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Influence of age on hand dominance

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Влияние на възрастта върху ръчното доминиране

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РЕЗЮМЕ

Цел

Целта на изследването е да проучи ефекта на възрастта върху ръчното доминиране и да го сравни при шизофренни пациенти и контроли.

Материали и методи

Изследвани са общо 165 случая (шизофренни пациенти и контроли) с 31 въпроса за ръчно доминиране, включващи 4 валидизирани въпросника. Участниците са разделени на 2 групи: >= 32 и <32 години.

Резултати

Има статистически значими разлики в полза на по-често недясно ръчно доминиране при по-младите участници (<32 години) в сравнение с по-възрастните (> = 32 години). Това се отнася също така и за шизофренната група, но не и за контролната група.

Заключение

Възрастта е фактор, който оказва влияние върху ръчното доминиране и шизофренните пациенти са подложени по-малко на ефекта на социалния натиск в сравнение с контролите.

Ключови думи: леворъчие, шизофрения, възраст

ABSTRACT

Aim

The aim of this study is to investigate the effect of age on hand dominance and to compare this effect between schizophrenic patients and controls.

Materials and methods

A total number of 165 cases (schizophrenic patients and controls) were tested by 31 questions for hand preference, including 4 validated questionnaires. The participations were divided into 2 groups: >=32 and <32 years.

Results

There is statistically significant differences in favor of more frequent non-right hand dominance in younger participants (<32 years) compared with older (>=32 years). This refers also to the schizophrenic group, but not to the control group.

Conclusions

Age is a factor that affects the hand dominance and schizophrenic patients suffer less from the effects of social pressure than healthy controls.

Key words: left-handedness, schizophrenia, age

INTRODUCTION

Human brain is lateralized, which means that it is asymmetric with respect to its functions. Consideration of left-handedness of epidemiological, neurobiological, and medical point of view gives an idea of brain lateralization and may answer some questions.

Left-handedness is a phenomenon that has existed for more than 200,000 years and occurs in about 10% of the population. The dominance of the hand has been studied in many aspects, one of which being its relationship with age. In the study of Gillbert and Wysocki [1] the authors indicate that 3–4% of the people, who were born before 1920, are left-handed, compared with 11–12% of those, born after 1950. An interesting study on old documentaries demonstrates controversial results [2]. The authors compared the number of people in these films who waved with their left and right hand. They found that left-handedness was more common among older individuals. McManus [3] summarized all historical data from the nineteenth and twentieth centuries. He concluded that the percentage of left-handedness in the late eighteenth century was 8–10%, then fell down to 3% in the nineteenth century to rise again in the first half of the twentieth century, reaching about 11% nowadays. The author tried to provide some explanation for these data. As with other natural variations, such as sexual orientation or skin color, left-handedness was also stigmatized. In Europe left-handedness has been considered undesirable and even a sign of inferiority until mid-20th century. Left-handers were subject to direct and indirect social pressure. Direct social pressure included the fact that they were forced to write with their right hand, as it was in Victorian schools [4] and under many different forms in other societies, but with the same purpose [5]. Indirect social pressure was much more subtle and acted to make left-handers more stigmatized and "taboo" so that they find it harder to have offspring. Therefore, their genes were less likely to be passed on, thus decreasing the percentage of left-handers in next generations. Another reason for decreased rates of left-handers is the Industrial Revolution in the 19th century. The new machines designed for right-handed individuals made left-handed people unskilled and also indirectly lead them to favor the right hand [6].

The aim of the current study is to investigate the effect of age on hand dominance and to compare this effect between schizophrenic patients and the control group.

MATERIALS AND METHODS

A total of 165 cases have been included in the study: 85 schizophrenic patients [48 men, 37 women] and 80 clinically [mentally] healthy subjects in the control group [29 men and 51 women]. The average age of the control group was 34.500, SD = 16.098 and for schizophrenic patients it was 43 392, SD = 10.668.

All tested patients and controls signed informed [written] consent to participate in the research after the goals, objectives and procedure of the research were clarified to them. The questionnaires that have been used include Edinburgh Handedness Inventory (EHI), Annett Hand Preference Questionnaire (AHPQ), Chapman and Chapman, and Hand Preference Demonstration Test (HPDT). The four validated questionnaires contain 23 various items. Eight more additional questions were added. Hence, the total number of questions for hand preference became 31. Subjects were asked to demonstrate how they perform certain actions. The evaluation was made as follows: "left hand"-2 points; "equally with both hands" - 1 point; "right hand"-0 points. The sum of the points is in the range 0–62.

The statistical analysis was carried out by applying the SPSS 22.0 programme. The evaluation of the data was made by using descriptive statistics, parametric, non-parametric and graphic analysis. $P < 0.05$, bilaterally, was accepted as the level of statistical significance.

RESULTS

The distribution of the sum of the responses for manual domination by age was made by using graphical analysis (Fig. 1).

As it can be seen in the left edge of the chart, young participants show a higher sum of questions for hand dominance, hence they are left-handed more often, compared with adult participants.

Study participants were divided into two groups according to their age: > = 32 years and <32 years.

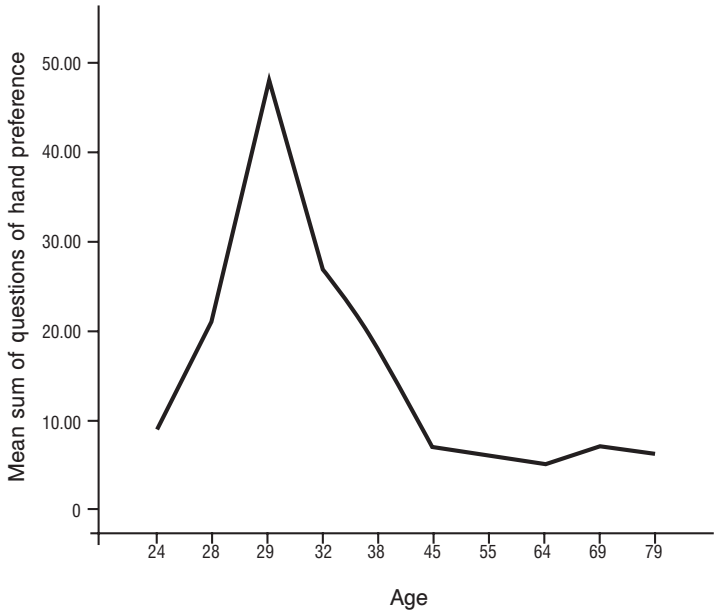

Figure 1
Disposition of the sum of the questions of hand preference by age

The two groups were compared by the sum of answers to questions about hand dominance with a T-Student test. There are statistically significant differences (F = 12.664; p = .000) in favor of more frequent non-right hand dominance in younger participants (<32 years) compared with older ones (tab.1).

Table 1

		Sum of questions of hand preference		
Age	N	Mean	SD	Min-Max
>= 32	92	8.206	7.815	0–46
< 32	65	11.907	14.940	0–62

The study individuals were divided into two groups: schizophrenic patients and healthy controls. Statistically significant difference were also found in the group of schizophrenic patients in favor of more frequently non-right hand dominance in subjects under 32 years, compared to those over 32 years (F = 25.781; p = .000) (tab.2). No statistically significant difference was found in the control group (F = 1.751; p = .190).

Table 2

		Sum of questions of hand preference		
Age	N	Mean	SD	Min-Max
>= 32	69	8.246	7.538	0–46
< 32	11	19.454	18.354	2–48

Discussion

Younger subjects (<32 years) were non-right-handers more often, compared with older subjects (> = 32 years). Age here is important in terms of the direct social pressure, exerted on different generations (born before and after '90), including forcing them to write with their right hand. Like in the rest of Europe, left-handed people in Bulgaria have been stigmatized as well and the generation, which has been educated prior to 1990, had been subjected to direct pressure to get accustomed to right-hand writing. This also applies to the subset of schizophrenic patients where younger people were also more often left-handed as compared to older ones. In the control group, however, statistically significant differences between the two age groups in terms of the predominance of the hand were not reached. One possible explanation is the fact that patients with schizophrenia drop out of the educational system earlier, which means that they have less years to acquire writing with the right hand. Furthermore, schizophrenic patients are socially more difficult to integrate, which gives them less opportunities to acquire the necessary skills to properly use objects, developed for right-handed people. This, in turn, allows manifesting their natural propensity for operating with their left hand. Namely, they are less exposed to social pressure, compared to the healthy population.

Conclusion

Age is a factor that affects hand dominance due to the different social pressure exerted on different generations. The schizophrenic patients suffer less from the effects of social pressure than healthy controls, because of the fewer years spent in the educational system and their difficult social inclusion.

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Peculiarities in the clinical manifestation and diagnostic approach in patients with Wilson disease

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Особености в клиничната проява и диагностичния подход при пациенти с болест на Wilson

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РЕЗЮМЕ

Болестта на Уилсон (БУ) е наследствено нарушение на медния метаболизъм. Атипичните прояви са предизвикателство за клиницистите. **Цел** на проучването е да се анализира клиничното представяне, ходът и диагностичния подход при пациенти с БУ.

