear transformations of the scores that produce a mean of 50 and a standard deviation of 10 for the cohort of 304 adults with epilepsy. Higher T-scores reflect a more favorable quality of life.

In the course of the study, we made a validation of the Bulgarian translation of QOLIE-89 and proved its reliability, internal consistency (the mean of Crohnbach's α was 0.9 ± 0.0; the coefficient of Spearman-Brown was 0.9; the mean inter-item correlation was 0.3; we calculated a high coefficient of correlation between the subscales scores and the overall score in two completions of the questionnaire [$r_{xy} = 0.8 – 1.0$]) and validity (strong correlations between the overall scores of QOLIE-89 and QOLIE-31 [$r_{xy} = 0.9$] and between their corresponding subscales were found [$r_{xy} = 0.9 – 1.00$]) /4/.

The collected primary information was checked, encoded, and entered into a computer database for statistical analysis. Data were processed using STATA Version 10 (Stata Corp., College Station, TX, U.S.A.) and SPSS (Statistical Package for the Social Sciences), version 14.0 (SPSS Inc., Chicago, IL, U.S.A.). The results for quantitative variables were expressed as the mean ± SE (standard error), and the results for qualitative variables were expressed as percentages ± SE. Age, gender, and the clinical findings of the patients with RE and PSE were compared by means of χ^2 – Test and Z – test. Fisher's criterion was used for the comparison of the assessments of the subscales and the overall score of QOLIE-89 of the patients with RE and PSE.

RESULTS

The clinical findings of the study participants are shown in Table 1.

The mean overall score of QOLIE-89 given by the patients with refractory epilepsy was 64.3 ± 17.1. In our data analysis, the T-scores were used for a more explicit comparison with the mean scores of the epileptic population. The obtained scores were accepted as very low (≤ 35), low (36 – 45), medium (46 – 55) and high (> 55). As a T-score, the mean overall score of QOLIE-89 was lower than the mean of the epileptic population (x = 47.8). Low mean scores were obtained for the subscales „health perceptions" (x = 39.4), „sexual relations" (x = 42.5) and „overall quality of life" (x = 42.8). The mean

Табл. 1. Клинични характеристики на изследваните пациенти
Table. 1. Clinical findings of the study participants

	RE		PSE		χ^2/Z	P
	N	P (%) ± SE	N	P (%) ± SE		
Type of epilepsy						
– partial	53	75.7 ± 5.1	24	34.3 ± 5.7	χ^2 = 26.4	< 0.001
– generalized	17	24.3 ± 5.1	46	65.7 ± 5.7		
Etiology of epilepsy						
– idiopathic	17	24.3 ± 5.1	30	42.9 ± 5.9	χ^2 = 5.4	> 0.05
– symptomatic	28	40.0 ± 5.9	21	30.0 ± 5.5		
– cryptogenic	25	35.7 ± 5.7	19	27.1 ± 5.3		
Type of seizures						
– partial	17	24.3 ± 5.1	24	34.3 ± 5.7	χ^2 = 52.7	< 0.001
– generalized	16	22.9 ± 5.0	46	65.7 ± 5.7		
– a mixture of partial and generalized seizures	37	52.8 ± 6.0	–	–		
Recent seizure frequency						
– without seizures in recent years	–	–	70	100.0		< 0.001
– 1/ several years						
– 1–11 seizures/ year	2	2.9 ±-	–	–	Z = 11.0	
– 1–3 seizures/ month	11	15.7 ± 4.35	–	–		
– 1–6 seizures/ week	21	30.0 ± 5.48	–	–		
– daily seizures	32	45.7 ± 5.95	–	–		
	4	5.7 ±-	–	–		

* SE – standard error
* P (%) – percentage of patients

scores of all other subscales were close to the mean for the epileptic population.

The subscales of QOLIE-89 were distributed in the following 5 groups: subscales associated with physical health, subscales associated with mental health, subscales associated with social health, subscales associated with epilepsy, and subscales associated with a more general assessment, e.g., „overall health“ and „overall quality of life“. For the purpose of data analysis, the overall QOLIE-89 score was also included in the last group.

Subscales associated with physical health: health perceptions, physical function, role limitations – physical, pain, energy/fatigue, health discouragement, change in health, sexual relations.

The mean T-values of the patients from both groups of these subscales are shown in Table 2. The lowest mean values were for the subscales „health perceptions“ and „sexual relations“ which suggested a lower perception of the quality of health and sexual relations of the patients with RE compared to the epileptic population. A significant difference between the scores of the patients with RE and PSE for all subscales associated with physical health with the exception of the subscale „change in health“ was found.

The distribution of the patients with RE according to the scores for the subscales associated with physical health is presented in Fig. 1. The greatest percentage of participants (72.83%), who gave very low and low scores, was for the subscale „health perceptions“. The lowest percentage of participants, who gave very low and low scores, was for the subscales „physical function“ (29.03%) and „role limitations – physical“ (36.17%). The greatest percentage of patients, who gave high scores, was for the subscales „physical function“ and physical limitations. 38.71% of the participants with RE were discouraged about their health and gave very low and low scores for the respective

Табл. 2. Средни стойности и съпоставка на оценките за подскалите свързани с физическото здраве на пациентите с РЕ и ФЧЕ
Table 2. Mean values and comparison between the scores for the subscales associated with physical health given by the patients with RE and PSE