Материал и методи: Анализирани са 65 пациента с БУ (43 мъже, 22 жени) на средна възраст 37,75 години и контролна група от 26 лица с други хронични чернодробни болести за периода от януари 2003 до декември 2013 година. Описани са клиничните прояви, лабораторните тестове, ехографското, хистологичното и офталмологично изследване. Приложена е Лайпцигската точкова система за диагностика на болестта.

Резултати: Тридесет и двама пациента (49,23%) се представят със смесена форма, 28 (43,08%) – само с чернодробна болест, 3 – с чиста неврологична изява и 2 пациента са безсимптомни. Чернодробната цироза е първа проява при 51,7% от случаите. Налице е средно 39 ме-

ABSTRACT

Background and aim: Wilson disease (WD) is an inherited copper metabolism disorder. Atypical presentations are a challenge for the clinicians. The aim of the study is to analyze the clinical manifestations, the course and the diagnostic approach in WD patients.

Material and methods: Sixty-five WD patients (43 males, 22 females) at a mean age of 37.75 years and control group of 26 subjects with other chronic liver diseases (CLD) were analyzed from January, 2003 to December, 2013. Clinical features, laboratory tests, ultrasound, histological and ophthalmological examinations were described. Leipzig scoring system for diagnosis was applied.

Results: Thirty-two patients (49.23%) presented with mixed form, 28 (43.08%) - with liver disease only, three - with pure neurological features and two patients were asymptomatic. Liver cirrhosis was the first presentation in 51.7% of the cases. There was a mean delay of diagnosis of 39 months. The low ceruloplasmin level was the main laboratory feature although it was normal in 15.4%. We

сеца закъснение при поставяне на диагнозата. Ниското ниво на церулоплазмина е главна лабораторна отличителна особеност, въпреки че в 15,4% е нормален. Установихме висока клинична значимост на стимулираната 24-часова куприурия. Съгласно Лайпцигската точкова система 89,2% от пациентите с БУ имат сбор от точки ≥4, което доказва диагнозата. Не са наблюдавани усложнения по време на бременностите. Общата преживяемост на пациентите с БУ е 90,77%.

Заключение: Болестта на Уилсон трябва да се има предвид в случаите на леки отклонения в чернодробните показатели, с нормален церулоплазмин, при съпътстващи заболявания и при липса на типичните за болестта белези. Високата степен на съмнение може да предотврати забавянето на диагнозата и води до навременно лечение.

Ключови думи: Болест на Уилсон, церулоплазмин, пръстен на Kayser-Fleischer, Лайпцигска точкова система, съпътстващи заболявания, преживяемост

found out a high clinical significance of the stimulated 24-hour cupriuria. According to Leipzig scoring system, 89,2 % of WD patients had score ≥4, which established the diagnosis. No complications during the pregnancies were noted. Overall survival rate of WD patients was 90.77%.

Conclusion: WD has to be considered in cases with mild liver tests changes, with normal ceruloplasmin level, in coexisting diseases and in cases with lack of typical signs of the disease. The high degree of suspicion can prevent the delay of diagnosis and lead to timely treatment.

Keywords: Wilson disease, ceruloplasmin, Kayser-Fleischer ring, Leipzig scoring system, comorbidities, survival

INTRODUCTION

Wilson disease (WD) is an inherited autosomal recessive disorder of the copper metabolism. It is related with copper accumulation and toxic action mainly in the liver, brain, kidneys, eyes and other organs (13,16). WD is characterized by broad clinical spectrum. It is easy to recognize in the classical cases of liver manifestation, overt neurological symptoms and presence of Kayser-Fleischer (KF) ring. Atypical cases, subclinical forms, lack of characteristic signs, and presence of concurrent liver disease are a clinical challenge and modify the diagnostic approach for the early recognition and timely treatment.

The objective of this study was to reveal the clinical manifestations, to describe the course of the disease and to optimize the diagnostic and therapeutic approach in WD.

PATIENTS AND METHODS

We performed a retrospective analysis of 65 adult patients with WD, 22 females and 43 males, at mean age of 37.75±12.98 years (range, 18–65 years) diagnosed and followed-up in the Clinic of Gastroenterology and Hepatology, St. Marina University Hospital of Varna, during the period from January, 2003 to December, 2013. A control group of 26 patients with other chronic liver diseases (CLD) – non-alcoholic steatohepatitis, viral hepatitis, autoimmune and alcoholic hepatitis and liver cirrhosis, were analyzed too. The following medical information was analyzed: demographic data, clinical profile, serum ceruloplasmin, 24-hour basal and stimulated urinary copper excretion, abdominal ultrasound and ophthalmological examination for KF ring and sunflower cataract, liver biopsy and brain MRI. All the patients were as-

sessed according to the Leipzig scoring system for WD diagnosis. Descriptive statistics, analysis of empirical distributions, *t*-test for equality of means, survival analysis by Kaplan-Meier method and Logit model were used. Statistical data processing was done by using of SPSS 16.0, MedCalc 11.6 and STATISTICA 5.0 for Windows.

RESULTS

Demographic data

The mean age at presentation was 37.75±12.98 years. Some 46 (70.77%) of cases were at age under 36 years at the initial symptoms. Six patients were over 50 years old as two of them - over 60 years (Figure 1).

The mean delay of diagnosis was 39 months. It was longer than one since the initial symptoms onwards in 43% and longer than six years in 18% of the cases. The longest period of late diagnosis was 30 years.

Clinical forms

Twenty-eight patients (43.08%) presented with liver disease only, 32 (49.23%) - with mixed hepatic and neurological features but three (4.62%) - with neurological features without any signs of a liver injury. Two (3.08%) patients at asymptomatic stage were diagnosed by family screening (Figure 2). The following hepatic forms were proved: cirrhosis - in 31 (51.7%), hepatitis - in 25 (41.7%) and steatosis only - in 6.6% (Figure 3). Fifteen out of 18 patients diagnosed in childhood were with clinically manifested hepatic involvement. Forty-seven subjects were diagnosed over the age of 18. Of them, 18 were with pure hepatic presentation and 26 ones with mixed form.

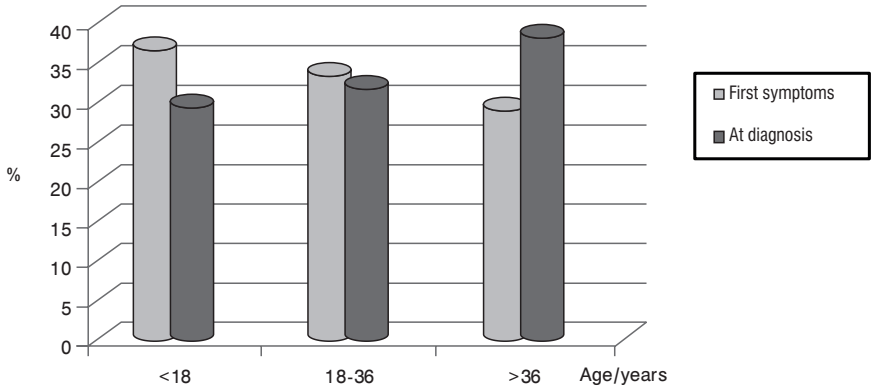

Fig. 1. Patients' age at onset of disease and at diagnosis.
Фиг. 1. Възраст на пациентите в началото на болестта и при диагнозата.

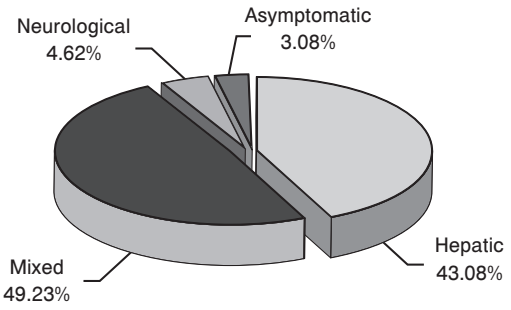

Fig. 2. Clinical forms of WD.
Фиг. 2. Клинични форми на болестта на Wilson.

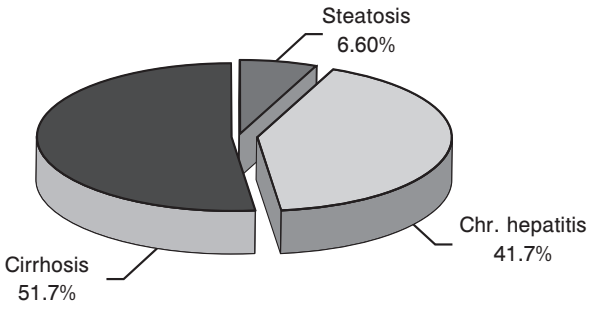

Fig. 3. Forms of hepatic involvement
Фиг. 3. Форми на чернодробно засягане.