QOLIE-89 subscale	Patients	N	x	Sx	95 % CI		F	P
Health perceptions	RE	70	39.43	1.42	36.60	42.25	45.79	< 0.001
	PSE	70	50.61	0.85	48.91	52.31		
Physical function	RE	70	50.17	1.16	47.86	52.49	28.95	< 0.001
	PSE	70	56.60	0.28	56.04	57.16		
Role limitations – physical	RE	70	50.05	1.36	47.34	52.77	36.96	< 0.001
	PSE	70	58.66	0.38	57.90	59.42		
Pain	RE	70	49.27	1.28	46.72	51.81	23.77	< 0.001
	PSE	70	56.39	0.71	54.97	57.80		
Energy/fatigue	RE	70	47.69	1.14	45.42	49.97	20.96	< 0.001
	PSE	70	53.89	0.73	52.44	55.36		
Health discouragement	RE	70	47.25	1.14	44.97	49.52	78.53	< 0.001
	PSE	70	58.34	0.52	57.31	59.37		
Change in health	RE	70	53.93	2.53	48.89	58.97	0.70	> 0.05
	PSE	70	56.43	1.58	53.27	59.59		
Sexual relations	RE	70	42.50	3.68	35.16	49.84	17.82	< 0.001
	PSE	70	60.71	2.25	56.22	65.20		

subscale, 21.51% were optimistic and gave high scores. 47.56% of the participants gave low score for the subscale „energy/fatigue". Regarding the subscale „pain" 41.86% gave very low and low scores, 19.77% – scores close to the mean for the epileptic population, and 38.37% gave high scores. 39.36% gave very low scores for the subscale „sexual relations", 37.23% – scores close to the mean for the epileptic population, the rest gave high scores.

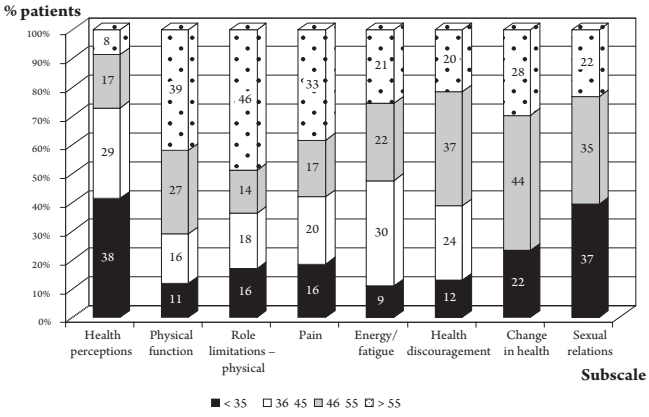
Subscales associated with mental health: role limitations – emotional, emotional well-being, attention/concentration, memory, language.

The mean T-values of the patients from both groups of these subscales are shown in Table 3.

The mean values of all subscales were close to the mean for the epileptic population. The lowest mean scores were for the subscales „emotional well-being" and „attention/concentration". Obviously RE has a greater negative impact on these aspects of mental health. The mean values of the other subscales were slightly above the mean for the epileptic population. The highest mean value was for the subscale „language". A significant difference between the

Фиг. 1. Разпределение на пациентите с РЕ според оценките им за подскалите свързани с физическото здраве

Fig. 1. Distribution of the patients with RE according to the scores for the subscales associated with physical health



scores of the patients with RE and PSE for all subscales associated with mental health was found.

The distribution of the patients with RE according to the scores for the subscales associated with mental health is presented in Fig. 2. Of the patients with RE 65.12% and 64.52% gave high scores for the subscales „role limita-

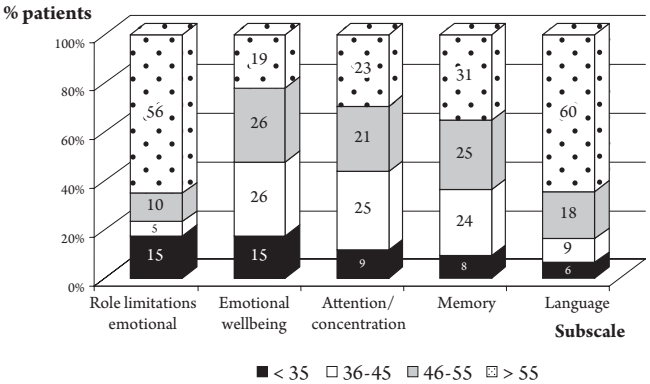
Табл. 3. Средни стойности и съпоставка на оценките за подскалите свързани с психичното здраве на пациентите с РЕ и ФЧЕ
Table 3. Mean values and comparison between the scores for the subscales associated with mental health given by the patients with RE and PSE.

Subscales	Patients	N	x	Sx	95 % CI		F	P
Emotional limitations	RE	70	51.62	1.34	48.94	54.29	29.44	< 0.001
	PSE	70	58.89	0.00	58.89	58.89		
Emotional well-being	RE	70	46.44	1.26	43.92	48.97	35.70	< 0.001
	PSE	70	55.13	0.72	53.70	56.56		
Attention/concentration	RE	69	48.30	1.22	45.87	50.73	87.61	< 0.001
	PSE	70	60.87	0.58	59.71	62.03		
Memory	RE	70	52.77	1.31	50.14	55.39	72.10	< 0.001
	PSE	70	65.73	0.77	64.18	67.27		
Language	RE	70	54.31	1.24	51.84	56.78	38.06	< 0.001
	PSE	70	61.96	0.08	61.79	62.13		

tions – emotional“ and „language“ respectively. As for the subscale „memory“ 36.36% gave very low and low scores. 35.23% participants with RE perceived their memory as good. With regards to the subscale „attention/concentration“, 43.59% gave very low and low scores, only 29.49% perceived these functions were not impaired. 47.67% of the participants with RE assessed their emotional well-being as disturbed by giving very low and low scores for the respective subscale. Only 22.09% gave high scores for the subscale „emotional well-being“.

Фиг. 2. Разпределение на пациентите с РЕ според оценките им за подскалите свързани с психичното здраве

Fig. 2. Distribution of the patients with RE according to the scores for the subscales associated with mental health



Subscales associated with social health: work/driving/social function, social support, social isolation

The mean T-values of the patients from both groups of these subscales are shown in Table 4. The lowest mean value was for the subscale „work/driving/social function“ – slightly below the mean for the epileptic population. The highest mean value was for the subscale „social support“, the mean value of the subscale „social isolation“ was close to the mean for the epileptic population.

A significant difference between the scores of the patients with RE and PSE for all subscales associated with social health was found.

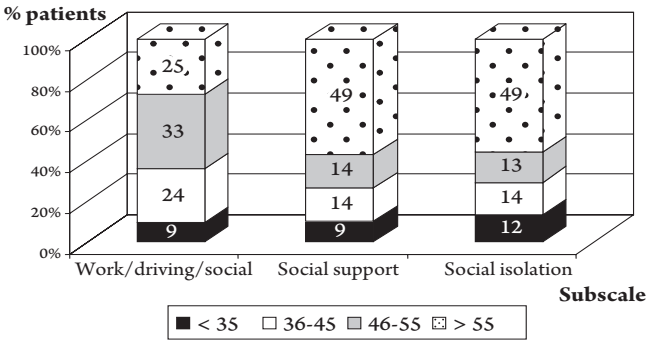
The distribution of the patients with RE according to the scores for the subscales associated with social health is presented in Fig. 3.

The greatest percentage of the patients with RE (40.74%) gave very low and low scores for their abilities of work, driving and social functions, 29.55% of the participants with RE gave similar scores for their social isolation, and 26.74% – for the social support. Respectively 56.98% gave high scores for the social support, 55.68% – for the social isolation, and only 27.47% – for the subscale „work/driving/social function“. We made the conclusion that the patients with RE perceived their social life as difficult, realized the social isolation, and gave medium to high scores for the social support.

Табл. 4. Средни стойности и съпоставка на оценките за подскалите свързани със социалното здраве на пациентите с РЕ и ФЧЕ
Table 4. Mean values and comparison between the scores for the subscales associated with social health given by the patients with RE and PSE

Подскали	Patients	N	x	Sx	95 % CI		F	P
Work/driving/social function	RE	70	48.40	1.13	46.14	50.65	114.79	< 0.001
	PSE	70	61.03	0.33	60.37	61.70		
Social support	RE	70	53.22	1.27	50.68	55.76	37.21	< 0.001
	PSE	70	61.33	0.38	60.57	62.09		
Social isolation	RE	70	50.30	1.38	47.55	53.05	39.04	< 0.001
	PSE	70	59.04	0.23	58.59	59.50		

Фиг. 3. Разпределение на пациентите с РЕ според оценките им за подскалите свързани със социалното здраве
Fig. 3. Distribution of the patients with RE according to the scores for the subscales associated with social health



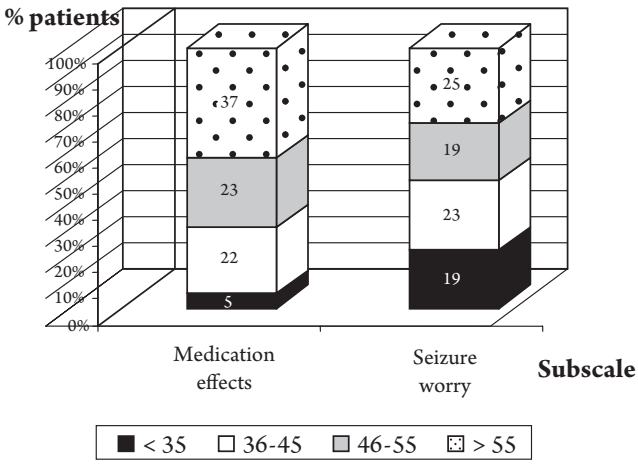
Subscales associated with epilepsy: seizure worry, medication effects

The mean T-values of the patients from both groups of these subscales are shown in Table 5.

The mean values of both subscales scores were close to the mean of the epileptic population. The mean value of the subscale „medication effects" was slightly higher, while the mean value of the subscale „seizure worry" was slightly lower than the mean for the epileptic population. We made the conclusion that the patients with RE were more worried about the seizure frequency and severity than the adverse drug events. A significant difference between the scores of the patients with RE and PSE for both subscales associated with epilepsy was found.

The distribution of the patients with RE according to the scores for the subscales associated with epilepsy is presented in Fig. 4.

Фиг. 4. Разпределение на пациентите с РЕ според оценките им за подскалите свързани с епилепсията
Fig. 4. Distribution of the patients with RE according to the scores for the subscales associated with epilepsy



The percentage of patients, who gave very low scores for the subscale „seizure worry", was higher (22.09%) than the percentage of patients with similar scores for the subscale „medication effects" (5.75%). 22 (25.29%) participants with RE gave low scores for the subscale „medication effects" and 23 (26.74%) – for the subscale „seizure worry". The percentage of patients with high scores was significantly lower – 29.07% for the subscale „seizure worry" and 42.53% for the subscale „medication effects".

OVERALL HEALTH, OVERALL QOL AND OVERALL SCORE OF QOLIE-89

The mean T-values of the patients from both groups of the subscales „overall health", „over-

Табл. 5. Средни стойности и съпоставка на оценките за подскалите свързани с епилепсията на пациентите с РЕ и ФЧЕ
Table. 5. Mean values and comparison between the scores for the subscales associated with epilepsy given by the patients with RE and PSE

Subscales	Patients	N	x	Sx	95 % CI		F	P
Medication effects	RE	70	51.59	1.26	49.09	54.10	47.98	< 0.001
	PSE	70	61.42	0.66	60.10	62.74		
Seizure worry	RE	70	45.66	1.34	42.99	48.34	99.96	< 0.001
	PSE	70	61.50	0.85	59.82	63.19		

Табл. 6. Средни стойности и съпоставка на оценките за подскалите „общо здраве“, „цялостно КЖ“ и общата оценка от QOLIE-89 на пациентите с РЕ и ФЧЕ
Table. 6. Mean values and comparison between the scores for the subscales „overall health“, „overall QOL“, and the overall score of QOLIE-89 given by the patients with RE and PSE