Laboratory parameters of copper metabolism

In 55 WD patients (84.62% of the cases), serum ceruloplasmin value remained below reference limits while in 10 one (15.38%) it was within the normal range. The descriptive analysis demonstrated that the mean spontaneous level of cupriuria in WD patients was 4.64 ±4.66 μmol/24 hours, while in the control group it was significant lower (0,87±0,4 μmol/24 hours).

The level of stimulated 24-hour cupriuria was 17.4±13.13 μmol/24 hours. It was significantly higher in the comparison with the control group (4,43±2,46 μmol/24 hours) (Figure 4). There was a fivefold increase over the normal range in 38 WD patients as well as a tenfold one in 10 of them. The elevation of copper excretion in comparison with its initial level by more than five times in 24 WD patients was of importance, too.

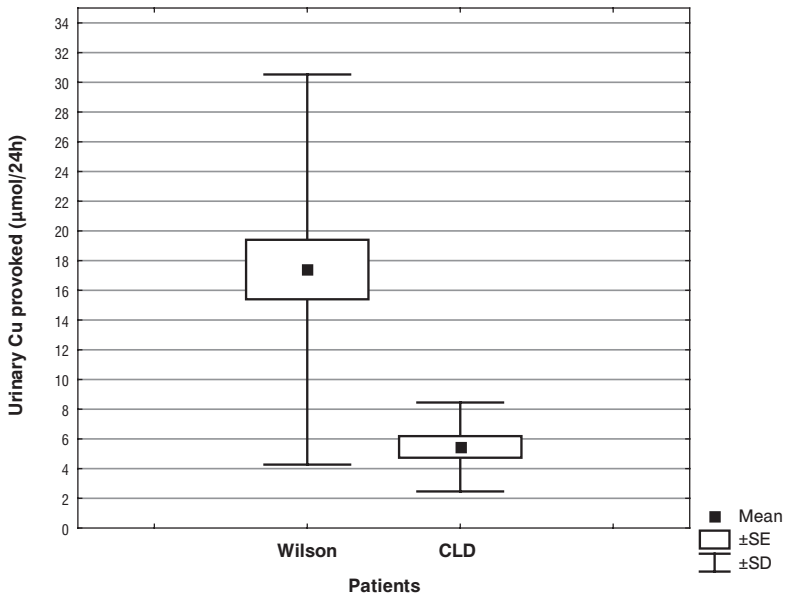

Fig. 4. Comparative analysis of the stimulated cupriuria in patients with WD and other CLD.

Фиг. 4. Сравнителен анализ на стимулираната куприурия при пациенти с болест на Wilson и други хронични чернодробни заболявания.

Ophthalmological findings

KF ring was detected in 23 (35.4%) of the patients, from which 17 presented with any neurological symptoms. It disappeared in 47.8% (11 of 23) cases. In two cases, KF ring decreased in size and intensity. Therefore, this sign regressed in a total of 13 (of 23) patients (i.e. 56.5% of the cases). Pathological findings on brain MRI were established in 12 cases with KF ring.

Sunflower cataract was detected in six cases.

Coexisting diseases

WD was diagnosed in patients with other liver diseases. Twenty-one patients were with a coexisting liver pathology such as nonalcoholic steatohepatitis (11), chronic viral hepatitis B and C (five and one, respectively), autoimmune hepatitis (three), and primary biliary cirrhosis (one patient). In some patients, the diagnosis was delayed. Initially, five patients with neurological symptoms were misdiagnosed.

Liver histology

Twenty-three (35.4%) patients underwent liver biopsy. The most common histological findings were the following: steatosis (16/23), inflammatory infiltration (11/23), fibrosis (12/23), and vacuolated nuclei (11/23). In 3 cases, there was transition to cirrhosis. Other findings were necrosis (5/13), regenerative nodes (2/23), and Mallory bodies (2/23). Rhodanine staining for hepatocyte copper deposits was positive in 10 cases.

Brain MRI

Brain MRI in 26 patients (40%) of which 22 with neurological symptoms identified pathological findings in 15 (57.7%) patients while 11 (42.3%) had normal image.

Leipzig scoring system

According to the Leipzig scoring system (6), 58 patients (89.2%) had score ≥4 and seven had score of three. In them, the viral, autoimmune and toxic reasons for chronic liver disease were excluded. Only four patients of the control group present with score of three while the rest 22 ones (84,6%) have a score that is less or equal to two.

WD and pregnancy

Four female were pregnant during the analyzed period. One patient ceased treatment until birth. The rest three patients with liver cirrhosis continued the treatment with a 50% reduction in the dose of D-penicillamine under gastroenterologist's control. There were no complications during pregnancy. The patients remained in firmly compensated liver disease.

Survival

The retrospective analysis showed overall survival rate of 90.77% in our patients. The cumulative 15-year survival rate was 80.3% (Figure 5). It was established that at each year of diagnosis delay, the probability of death increased approximately by 10% per year delay.

DISCUSSION

The estimated WD prevalence in Northeastern Bulgaria is 3.96/100000 inhabitants (1).

The study shows the male predominance (66,2%) in our cohort like in other reports (10,18).

The retrospective analysis of our patients finds delays in diagnosis by an average of about three years. These data about the late diagnosis of WD are consistent with other studies (4,7,10,19). The following causes are discussed: 1) The triad of low ceruloplasmin, KF ring's presence and extrapyramidal symptoms is too inaccurate to detect WD. Serum ceruloplasmin is normal in 15% of our cases. KF ring is absent in one third of patients. Neurological symptoms are missing in a considerable proportion of patients. 2) Another reason for the delay in diagnosis is that WD is a slowly progressive disease. 3) The diagnosis is easily overlooked in symptomatic patients due to the variable clinical presentation as sometimes the presence of symptoms that mimic other diseases leads to an incorrect initial diagnosis.

Most patients diagnosed in childhood are with liver pathology. Our own data confirm the widespread opinion that hepatic dysfunction is the most common WD manifestation in child-

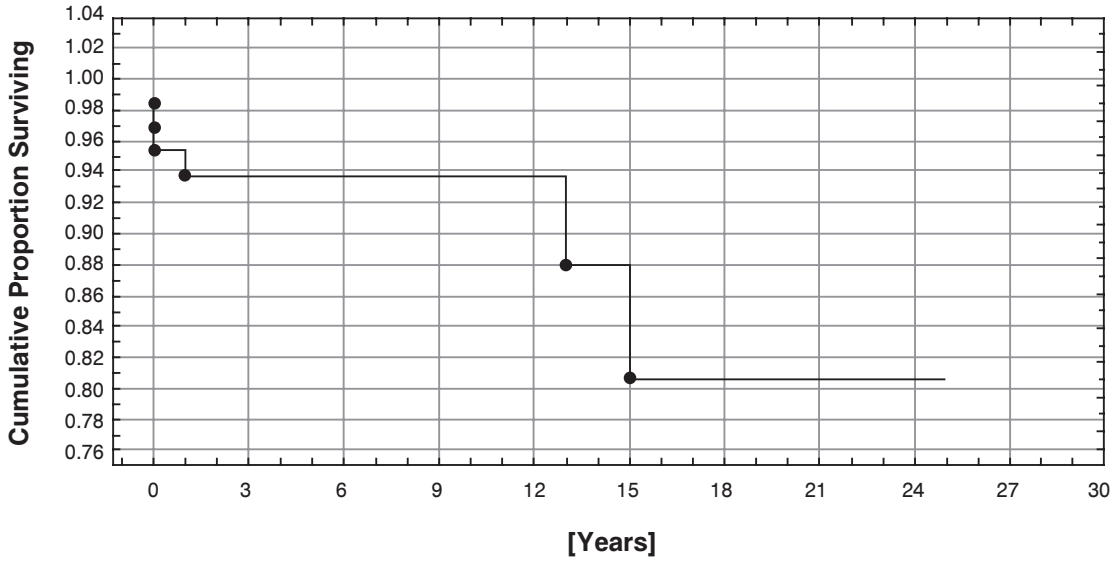

Fig. 5. Survival of patients with WD after diagnosing.
Фиг.5. Преживяемост на пациенти с болест на Wilson след поставяне на диагнозата.

hood (8,14,17). It is noteworthy that in children, WD is diagnosed soon after the first symptoms. The prevalence of hepatic involvement in children and the clinical focus on this disease contribute to early diagnosis and treatment.

In the group of patients diagnosed at the age over 18 years, almost all but three are with liver disease, which proves that WD is mainly a liver disease. Nearly 52% have cirrhosis at presentation, which is close to the data of other authors (5,9). Neurological symptoms commonly develop later in comparison with liver disease, most commonly, in the second and third decade (14). We observe the same development as in some patients, the neurological symptoms have appeared more recently, in the fourth or fifth decade. Our observation supports the need for neurological examination of patients with hepatic form once a year in order to assess the development and progression of the disease.