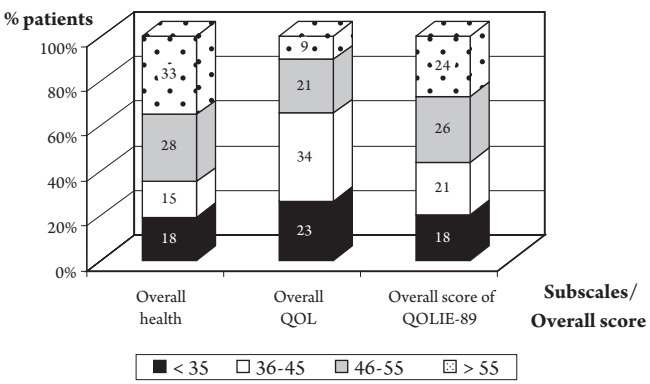
Subscale/ Overall score	Patients	N	x	Sx	95 % CI		F	P
Overall health	RE	70	52.79	2.46	47.88	57.69	68.67	< 0.001
	PSE	70	77.79	1.75	74.30	81.27		
Overall QOL	RE	70	42.79	1.24	40.31	45.27	49.84	< 0.001
	PSE	70	53.67	0.91	51.85	55.48		
Overall score of QOLIE-89	RE	70	47.80	1.37	45.07	50.54	86.02	< 0.001
	PSE	70	61.89	0.65	60.59	63.20		

all QOL“, and the overall score of QOLIE-89 are shown in Table 5.

The mean score of the subscale „overall health“ was slightly higher than the mean for the epileptic population. The mean value of the subscale „overall QOL“ was significantly lower, while the mean value of the overall score of QOLIE-89 was slightly lower than the mean for the epileptic population. A significant difference between the scores of the patients with RE and PSE for both subscales and the overall score of QOLIE-89 was found.

The distribution of the patients with RE according to the scores for the subscales „overall health“, „overall QOL“, and the overall score of QOLIE-89 is presented in Fig. 5. The greatest percentage of patients with RE, who gave very low and low scores (65.51%), was for the subscale „overall QOL“. Only 9 (10.34%) participants with RE gave high scores for this subscale. Regarding the subscale „overall health“ 33 (35.11%) patients gave very low and low scores; the same percentage gave high scores. The analysis of the overall score of QOLIE-89 demonstrated that 18 (20.22%) participants assessed their QOL as very low, 21 (23.60%) – as low, the assessments of 26 (29.21%) were close to the mean for the epileptic population, and 24 (26.97) perceived their QOL as high. We made the conclusion that the RE had a negative impact on the subscales „general health“, „overall QOL“, and the overall score of QOLIE-89.

Фиг. 5. Разпределение на пациентите с РЕ според оценките им за подскалите „общо здраве“, „цялостно КЖ“ и общата оценка от QOLIE-89
Fig. 5. Distribution of the patients with RE according to the scores for the subscales „general health“, „overall QOL“, and the overall score of QOLIE-89



DISCUSSION

The purpose of this study was to assess the quality of life of adult patients with refractory and pharmacosensitive epilepsy and to determine the specific aspects of the quality of life of the patients with refractory epilepsy. The overall score of QOLIE-89 calculated for the patients with RE was slightly below the mean for the epileptic population (47.80). Low mean scores were calculated for the subscales „health perceptions“ (39.43), „sexual relations“ (42.50), and „overall QOL“ (42.79). The mean scores of all other subscales were close to the mean for the epileptic population (46–55). A significant difference between the scores of the patients with RE and PSE for all subscales with the exception of the subscale „change in health“ was found – the scores given by the patients with RE were lower. RE has a negative impact on all aspects of QOL (with the exception of the change in health in the last year) and mostly on health perceptions, sexual relations, and the overall QOL.

The results from our study are in conformity with the data from other studies. Wagner et al. (1996) have proven that the QOL of adult patients with epilepsy is lower than the QOL of healthy people, especially with regards to the following aspects: health perceptions, mental health, energy, daily activities, and emotions (5). In 2000 Stavem et al. have demonstrated that the QOL of patients with controlled seizures is comparable to the QOL of the general population, except for slightly lower results in the emotional domain (6). In cases with refractory epilepsy however, the results for the social aspects (marriage, education, work) of QOL are lower (7). Loring et al. (2004) have calculated a mean overall score for QOLIE-89 of 41.9 ± 11.0 as a result from their study of patients with RE (8). The mean overall score of QOLIE-31 obtained by Guekht et al (2007) from their study was 42.13 ± 4.14 (9). Both results seem lower than the real value obtained from our study (64.30 ± 2.04), T-value of 47.80 ± 1.37, but a significant difference among the three results was not found $P>0.05$, u (Loring) = 0.56; u (Guekht) = 1.30.

LIMITATIONS

The first limitation of our study is that with the purpose of an adequate completion of QOLIE-89, we excluded patients older than 65 years, as well as those having cognitive impairment, progressive neurological disease, and those with either simple or complex partial seizures in the last 4 hours or generalized tonic-clonic seizures in the last 24 hours. The participation of only those patients that had access to the University Clinic of Neurology, as they usually attended it for either a regular examination or in cases of unsatisfactory seizure control or adverse events from treatment, is also a limitation. These limitations do not devalue the results from our study. Further investigations of patients having different demographic, clinical and social characteristics are needed.

IN CONCLUSION

In conclusion the demonstration of the significant decrease in most aspects of the QOL of patients with RE contributes to the attention that this problem has drawn in Bulgaria, as well as all over the world, and illustrates the necessity of a multidisciplinary approach in dealing with these patients. Doing so will give us opportunities for a broader campaign, with the aim of „getting epilepsy out of the shadows“ by increasing the role of epileptic patient associations, media, improving the education of medical and non-medical staff, and encouraging the government to give additional financial aid to patients and their families and invest in educational programs and research.