Both serum ceruloplasmin and urinary copper excretion represent the basic parameters of copper metabolism assessment. Ceruloplasmin is an acute-phase protein and its concentration increases in response to inflammation, infection and injury. That is why its falsely-elevated as well as normal values can occur in some patients. In 15.38% of our cases, ceruloplasmin is within the normal range that is close to the results reported by other authors (8,9,11). Therefore, we accept the use of this test not alone, but in combination with other parameters of copper metabolism.

D-penicillamine-induced cupriuria, i.e. D-penicillamine challenge test, is an important and useful additional diagnostic test. It is significantly increased over the upper limit of normal value as well as when compared to the basal level. This parameter is significantly higher in WD patients in comparison with the control group. This fact enables us to consider the test useful, necessary and applicable diagnostic parameter, especially in cases of unexplained hepatomegaly, vague liver disease, elements of hemolysis, or unspecified neurological disease.

Although the KF ring is considered pathognomonic for WD, it can be found under other conditions, too, mainly in diseases with prolonged cholestasis (6,15). On the other hand, its absence does not exclude the diagnosis. This is the case in two patients of ours with overt neurological symptoms and cirrhosis. There is no KF ring in the presence of the typical MRI changes in the cerebral structures. In a recent study (20), the authors report that 26.7% of neurological patients are without any KF ring. Moreover, cirrhosis and typical changes in the brain are less common in patients with a neurological form without any KF ring. Our data are similar. These results indicate that the lack of a KF ring can be regarded as a form of neurological disease with less expressed copper involvement and emphasize the need for further tests if in a patient without KF ring, a neurological form is suspected.

Fatty degeneration and vacuolated nuclei are some of the earliest changes in the liver cells in WD. They are most commonly seen in the periportal areas. Despite the small number of our biopsied patients, the data from the morphological study match with those findings in WD described in the literature available (2,4,10). The positive result of Rhodanine staining is pathognomonic for WD. Similar to that described in the literature, in our biopsies copper is distributed in periportal areas. The negative result does not exclude the diagnosis, as copper is distributed unevenly in the parenchyma (12).

The majority of our patients with changes in brain structures present with neurological manifestations of WD. In the patients with a predominantly hepatic form, however, more often, there are no changes. Furthermore, some of our patients with neurological symptoms have no changes in the brain. We should note that in the proportion of patients, MRI has been done months or years after the start of chelation therapy, which has, probably, led to a reversal of the pathological changes. Because of these facts, we assume that the absence of abnormalities in the brain does not exclude the diagnosis.

The coexistence of two liver diseases can lead to delays in diagnosis, as in some of our 21 cases with comorbidities. The presence of neurological symptoms may result in an incorrect initial diagnosis, too. WD has a wide clinical spectrum and represents diagnostic challenge. Because of its relative rarity and lack of knowledge, the correct diagnosis is often delayed. Our experience shows that the clinicians should obligatorily suspect and test for the disease in the cases of neurological symptoms, regardless of other accompanying diseases and aggravating cofactors such as alcohol, drugs etc. In any patient with liver disease, a comprehensive liver screening should be performed for the early diagnosis and treatment of WD.

Our observation confirms the comprehension that continued treatment with chelating agents during the pregnancy is safe, well-tolerated and associated with good outcome for both mother and infant.

According to the Leipzig scoring system, 89.2% of WD patients have score ≥4 that establishes the diagnosis. In cases of score of three, other causes of liver diseases are excluded. Clinical observation and favourable therapeutic influence on laboratory parameters confirm the diagnosis in these patients. Our results support the high diagnostic significance of this score and demonstrate that the parameters included in the Leipzig scoring system are a reliable combination of criteria for the diagnosis of disease.

We find out a good overall survival rate in the study cohort. The cumulative survival rate in our patients is lower than in other studies (3,5). This is, probably, due to the shorter period of observation and the smaller number of patients in our group.

Conclusion

The diagnosis of WD is based on the complex assessment of a combination of various clinical signs and laboratory parameters. The special forms with subclinical manifestation are true difficulty and challenge. WD has to be considered in cases with mild changes in liver tests, even with normal level of ceruloplasmin as well as in patients with coexisting liver or neurological diseases. Our study demonstrates that the lack of typical findings of the disease such as KF ring, identifiable mutations, MRI changes in the brain, and overt neurological symptoms does not exclude the disease. In cases of atypical presentation, WD is diagnosed by exclusion of other liver diseases, following-up the parameters and, in some cases, by carrying out a therapeutic test with D-penicillamine. The high degree of suspicion can prevent the delay of diagnosis and lead to timely treatment.

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Blood-borne infections resulting from dental treatment

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Кръвнопреносими инфекции, резултат от дентално лечение

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РЕЗЮМЕ

Епидемиологичните изследвания на инфекции, свързани с дентално обслужване (ИСДО), са голямо предизвикателство, особено за имунокомпрометираните пациенти и дентален персонал, потенциален контингент за инфекционисти, гастроентеролози и др. Денталната практика би следвало да поддържа безопасността към инфекции, спазвайки Стандарта за процедурите по инфекциозен контрол за всеки пациент. Медицината базирана на доказателства очертава риск за ИСДО в 6 направления:1) кръвно преносими инфекции (КПИ); 2) инфекции, свързани с водната система на денталния юнит;3) инфекции, свързани с микроорганизмов дентален аерозол; 4) неинфекциозни масови заболявания; 5) вирусни хепатити А и Е

ABSTRACT

Epidemiological studies of dental healthcare associated infections (DHCAI) are challenging, especially for immunocompromised patients and dental staff members, potential contingent of infectionists, gastroenterologists, etc. Dental practice should follow the Standards of infection control procedures for each patient. Evidence-based medicine, however, outlines the risk of DHCAI in six areas: 1) blood-borne infections (BBI); 2) infections related with dental unit water system; 3) infections related with bacterial aerosols in dental practice; 4)common non-communicable diseases; 5) viral hepatitis A and E (infrequently) and 6) prions. BBIs are the most important and the transmission evidences are based on prevalence studies, epidemiological studies and case reports. Topicality and the lack of data

(рядко) и 6) приони. КПИ са най-значимите и доказателствата за предаването им се основават на проучвания за серопозитивност, епидемиологични проучвания и доклади за отделни случаи. Актуалността и липсата на данни в България за ИСДО са стимули, чрез медицина базирана на доказателства, да представим хронологично доказаните случаи. За периода 1974 г. до 2014 г. са анализирани онлайн бази данни относно маркерите и специфичните аспекти на кръвнопреносимите вирусни хепатити B, C, D и ХИВ. Въпреки многото публикации за програми и стратегии за предотвратяване на предаване на КПИ, те остават основен проблем на общественото здраве и има нужда от повече правилно планирани, контролирани и анализирани проучвания.

Ключови думи: *кръвнопреносими инфекции, дентално лечение, медицина базирана на доказателства*

for DHCAI in Bulgaria are the incentives to present chronologically documented cases derive from the evidence-based medicine. We analyzed online database on markers and specific aspects of blood transmitted viral hepatitis B, E and C and HIV for the period 1974–2014. While many publications include prevention programs and strategies, BTIs remain major public healthcare problem that need more properly planned, supervised and analyzed studies.

Keywords: *blood transmitted infections, dental treatment, evidence-based medicine*

Provision of oral health care by dentists is associated with working very close to the dental patient facilitating easy transmission of pathogenic microorganisms through the droplets or aerosols generated by high speed dental turbines. The importance of the problem of dental health care associated infections (DHCAI) and the lack of data for them in Bulgaria are the reason we set out to present some published cases in a chronological order using principles of evidence based medicine.

Evidence based medicine predicts that risks for DHCAI are mainly found in 6 areas:

- blood-borne infections – HBV (hepatitis B virus), HCV (hepatitis C virus), HIV (human immunodeficiency virus);
- infections associated with the dental unit waterlines – Legionellosis, etc.;
- infections associated with microbacterial dental aerosols:
  - viruses causing rubella, mumps, measles, influenza A and B, types 1 and 2 herpes viruses, varicella-zoster virus, Epstein-Barr virus, CMV, human papillomavirus, adenoviruses, coxsackie virus, human parvovirus B19 and RSV.
  - bacteria causing acute infectious diseases
  - Corynebacterium diphtheriae, Neisseria meningitidis, Bordetella pertussis, Mycobacterium tuberculosis, MRSA and MSSA.
  - associated with common non-communicable diseases
  - more rarely, associated with viral hepatitis A and E
  - prions.

The evidence for transmission of blood borne viruses, HBV, HCV and HIV, comes from studies on seropositivity, epidemiologic studies, including studies on genetic markers and case reports (6,11,13,15,16,26,27,34). In the present study, based on data from literature in English available between 1974 and 2014, we discuss information concerning nosocomial transmission and epidemiological aspects of

hepatitis B, hepatitis C and HIV/AIDS in dental healthcare settings.