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РЕЗЮМЕ

Маркетингът е управленски процес на идентифициране, реагиране на промени и задоволяване изискванията на потребителите, като се реализира печалба. В областта на медицината единствено рекламата на лекарствени средства е разрешена и то под строги регулации. **Цел** на проведеното проучване е анализ на нормативната база за осъществяване на рекламна дейност и реклама в здравеопазването и определяне на вида рекламна стратегия и използваните рекламни материали на мястото на продажбите в аптеки на територията на гр. Пловдив. **Метод и материали**: Проведеното проучване включваше документен анализ на правни и нормативни документи, касаещи рекламата в здравеопазването анкетно проучване на място в аптеки на територията на гр. Пловдив. **Резултати**: Резултатите от проучването показват, че най-често използваните рекламни продукти са химикалките, брошурите и диплянки, като те заедно формират над 50% от използваната реклама. Най-често рекламирани са продуктите повли-

ABSTRACT

Marketing is a managerial process of identification, reaction to changes and meeting customers' needs, while making profits. In the field of medicine in Bulgaria it is only medicinal products advertizing that is permitted and it is a subject to strict regulations. The **goal** of the study is to analyze the normative basis for advertizing in healthcare, and to determine the types of strategies and materials used at the point of sale in the pharmacies at the territory of the city of Plovdiv. **Methods and Materials**: The study included documentary analysis of legal and normative documents, concerning advertizing in healthcare and survey of pharmacies in Plovdiv. **Results**: The results from the study revealed that the advertising materials most often used were pens, brochures, folders (in more than 50% of the cases). The most frequently promoted products were those acting on metabolism and the alimentary system targeting people over 15 years of age. **Conclusion:** The existing legislation frameworks leave legal vacuum in the area of advertizing leading to ineffective regulation. There is a need of modernization and

яващи метаболизма и храносмилателната система, а целевата група към която е насочена рекламата е във възрастта над 15 години. **Изводи**: Съществуващите законовите рамки към настоящият момент създават правен вакуум в областта на рекламата, което води до неефективно използване на рекламните стратегии. Необходимо е осъвременяване и промяна на законите третиращи използването на реклама изобщо, и в частност рекламата в здравеопазването.

Ключови думи: маркетинг в здравеопазването, рекламни стратегии, аптеки

change of legislation concerning the advertizing in general and in healthcare in particular.

Keywords: health marketing, advertisement strategies, pharmacies

INTRODUCTION

Promotion is a major part of the marketing mix involving all forms of communication, used by the manufacturers for informing, influencing attitudes, persuading or reminding the consumers of the goods or services produced and offered through public activities or work in the communities. Promotion includes various marketing tools delivering information to the targeted groups, namely advertizing, public relations, sale incentives, personal sales [2,3].

Publicity (from Latin *reclamo* – evoke) implies distribution of positive information related to physical or legal entity, goods, ideas or initiatives, in any form and by all means, directed to specific groups of customers, with the aim of provoking, direct or maintain their interest in the entities, goods, ideas and initiatives under question so that they may be chosen, bought, visited, followed etc [2,4].

With regards to the medical services, the Health Act (HA***)*** contains two articles regulating the use of advertising [6]. The first one refers to non-conventional methods used in the medicine. Art. 169 prohibit all forms of advertising non-conventional methods, including their relations to prophylactics, diagnostics, treatment and rehabilitation. The second article (art.190 (2)) doesn't allow to the medical specialists as well as to medical institutions and establishments the use of commercial advertising of their activity. The BHA however doesn't contain definition of „commercial advertisement". The same act doesn't contain definition of „advertising" in general.

At present in the field of medicine and healthcare in Bulgaria only the advertizing of medicinal products is allowed and it is strictly regulated. Commercial advertizing of medical specialists and medical institutions or establishments is prohibited, yet the respective laws do not define or refer to the interpretation of „advertisement", allowing misinterpretation. In current Bulgarian legislation there are no particular acts treating advertizing [6,7,8,9,10,11].

AIM

The goal of this study was to analyze the legislation on advertizing in the field healthcare and to determine the types of advertizing strategies and advertisement material used at the point of sale in pharmacies (registered with the Ministry of Health) in Plovdiv [12].

METHODS AND MATERIALS:

30 pharmacies (20 % random sample of the pharmacies registered in Plovdiv) were studied using direct, individual surveys in the period 19.03.2012 – 24.03.2012.

For the purposes of the study an original tool was developed which included the following panels of questions: 1) main characteristics of study objects (location, size, contract with

NHIF, type of advertisement used at the point of sale); and 2) pharmacy supply with advertisement materials, preferences of the pharmacy employees with regards to the advertising materials, the knowledge of regulations with regards to the advertizing medicinal products as well as a questions related to the ownership of the pharmacy.

The study is representative for the studied group (pharmacies in Plovdiv). Referring to the size (number of the employees) majority of the pharmacies had one employee (n=14, 33.33%), followed by the big pharmacies with more than 3 employees (n=10, 46, 67 %) and the smallest number were those with 1 to 3 employees – medium size pharmacies (n=6, 20 %). The majority were separate 23 (n=76, 67%), and only 7(n=23, 33%) are part of chains.

The data processing and graphical presentation was performed by means of software packages MS Excel 2003 and Open office ver. 3.3.0.

RESULTS AND DISCUSSION

location is of great importance to attendance frequency and commercial profits. The survey results revealed that most of the pharmacies– 12 (40%) were located close to places with intensive traffic, 11 (37%) were located close to hospitals and only 7 (23%) were outside of these settings.

Since the use of advertisement materials in the pharmacies is related to use of space, parts of windows or internal space, it always requires the consent of the pharmacy owners. Usually when pharmacies are part of a chain the owners follow one and the same approach towards the advertising in the different commercial places.

The sale of drugs which are reimbursed by the NHIF may be an important factor determining frequency of attendance to a pharmacy. In our survey 21 (70%) of the pharmacies that took part fulfilled prescriptions for medications reimbursed by NHIF.

Advertising ball-point pens proved to be the most frequently used materials. However these pertain to souvenir advertisements, which are rather company image advertising, promoting the name of the company and/ or the name of one product instead of providing actual information of a product itself. Second by frequency of use came the brochures– 18%, followed by advertisement displays – 12% and stands – 12%. Posters, folders, windows brandings with PVC-foil in total made up to 18%. (See fig.1)

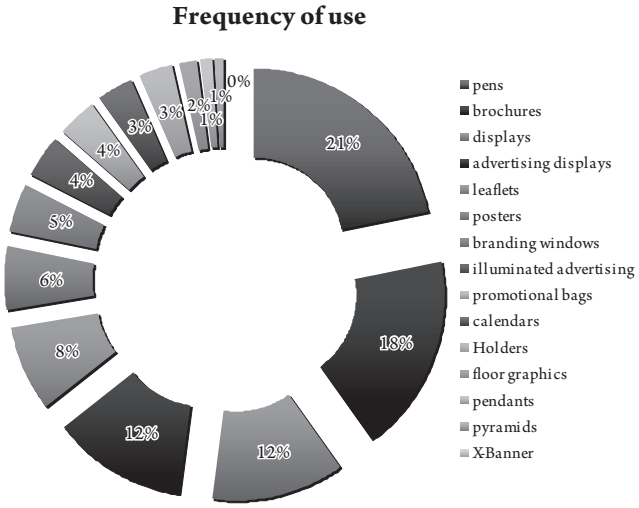

Fig. 1 Frequency of use of promotional materials

When it comes to addressing the consumer we must note that although ball-point pens were the most frequently used they are not the most effective way of advertisement.

On one hand it is a result of the number of consumers reached by the advertisement – most often one, two or three and on the other the commercial brochures which are also directed to one or two or three customers provide much more comprehensive information.

With regards to the space used for advertising provided by the different types of advertisement materials the distribution is quite different. The advertisement ball-point pens rank last with smaller space – just about 0.0005m2 per piece. Although this is the most frequently used material the total area used in all pharmacies is only 0.15 m2. (See fig.2).

Largest advertisement space is provided in the window brandings with PVC foil, followed

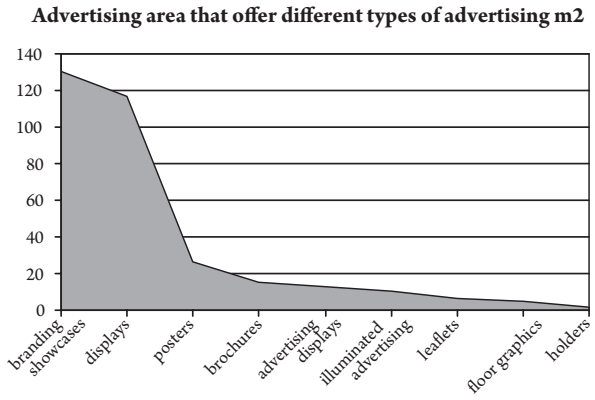

Fig. 2 Advertising area that offer different types of advertising

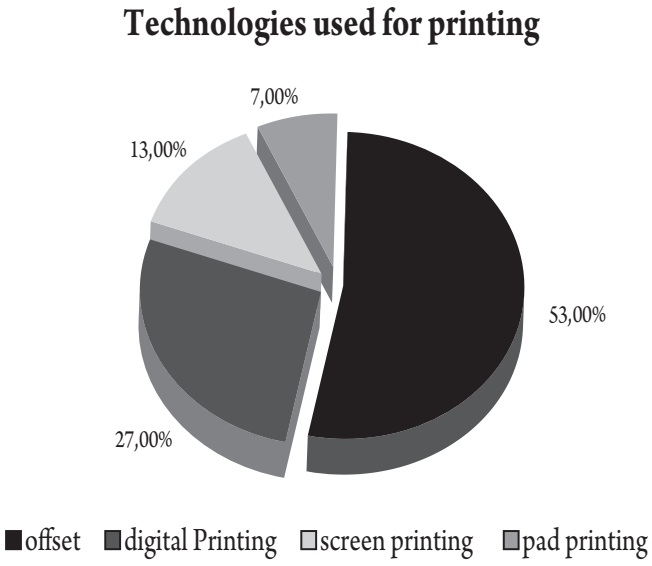

Fig. 3 Technologies used for printing promotional materialse

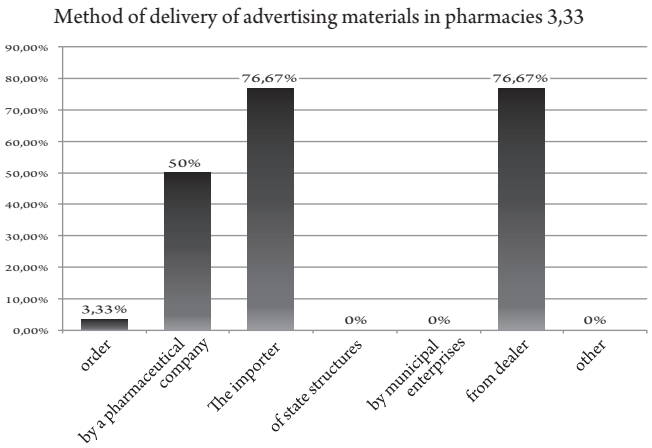

Fig. 4 Distribution of advertisement materials in the pharmacies

by the use of displays. The wide use of PVC foil in the last decade is a result of the significant development of digital printing technologies.

Nowadays there are numerous and various types of printing technologies used for production of advertisement materials. The most frequent are offset, inkjet printing, screen printing, pad printing, gravure printing, flex printing, electro-photographic (xerographic, also known as laser) printing, metallographic, thermography, etc. Each of these types has specific characteristics and application area.

After the analysis of the data in the current study we found that in production of advertisement materials mainly used is offset printing – 53%, followed by digital printing –27%, screen printing – 13% and pad printing 7 %. (See. Fig. 3)

The findings reveal that the advertisement materials in pharmacies were provided mainly by the importing companies and the distributors of the respective medicinal product. On second place were the manufacturers of the pharmaceutical products (the results exceed 100% due to the possibility of more than one answer). (See. Fig 4)

THE RESULTS SHOW THAT THE VOLUME OF INFORMATION MATERIALS PROVIDED BY MUNICIPAL OR STATE INSTITUTIONS IS ZERO.