Some key epidemiological data for risk

The major factor of infection with HBV and HCV in dental practice is the blood of infected persons. The HBV and HCV concentrations in other body fluids and their role as transmission vectors is also of essence in this respect. The HBV titer in saliva has been found to be about 1,000 to 10,000 times lower than the corresponding titer in serum (5), while the presence of HCV RNA in the saliva of patients with chronic liver disease (anti-HCV and HCV RNA positive) was 48% (17). Saliva and nasopharyngeal secretions, especially if mixed with blood, are acknowledged to play a role in transmission (20,23). It has been also found that the concentration of HBV in the mouth is the greatest in the gingival sulcus (14), and 77% of HCV patients have higher levels of HCV RNA in the gingival sulci than in saliva (8,31).

Some of the significant indirect evidence for nosocomial transmission are associated with:

1. the higher incidence of HBV in dental health care workers, especially in oral surgeons and endodontists than in other health care medical staff (25).
2. the higher seroprevalence of HBV in patients with paradontosis, sever gum bleeding and poor oral hygiene (12).
3. the high rate of detection of HBsAg, anti-HBc, anti-HCV or anti-HCV and/or anti-HBc in non-stimulated saliva of patients with periodontal disease compared with controls (12).

HCV RNA has been detected also in saliva and the salivary glands of patients with sialadenitis (1,3,21). Therefore, presence of HCV RNA in saliva makes it a possible factor of transmission of HCV infection, which does not necessarily mean transmission of infection. Also, Lock G et al. have demonstrated contamination with HCV RNA of a considerable portion of toothbrushes used by hepatitis C patients, which is a factor conducive to the domestic transmission of viruses between household members when sharing these objects (18).

The risk of transmission of infections in dental care settings is related to the existence of sources of infections, respectively patient – infected or infection carrier and attending dental health care staff – infected or infection carrier. Finding exactly how much risk people face requires taking an epidemiologic history of dental practitioners. In all cases, however, you need to bear in mind the ground rule that every patient is potentially infected, and that each member of the staff should take care to protect themselves personally (2,22). Moreover Ordinance No.3 of Bulgarian Health-Care Ministry of 8.05.2013 for the promotion of medical standard to prevent and control infections associated with medical care/ nosocomial infections regulates the statutory nature of the protection of patients and staff (22).

EVIDENCE FOR NOSOCOMIAL TRANSMISSION OF HBV – CHRONOLOGICAL PRESENTATION OF CASES

1. Transmission from dental doctor to patient Levin ML, et al. JAMA, 1974 (16).

A dental surgeon who was HBsAg positive infected 55 patients with HBV over a period of 42 months. Reason: The surgeon did not use gloves. At that time the standard guidance for preventive measures issued from the Center for Disease Control and Prevention (CDC, Atlanta) were not yet formulated and published. The results of a study we conducted in 2011 in individual dental practices suggested that the percentage of dentists using gloves at work was unacceptably low (20–40%), and this against the proven finding that gloves reduce the amount of blood by at least 50% (30).

Hadler SC, et al. Ann Intern Med 1981 (13).

In September, 1978 in Baltimore, Maryland two dental patients were diagnosed with HBV infection. These patients were both treated by the same dental health care worker who had had acute hepatitis B in June 1978 and had remained positive for HBsAg and HBeAg over the

ensuing 6 months. Reason: The dental practitioner who was the source of infection kept on working without using surgical gloves to minimize the risk of transmitting the infection. A serological follow-up study of 764 patients divided into two groups, of which 395 were treated by the dentist before he began wearing gloves and 369 treated by the same dentist wearing gloves, was the basis for the conclusion. The follow-up identified a total of six patients with HBV after dental treatment three of whom were symptomatic. All infected patients were from the group treated by the dentist without gloves. The patients in this group had in addition highly traumatic dental work (attack rate 6.9 percent). They were at significantly higher risk than patients having either less traumatic dental procedures (attack rate 0.5%) or non-traumatic work (attack rate = 0, $p<0.02$). The definitive conclusion was that gloves could reduce the risk of transmitting the virus from the dentist to the patient since none of the 369 patients treated by this dentist with protective gloves become infected.

Shaw FE Jr et al. JAMA 1986 (29).

Between April 1984 and February 1985, 9 cases of HBV were reported to have occurred among dental patients in a rural county in Indiana (United States) with a population of 35,000. This incidence was over 20 times higher than the mean annual rate for the county in the previous decade. All patients had been treated by a dentist 4–5 months before illness, and the dentist was HBsAg and HBeAg positive and anti-HBc-IgM antibodies negative.

2. PATIENT-TO-PATIENT TRANSMISSION

Redd JT, et al. 2007 (27).

In October 2001 in the United States a 60-year-old woman with no history of hepatitis B vaccination and none of the traditional hepatitis B risk factors in her medical history, became symptomatic with acute VHB. A few months before this she had had an oral surgery (extraction of 7 teeth). The serological tests of the dentists working in this settings were negative for HBsAg, and 93.3% of them had a receipt of 3 doses of hepatitis B vaccine documented from vaccination records. Detailed epidemiological investigation shows: 161 min before the index patient had her teeth extracted, another patient, a 36-year-old woman that was HBsAg positive since the end of 1997, had three teeth extracted. Virus identity of the two patients was proven by sequencing. The serological tests of 25 (93%) of the 27 patients operated after the source patient showed 19 (76%) of them to be immune to HBV, and no other cases were detected. Conclusion: Virus transmission is limited because of the high percentage of patients vaccinated against HBV.

CDC. Patient-to-Patient Hepatitis B Virus Transmission in an Oral Surgery Practice 2001 (10).

In 2001 a patient with HBV was reported to a state health department in USA. The epidemiological investigation found that the patient had only a recent oral surgery in a dental setting, where earlier that same day another patient with chronic HBV had received dental care. Molecular epidemiologic techniques indicated transmission of HBV between the two patients. A control visit in the dental office found strict compliance with the standard infection control practices and regular vaccination of the entire staff who all were negative for HBV. The only assumption the control officers could make was that there was only a single lapse in the decontamination procedures in which the source patient had contaminated the dental area with blood.

Radcliffe RA, et al. J Am Dent Assoc 2013 (26).

In 2009, five cases of HBV infection were reported in a two-day, portable dental clinic in West Virginia, United States. The clinic was organized in a charity campaign and was held in a gymnasium staffed by 750 volunteers, including dental care specialists, who provided dental care to 1137 adults. Retrospective investigation

was conducted by using treatment records and history, volunteer logs, interviews of patients and volunteers with acute HBV infection, and other clinic volunteers. Molecular sequencing of the virus from the persons with acute HBV infection was also conducted. There were five people with acute HBV infections (3 patients and 2 volunteers) identified by the local and state health department. Four of the viral isolates were genotype D - three patients had tooth extraction and one had a dental cleaning. None of these cases reported having any behavioral risk factors for hepatitis B. Neither were they treated by the same dentist. Only a few infection control breaches were found by the investigators:

- the outer surfaces of the dental handpieces and mirrors were cleaned with disinfectant wipes without any subsequent heat sterilization between patient uses.
- used instruments were sterilized unwrapped,
- some of the patients were allowed to carry their partially used anesthetic carpules in the metal syringes on a tray next to other consumables if needed for later reuse,
- no training was provided to the dental staff in the standards for prevention of blood-borne pathogens transmission,
- there was only limited written information on individual patient procedures and consumables.

Analysis of the results of the surveys we conducted previously in individual dental practices has shown that (30):

- only 48.15% of all dental practitioners comply with the requirements of the Standard for Prevention and Control of Medical/Dental Care Associated Infections, which is far from a satisfactory result.
- about 40% of dental doctors have an insufficient number of handpieces, which makes it difficult to disinfect them, and 8.64% of the dentists attempt no decontamination procedures with the handpieces after each patient, which is to say that they do not consider them potential factors for the transmission of infections.
- 86.86% of the dentist blow out their turbines only at the beginning of their working day and only 53% do this after each patient. To reduce the risk of sucking back secretions from the oral cavity by the turbine which creates conditions for potential cross-infection of subsequent patients, air needs to be discharged from the turbine for 3 minutes. Only 26.97% of the respondents reported doing this which indicates ignorance and misjudgement of turbines as a factor for dental care associated infections transmission.
- disinfection in the vicinity of the dental chair is carried out by 85% of dentists, while 11% report directly that they do not do such disinfection.
- hand disinfection using the six steps method is performed incorrectly in 30% of cases. The survey results did not convince us that all other respondents with positive response follow strictly this method and not just knowing it in theory.

In terms of the risk of transmission of HCV, medical and dental procedures occupy the last place in the list of risk factors/groups for infection:

- recipients of clotting factor before 1987
- recipients of blood or organ transplant before July 1992,
- current or former intravenous drug addicts,
- patients on chronic hemodialysis,
- persons who are already infected with HIV,
- children born to mothers positive for HCV,
- health workers involved in high-risk exposures
- medical and dental procedures.

EVIDENCE FOR NOSOCOMIAL TRANSMISSION OF HCV - CHRONOLOGICAL PRESENTATION OF CASES

1. Transmission from dentist to patient

No cases of transmission of HCV from a dental physician to patients have been reported in the available English-language literature, and the risk for such transmission appears rather limited (8). However, there have been reports of transmissions from HCV-infected surgeons occurring during invasive procedures, the overall risk for such infection being 0.17% (8).