The regulation of advertizing of the medicinal products is subject to chapter eleven of the Law for medicinal products for Human use and EU directive 2001/83/EU of the European Union and Council [7,1]. Article 244 (1) defines the advertisement of the medicinal products as any type of information, presentation, promotion or proposal aiming to stimulate the prescription, sale or use of a medicinal product. According to this law the advertisement is classified by the product that is being subject to advertisement namely advertisement of medicinal products without prescription, with prescription and advertisement of products for over the counter sale (OTC products).

We found that the main group (n=53, 88.14%) of advertised drugs were hard forms (tablets), in 6.78 % semi-hard (or soft) forms –

and lest common (n=3, 5.08%) – the liquid medicinal products (See Fig. 5).

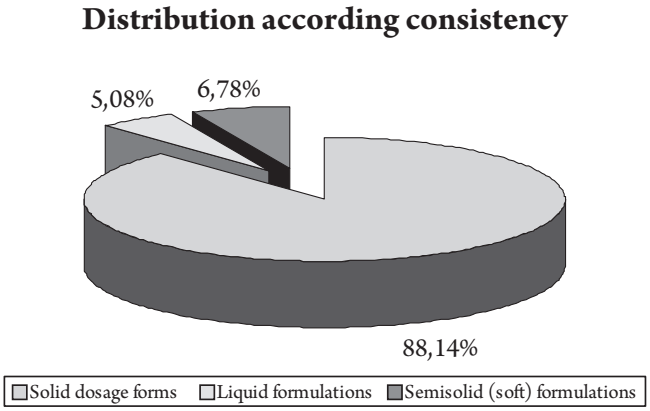

Fig. 5 Type of drug forms advertised

According to Anatomical Therapeutic Chemical classification (ATC) drugs are classified according to their therapeutical and chemical characteristics and or/ human organ or system on which they act [5,11,14].

The data collected showed that the most of the advertised products acted on the alimentary tract and metabolism (31%), followed by those acting on the respiratory and muscular-skeletal system (19% each). It is not by chance that products acting on the alimentary tract and metabolism rank higher in percentage of advertizing. Over the last few years frequency of the diseases that result from lifestyle (lack of physical activity) and irrational nutrition increased. (see fig.6)

The classification of advertisement materials may be done also according to the targeted groups. The target groups may be determined by expert assessment or by the description of the products – which is the target that will be using it. We divided the products conditionally to such intended for use by children and such intended for use by adults. (See. Fig.7)

The relativity of segmentation refers to the fact that some of the drugs intended for use by adults may be used by children and the difference is only in the dosage of the active substance. Naturally the group of the medicinal products intended for use by adults is the biggest one corresponding to the population structure in the country [13].

A: Alimentary tract and metabolism
B: Blood and blood-forming organs
C: Cardio-vascular system
D: Dermatologicals
G: Genito-urinary system and sex hormones
H: Systemic hormonal preparations
J: Anti-infectives for systemic use
L: Antineoplastic and immunomodulating agents
M: Muscular-skeletal system
N: Nervous system
P: Antiparasitic products, insecticides and repellents
R: Respiratory system
S: Sensory organs
V: Various

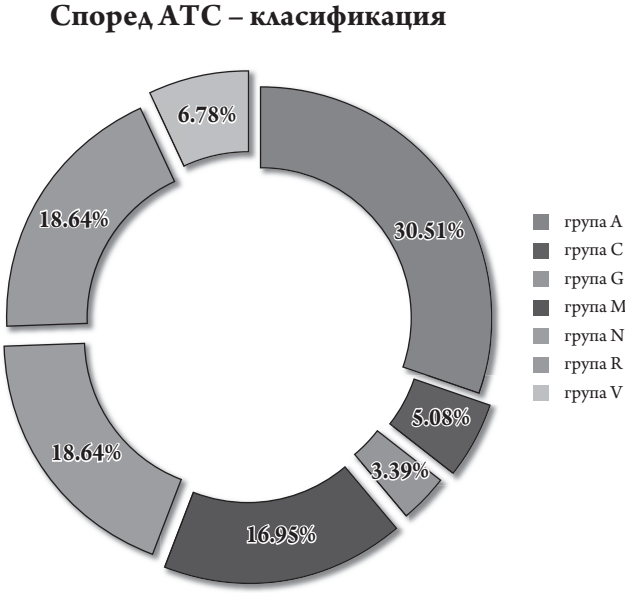

Fig. 6 Distribution of the advertisement materials according to the ATC classification

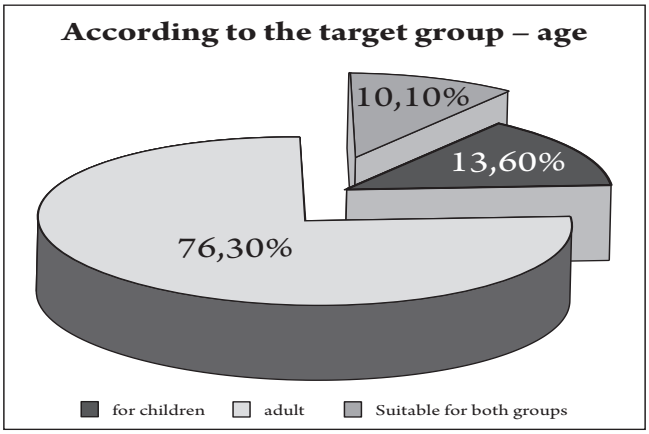

Fig. 7 Distribution of the advertisement materials according to the targeted groups

The assessment of the effect of advertising on sales or the so called advertisement effectiveness is related to the development of financial analysis of the investments and the generated sales' revenue. In this study was analyzed the subjective opinion of the employees regarding the efficacy of the used advertisements of the medicinal products at the point of sale.

The results showed that every second participant in the survey believed that advertisements affected sales positively.

In general people working in the pharmacies were familiar with the regulations related to advertising (43, 33%), but half of them acknowledged that they may need to extend their knowledge about the legislation in this area even further.