2. Transmission from a patient to a dentist

There has been evidence of seropositive dental health care personnel (presence of anti-HCV) which indirectly indicates occupational exposure.

Klein RS, et al. Lancet 1991 (15)

Assessment of occupational risk for HCV infection among dentists in New York City area, the United States, found that 8 (1.75%) out of 456 dentists were anti-HCV antibodies positive compared with 1 (0.14%) of 723 controls (OR 12.9, 95% CI 1.7–573). Anti-HCV was found in even higher concentrations in oral surgeons - 4 (9.3%) of 43 compared with 4 (0.97%) out of 413 other dentists (OR 10.5, 95% CI 1.9–58). Seropositive dentists claimed that they had treated more intravenous drug addicts in the week (p =0.04) or month (p = 0.03) before the study than did seronegative dentists. Conclusion: Dental health care workers are at increased risk for HCV infection, and all health care workers should regard patients as potentially infected.

Lodi G, et al.Br Dent J 1997 (19).

The cases of acute HCV infection are commonly associated with incidents involving exposure to blood. In some dental practices in the UK, 1.2% of their dental health care workers have been found positive for anti-HCV antibodies (these were not dental physicians). Since the prevalence of HCV infection in the UK general population is between 0.08% and 0.55%, this means that dental health care workers are at a significantly higher risk for HCV infection than is general population.

3. Transmission of HCV infection from patient to patient

Jean Williams, et al. ADA News 2013 (35) and Weaver JM, Anesth Prog 2014 (34).

The first documented case of patient-to-patient transmission of HCV infection during dental treatment occurred in Tulsa, Oklahoma in 2013. The oral surgeon there was investigated by the State Dental Board and epidemiologists from the CDC. Many violations were found in this dental practice of the standard disinfection and sterilization control procedures, as well as of the proper use of disposable materials (reuse of disposable needles). 4202 patients were tested and 89 of them were found positive for HCV, 5 for HBV and 4 for HIV, but still the investigators could not find evidence to link the cases with the dental office in question, as their incidences were close to the expected ranges in the general population. The comment of R.A. Faiella (the ADA President) in this respect is worth including here: "While this is an isolated case, it raises questions about infection control in the dental office. The ADA encourages people to talk with their dentists, who will be glad to explain or demonstrate their infection control procedures."

The most recent CDC data on nosocomial outbreaks of HBV and HCV infection in the United States pose serious questions for the supervision and control of infections (9). There were 44 outbreaks of these diseases between 2008 and 2014; of these 42 (95%) occurred in non-hospital settings. Twenty-three outbreaks of HBV were reported with 175 outbreak-associated cases in which 10,700 persons were notified for screening and 22 outbreaks of HCV with 239 outbreak associated cases and 90,400 at-risk persons notified for screening. There was only one outbreak of HBV infection in a dental clinic in non-hospital settings.

EVIDENCE FOR NOSOCOMIAL TRANSMISSION OF HIV - CHRONOLOGICAL PRESENTATION OF CASES.

1. Dentist-to-patient transmission of HIV

C. Ciesielski, et al. Ann Intern Med 1992 (11).

These authors reported the first documented cases of HIV infection from dental worker which were confirmed by molecular analyses

in Florida, United States. The investigation was initiated in connection with 6 diagnosed cases of HIV (without any other risk exposures) among 650 patients treated by the same dentist. Four of these patients visited his dental office on the same day. The exact mechanism of transmission was unclear: the more likely pattern was dentist-to-patient transmission rather than patient-to-patient transmission. The dental doctor was not aware of his condition and was not receiving any antiretroviral therapy at the presumed time point of infection of his patients. Statistically, the estimated risk of HIV infection is calculated to be 1.98 cases of HIV per 1,000,000 dental procedures with strict adherence to universal precautions.

To assess the risk for patients treated by HIV-infected health-care workers (doctors and dentists), CDC (United States) conducted a retrospective study (28):

- as of January 1995 CDC received reports of investigations of a total of 64 HIV-infected health-care workers.
- CDC obtained the results of the investigation of 22,171 patients with invasive procedures treated by 51 of the 64 HIV-infected health-care workers.
- only 113 (0.5%) patients were reported to be seropositive – most were unaware of their status prior to the investigation.
- DNA sequencing was not able to detect any genetic similarity of viruses from the patients and the infected health-care workers.
- the risk of transmission of infection from infected health-care workers to patients during surgical procedures is low, in the order of 2.4 to 24 per million procedures.

2. Patient-to-patient transmission via contaminated dental instruments

Bautista LE, et al, Rev Panam Salud Publica, 1997 (4).

An outbreak of 14 cases of HIV infection was discovered by chance in May 1993 among hemodialysis patients at the university hospital in Bucaramanga, Colombia The outbreak occurred in 1992. Stored sera were used to establish the probable period of infection (PPI) for 10 of the 14 cases. A nested case-control study was carried out to evaluate possible transmission mechanisms. The health care experience of each HIV-positive patient during that patient's PPI was compared to the experience of time-matched controls. It was found that 7 out of 9 cases of HIV infection with known PPI in 1992 had had an invasive dental procedure performed 1 to 6 months before seroconversion. None of the dental health-care personnel were found to be a source of infection. Most likely, the infection was transmitted from patient to patient by contaminated dental instruments during invasive dental procedures strongly associated with risk of infection. Such patients have an average risk of HIV infection 8.15 times greater than comparable controls (P = 0.006).

Occupationally acquired HIV infection worldwide

Worldwide, 4.4% (0.8%–18.5%) of HIV infections among healthcare workers may be attributable to occupational injuries (32). More than 90% of the infections occurred in low income country, most of which could have been prevented (7). The last big report of the Public Health Laboratory for 1999 (24) reported 319 cases worldwide of HIV infection acquired as a result of occupational exposure among health care workers (of these 102 were definite and 217 were considered possible and probable). Until 2005 there had been 25 new cases of occupationally acquired HIV, of which 106 were definite and 238 - possible (33). Most of the definite cases occurred after percutaneous exposure (91%, 96/106). There were no dental workers among the definite cases, but among those classified as possible there were 8 dentists and dental workers, which is 3% of the possible 238 cases.

Conclusions: 1. Dental treatment is among the risk factors for HBV, HCV and HIV/AIDS. At present, this risk for patients and dental staff may be eliminated by using standard precautions and by developing new tools, protective

equipment and techniques. 2. Development of recommendations for risk management of transmission of blood-borne pathogens from health care workers to patients during invasive procedures is difficult, mostly due to incompleteness of the available scientific data. 3. The best strategy to reduce blood-borne infections is to have a very strict specification of the possible risks of acquiring them. To achieve this there should be conducted more properly planned, case-control and better analysed studies.

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Comparative investigation of neonatal effects of levetiracetam and valproic acid on the behavioural changes and levels of pro-inflammatory cytokines in neaonatal kainat model of epilepsy

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РЕЗЮМЕ

Епилепсията е хронично неврологично заболяване. В 30% от пациентите се установява незадоволителен контрол над гърчовете. Тези пациенти се приемат за фармакорезистентни. В последните 10 години голям брой научни доказателства дават доказателства за участието на невровъзпалението в патогенезата на епилепсията. Експериментални доказателства потвърждават клиничните наблюдения за активация на проинфламаторни молекулярни механизми при епилепсията. Макар, че участието на възпалението в клиничния ход на епилепсията е хипотетично, научни доказателства възможността чрез блокиране на възпалението да се спре прогресията на болеста. В нашето изследване сравняваме ефекта на леветирацетам и валпроева киселина върху епилептичната активност и нивата на проинфламаторните цитокини.

ABSTRACT

Epilepsy is a chronic neurological disorder. 30% of the patients have poor control on seizures. They are considered pharmacoresistant. In the last 10 years mounting body of evidence provided proofs for involvement of neuroinflammation in the the pathogenesis of epilepsy. Experimental facts support clinical observation of activation of proinflammatory pathways in epilepsy. Although the participacion of inflammation in the course of epilepsy is hypothetical, scientific evidence reveals the possibility by blocking neuroinflammation to halt the progression of the disease. In our study we compare the effects of levetiracetam and valproic acid on seizure activity and levels of proinflammatory cytokines in experimental pilocarpine model of epilepsy. The molecular effects of levetiracetam are contaversial, but in our work we observed that the drug suppresses simulteniously the seizures and

Молекулярните механизми на леветирацетам са противоречиви, но в нашето изследване ние наблюдавахме едновременно подтискане, както на на гърчовете, така и на нивата на проинфламаторните цитокини. Това ни дава основание да предположим, че антиинфламаторните ефекти на леветирацетам са в основата на неговия антиепилептичен ефект. Това подкрепя хипотезата, че невровъзпалението е ключов фактор в епилептогенезата.

Ключови думи: епилепсия, леветирацетам, невровъзпаление, проинфламаторни цитокини.

the levels of TNF-alpha and IL-1beta. This data gave us a reason to suggest that the anti-inflammatory effect of levetiracetam is responsible for its aniepiletic effect. This data corroborate the hypothesis that neuroinflammation is key factor in epileptogenesis.

Key words: epilepsy, levetiracetam, neuroinflammation, proinflammatory cytokines.

1. INTRODUCTION

Epilepsy is a neurological disease with the clinical manifestation of persistent seizures, affecting 50 million of the world population (6). Pharmacotherapy is considered to be the conventional approach in antiepileptic therapy [3]. However, existent AED's provide mediocre seizure control in 70% of epileptic patients (10,11). Drug-resistant epilepsy (DRE) patients present with uncontrolled seizures in 1/3 of epileptic cases (12).

Preventive measures in the treatment of epilepsy with existing medications have shown low efficacy in targeting the mechanisms of spontaneous recurrent seizures. Anticonvulsants failed to respond to approximately half of epileptic patients, only providing relief of symptoms. Therefore, development of effective pharmacotherapies with disease modyifing effects is required to treat the root of the pathological mechanisms.

Emerging body of evidence suggests that a relationship between the immune system and epilepsy exists. Increased expression of proinflammatory cytokines and activation of glial cells were observed in epileptic patients and animal models of epilepsy (13, 17, 31). Further, epileptogenesis is suggested to occur in the presence of a compromised immune system and its associated inflammatory processes in the brain (4, 7, 8). Data have indicated that cytokines are mediators of unprovoked epileptic seizures (4, 21, 22).

Various brain insults in the childhood—such as neurotraum, infection, perinatal injury, febrile seizures, and status epilepticus can induce inflammation in the brain (28), and these injuries in humans represent risk factors for the development of epilepsy. This evidence suggests that an epileptogenic event, even if subclinical, occurring at birth or during the lifetime may initiate a cascade of chronic inflammatory processes in the CNS that contributes to the onset of epilepsy.

Upon the initiation of local inflammation in the brain as a result of injury or status epilepticus in the childhood, microglia is activated releasing various pro-inflammatory mediators including TNF-alpha and IL-beta. Such mediators are key factors in epileptogenesis and can have major effects on neuronal excitability.

Development of drugs that influence neuroinflammation and in this way restrain activation of microglia and process of epileptogenesis is from crucial importance.

Levetiracetam (LEV) is a newer antiepileptic drug (AED) wich more than 15-y history of clinical approval for the treatment of parcial onset and generalized seizures (23, 31). Multitude of studies have proposed that LEV has neuroprotective properties in both epileptic and nonepilpetic disorders (1, 7, 22, 24, 31). The molecular effects of LEV remain uncharacterized, although a specific protein binding site, synaptic vesicle protein 2A (SV2A), has been identified (11). Therefore, LEV acts in a unique manner

distinguished from other AEDs. Interestingly, anti-inflammatory properties of LEV have been recently reported using in vitro bioassay (5).

Pilocarpine is a muscarinic cholinergic agonist. Numerous research on pilocarpine-induced SE has been conducted with regard to the pathophysiology and neuropathology (26). Intraperitoneal administration of high doses of pilocarpine (300–400mg/kg) in rodents results in mimicking of behavioral and electrographic seizures in humans (25).

In SE animal models for epilepsy research, pilocarpine and kainic acid-induced SE are suppressed by diazepam (DZP), controlling seizure activity due to the high effectivity against the aforementioned convulsants.

The present study aimed to compare effects of LEV, valproic acid (VPA) and DZP on the behavioral changes (seizure score and wet dog shakes-WDS) and levels of proinflammatory cytokines TNF-alpha and IL-1beta in hippocampus of immature rats in neonatal kainite-inuced model of epilepsy. Elucidating of these changes would reveal the role of neuroinflammation and glia as a key factors in epileptogenesis, as well as opportunities for influence on it.

The investigation would shed light on whether glial activation and neuroinflammation are the link between seizures in childhood and ensuing long lasting neurological dysfunction and development of spontaneous reccurent seizures.

2. MATERIALS AND METHODS

The study was performed using neonatal kainite-induced experimental model of epilepsy and scoring severity of seizures and levels of TNF-alpha and IL-1beta. The same design was used, but with pretreatment of animals with LEV, DZP and VPA.

2.1. Animals

A total of 25 male immature rats (body weight 30 to 50 g) were obtained from the Central Vivarium, MU-Sofia, and housed in groups of four per cage under standard laboratory conditions. They were maintained at constant room temperature (21 °C ± 2 °C) under a 12:12 h light-dark cycle with free access to food and water. All animal experiments were performed in accordance with the animal care guidelines of the Ethic Committee of Medical University, Sofia, so as to minimize the number of animals used and their suffering.

2.2. Drugs

- Levetiracetam (USB, Belgium, 50mg/kg, i.p)
- Valproic acid (Elta-90, Bulgaria, 50mg/kg, i.p.)
- Kainic acid (Elta-90, Bulgariua, 10mg/kg, i.p.)
- Ketamine (Gedeon Richter, Hungary, 100mg/kg, i.p.)
- Diazepam (Elta-90, Bulgaria, 5mg/kg, i.p.)

2.2. Kainic acid administration and experimental design

The rats were allocated into 5 groups of 5 animals each.

1. In the first group, animals were given saline, 0,9% (i.p.) to serve as negative control .
2. Animals from second group were administered kainic acid-10mg/kg, i.p. to serve as positive control.
3. Animals from third group were administered diazepam to serve as negative control.
4. In the fourth group animals received LEV (50mg/kg, i.p.) for 10 days via gavage, and on the last day kainic acid (10mg/kg, i.p.) was injected 30 min after after last administration of LEV.
5. In the fifth group animals received VPA (50mg/kg, i.p) for 10 days via gavage, and on the last day kainic acid (10mg/kg, i.p.) was injected 30 min after after last administration of LEV.

2.3. Behavioral tests

Following administration of KA, rats were placed singly in plexiglas cages and were observed for behavioural changes over a period of 2 h. During the observation period, convulsive attacks were measured on Racine scale as follows: 0-behavioral arrest (motionless), hair raising, excitement, and rapid breathing; 1-mouth movements (lips

and tongue), vibrissae movements, and salivation; 2-head and eye clonus; 3-forelimb clonus; "wet dog shakes"; 4-clonic rearing; 5- clonic rearing with loss of postural control and uncontrollable jumping. Finally, 1d after KA administration, rats were anaesthetized with i.p. injection of ketamine (100 mg/kg, i.p. and sacrificed. The hippocampi of animals were immediately removed, cleaned with chilled saline (0.9%) and used for biochemical analysis.

2.4. TNF-a and IL-1beta Assay in Rat Brain by Enzyme Linked Immunosorbent Assay (ELISA)

The brain was homogenized in 1 ml of ice-cold lysis buffer (radio-immunoprecipitation assay, RIPA) containing 50mM Tris–HCl (pH 8.0), 150mM sodium chloride, 1.0% Iepal CA-630 (NP-40), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% phosphatase inhibitor cocktail and a protease inhibitor cocktail. The lysate was centrifuged (15000g 4 °C) for 15 min, and the supernatant was added to 96-well ELISA plates. The TNF-a and IL-1beta concentration were then determined by reading the ELISA plate.

3. RESULTS

3.1. Behavioral tests.

In the present study, we observed seizure behaviour on Racine's scale and WDS, after KA-treatment, and also after pretreatment with LEV, DZP, VPA (**Table 1**).

Group 1. In the first group we did not observed seizure activity.

Group 2. Effect of kainic acid on behavioral symptoms and convulsions.

All the rats in the KA group exhibited behavioral signs like grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, and head nodding within 5 min after kainic acid administration (10 mg/kg, i.p.).

Group 3. Effect of diazepam on KA-induced seizures (negative control group) showed substantial decreased level of seizure activity.

Group 4. Effect of levetiracetam on KA-induced seizures.

In the levetiracetam+KA group, the rats were pretreated with 50mg/kg, i.p. for 7 days prior to KA(10mg/kg, i.p) administration. Seizure scores in this group were significantly lower than those in the control group (Table 1).

In this group valproic acid also was administered for 7 consecutive days, prior to KA(10mg/kg, i.p.). Valproic acid also attenuated the seizure score in this group, but less then levetiracetam+KA-group.

We also counted the number of wet dog shaking (WDS) behavior at 0,5h. after KA administration (**Fig 1 and Fig 2**). During the 2-hour observation period after KA administration, the number of WDS in the groups 3 and 4 were significantly lower than that in the control groups, but in different degree (**Fig 2**). The lowest number and onset of WDS were detected in levetiracetam+KA group.