CONCLUSION

The advertisement materials most often used at the points of sale of the medicinal products were ball-point pens, brochures, folders (50%). The most frequently promoted products were those acting on the metabolism and alimentary system and the targeted group were people above 15 years of age.

The pharmacies' staff preferred the advertisements of new medicinal products and they were convinced that had positive effect on the sales. In the sometime big part of these people were not fully familiar with the legislation in this area in spite of the fact that at present in the field of healthcare in Bulgaria the advertising of medicinal products is allowed under strict regulations.

The existing legal frameworks create legal vacuum in the area of advertising, which leads to ineffective use of advertisement strategies. Modernization and change of legislation is needed concerning the general use of the advertisements and in healthcare in particular.

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The abstracts are not included in the size of the paper and should be submitted on a separate page with 3 to 5 key words at the end of the abstract. They should reflect the most essential topics of the article, including the objectives and hypothesis of the research work, the procedures, the main findings and the principal conclusions. The abstracts should not

Списание „Българска медицина“, издание на Българската Академия на Науките и Изкуствата, Отделение за наука, Научен център по медицина и здравеопазване, излиза в четири книжки годишно. „Българска медицина“ е достъпна онлайн на сайта на БАНИ, раздел издания.

В него се отпечатват оригинални научни статии, казуистични съобщения, обзори, рецензии и съобщения за проведени или предстоящи научни конгреси, симпозиуми и други материали в областа на клиничната и фундаменталната медицина. Списанието излиза на английски език с подробни резюмета на български и английски. Изключения се правят за обзорни статии по особено значими теми. Заглавията, авторските колективи, а също надписите и означенията на илюстрациите и в таблиците се отпечатват и на двата езика.

Материалите трябва да се предоставят в два еднакви екземпляра, напечатани на пишеща машина или на компютър, на хартия формат А4 (21 х 30 см), 60 знака на 30 реда при двоен интервал между редовете (стандартна машинописна страница). Освен това могат да бъдат изпратени като прикачени файлове по електронната поща на адресите, посочени по-долу.

Обемът на представените работи не трябва да превишава 10 стандартни страници за оригиналните статии (или 5000 думи според стандарта на англосаксонските издания) 12 страници (7 500 думи) за обзорните статии, 3–4 страници за казуистичните съобщения, 4 страници за информации относно научни прояви в България и в чужбина, както и за научни дискусии, 2 страници за рецензии на книги (монографии и учебници). В посочения обем се включват книгописът и всички илюстрации и табли-

exceed one standard typewritten page of 200 words.

The basic structure of the manuscripts should meet the following requirements:

Title page

The title of the article, forename, middle initials (if any) and family name of each author; institutional affiliation; name of department(s) and institutions to which the work should be attributed, address and fax number of the corresponding author.

Text of the article

Titles and subtitles should be standardized.

The original research reports should have the following structure: introduction (states the aim, summarizer the rationale for the study), subjects and materials, methods (procedure and apparatus in sufficient detail, statistical methods), results, discussion, conclusions (should be linked with the aims of the study, but unqualified statements not completely supported by research data should be avoided). These requirements are not valid for the other types of manuscripts. Only officially recognized abbreviations should be used, all others should be explained in the text. Units should be used according to the International System of Units (S. I. units). Numbers to bibliographical references should be used according to their enumeration in the reference list.

Illustrations

Photographs should be presented both in the text body to indicate their location and in separate files as saved in jpeg, tif or bitmap formats.

The figures, diagrams, schemes, photos should be submitted in a separate file with: consecutive number (in Arabic figures); titles of the article and name of the first author. The explanatory text accompanying the figures should be presented along with the respective number of the figure in the main text body with space left for insertion of the figure.

ци. В същия не се включват резюметата на български и английски, чийто обем трябва да бъде около 200 думи за всяко (25–30 машинописни реда).

Резюметата се представят на отделни страници.Те трябва да отразяват конкретно работната хипотеза и целта на разработката, използваните методи, най-важните резултати и заключения. Ключовите думи (до 5), съобразени с „Medline“, трябва да се посочат в края на всяко резюме.

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а) заглавие, имена на авторите (собствено име и фамилия), название на научната организация или лечебното заведение, в което те работят. При повече от едно заведение имената на същите и на съответните автори се маркират с цифри или звездички;

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Заглавията и подзаглавията следва да бъдат уеднаквени и различими.

Оригиналните статии задължително трябва да имат следната структура: увод, материал и методи, собствени резултати, обсъждане, заключение или извод.

Методиките следва да бъдат подробно описани (включително видът и фирмата производител на използваните реактиви иапаратура). Същото се отнася и за статистическите методи.

Тези изисквания не важат за обзорите и другите видове публикации. В текста се допускат само официално приетите международни съкращения; при използване на дру-

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The references should be presented on a separate page at the end of the manuscript. It is recommended that the number of references should not

Exceed 20 titles for the original articles and 40 titles for the reviews; 70 % of them should be published in the last 5 years. References should be listed in alphabetical order, English first, followed by the Bulgarian ones in the respective alphabetic order. The number of the reference should be followed by the family name of the first author and then his/her initials, names of the second and other authors should start with the initials followed by the family names. The full title of the cited article should be written, followed by the name of the journal where it has been published (or its generally accepted abbreviation), volume, year, issue, first and last page. Chapters of books should be cited in the same way, the full name off the chapter first, followed by"In:" full title of the book, editors, publisher, town, year, first and final page number of the cited chapter.

Examples:

Reference to a journal article:

1. McLachan, S. , M. F. Prumel, B. Rapoport. Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? J. Clin. Endocrinol. Metab., 78, 1994, 5, 1070–1074.

Reference to a book chapter:

2. Delange, F. Endemic Cretenism. In: The Thyroid (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942–955.

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Примери:

Статия от списание:

1. McLachlan, S., M. F.Prumel, B. Rapoport. Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? J. Clin. Endocrinol. Metab., 78, 1994, 5, 1070–1074.

Глава (раздел) от книга:

2. Delange, F. Endemic Cretenism. In: The Thyroid (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942–955.

Адрес за кореспонденция с авторите

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