	C-group (n=5)	KA-group (n=5)	DK-group (n=5)	LK-group (n=5)	VK group (n=5)
Grade 0	5	0	3	0	0
Grade 1	0	0	2	1	3
Grade 2	0	0	0	3	2
Grade 3	0	1	0	1	1
Grade 4	0	2	0	0	0
Grade 5	0	2	0	0	0

Table 1: Effect of pretreatment with diazepam (DK group), levetiracetam (LK group) and valproic acid (VK group), prior to kainic acid-induced seizures, on seizure scores. C-control rats group, KA-kainic acid treated rats, DK-diazepam+kainic acid treated rats, LK levetiracetam+kainic acid treated rats, VK-valproic acid+kainic acid treated rats. Motor seizures severity were measured on Racine's scale. Data are expressed as mean ± standart error of mean.

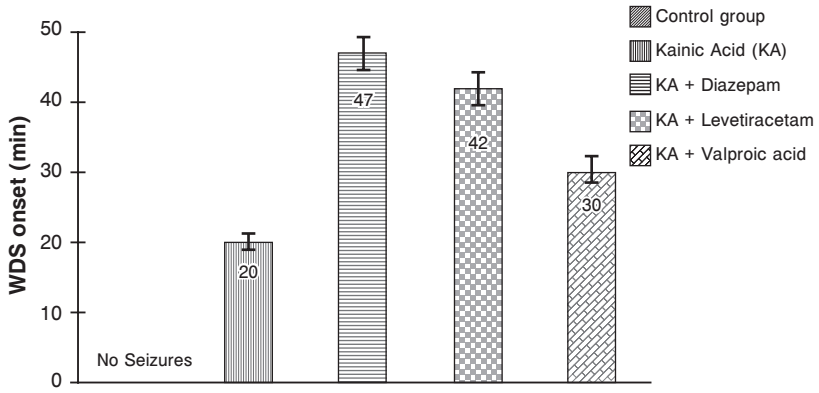

Figure 1. The effect of diazepam, levetiracetam and valproic acid on onset of WDS in immature rats with kainic acid-induced seizures. Data are expressed as mean ± standart error of mean.

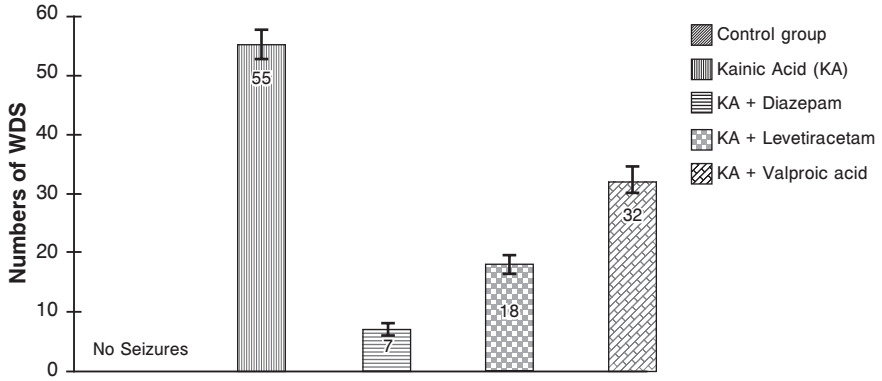

Figure 2. The effect of diazepam, levetiracetam and valproic acid on the number of WDS in immature rats with kainic acid-induced seizures. Data are expressed as mean ± standart error of mean.

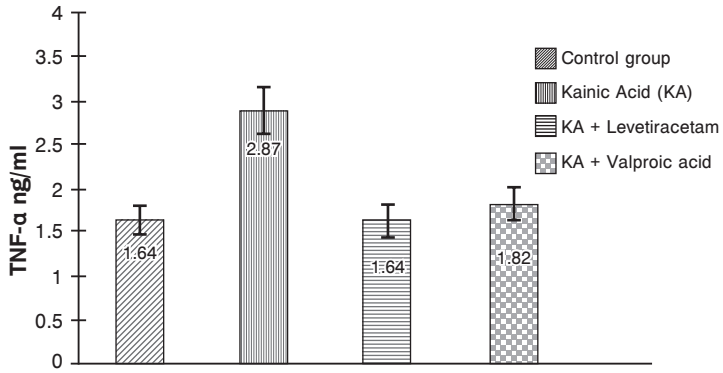

Fig3. The effect of, levetiracetam and valproic acid on the hippocampal levels of TNF-alpha in immature rats with kainic acid-induced seizures. Data are expressed as mean ± standart error of mean.

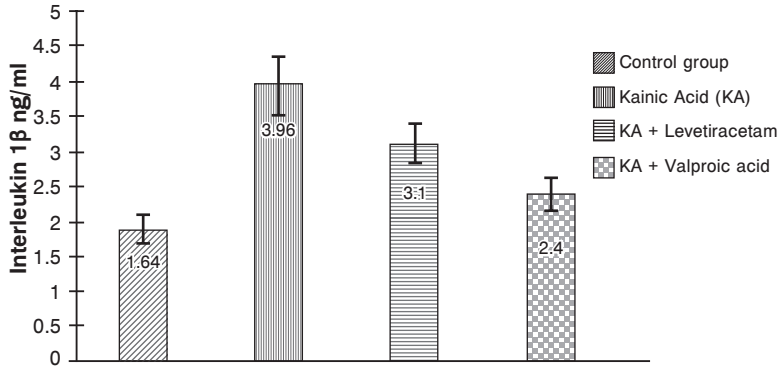

Figure 4: The effect of, levetiracetam and valproic acid on the hippocampal levels of IL-1beta in immature rats with kainic acid-induced seizures. Data are expressed as mean ± standart error of mean.

3.2. Effect of Levetiracetam and Valproic acid on the hippocampal levels of TNF-a and IL-1beta in kainic acid induced seizures

The brain level of TNF-a and IL-1beta were significantly raised after KA-administration as compared to the control group rats. In the levetiracetam+KA groups, levetiracetam pretreatment attenuated the KA- induced rise in brain levels of TNF-a (**Fig. 3**). This reduction was more pronounced as compared to valproic acid+KA group. Importantly, there was coincidence between this results and behavioral test, described above.

4.DISCUSSION

In the present study, we investigated the possible effects of 10-days levetiracetam and valproic acid pretretment on seizure score and levels of pro-inflammatory cytokines in KA-induced seizure in immature rats. Pretreatment with these drugs reduced the severity of seizures and levels of these cytokines, relative to control animals.

There are currently no therapies that target the mechanisms of epileptogenesis after neurologic injury.We used Levetiracetam and Valproic acid, to test the hypothesis that inhibition of seizure activity is a result of influence of these drugs on the neuroinflammation and to compare their effects in this context.

The role of inflammatory responses in the pathogenesis of epilepsy and seizure-induced brain damage has been appreciated only recently. Inflammatory processes including activation of microglia and astrocytes and production of proinflammatory cytokines like TNF-a , IL-1b , IL-6, and related molecules have been described in human epilepsy patients as well as in experimental models of epilepsy (29).

In the present study, we found that LEV and Valproic acid effectively decreased levels of TNF-alpha, but without significant differences between two drugs. Levels of IL-1beta were significantly lowered, but LEV had much more pronuenced effect as compared to VPA. We hypothesized that one potential mechanism by which seizures in the immature brain may contribute to increased vulnerability to a subsequent process of epileptogenenesis is through a glial activation cycle that leads to neuroinflammation, increased levels of IL-1beta and neuronal dysfunction.

Several studies strongly suggest that brain pro-inflammatory cytokines may play a significant role in the generation and/or maintenance of seizures, and also in the establishment of chronic epileptic focuses (9, 16, 18). Therefore, the putative brain anti-inflammatory action of LEV suggested by present findings, might also contribute importantly to its anti-seizure effect.

LEV and PEV pretreatment was found to significantly reduce KA-induced WDS, characteristic of experimental convulsive seizures (19). Further, the extent of inhibition of WDS correlated with the degree of suppression of proinflammatory levels, as mentioned above. Neuronal hyperactivity in limbic structures that spread to the midbrain and motor system were as a result of KA-induced WDS (14). An increase of GABA receptors has been documented after limbic stimulation (20) suggesting that WDS is a sign of the progression of limbic seizures towards generalized seizure (19). The data suggests that neuronal hyperactivity could be prevented in certain areas of the brain with the utilization of levetiracetam.

The data in this study supports the hypothesis that activated glial responses play a role in the mechanisms of childhood seizures initiation of the epileptogenic process, where there is an increased susceptibility to consequent secondary neurologic damage. The inhibited levels of seizure scores with LEV application suggest that therapies that targets epilepsy course disease and affects the pathogenic substrate of epilepsy could be more effective as compared to common antiepileptic treatment.

Glial activation and neuroinflammation as a result of acute brain injury, if selectively targeted with therapies, consequently, may assist

in prevention of neurologic disorders in adulthood. The implications of this study infer to the possibility of intervention following seizures in childhood that control acute microglial activation and proinflammatory cytokine response, that may minimize long-term neurologic effects and vulnerability to seizures in adult years.

CONCLUSION

In conclusion, seizures in early-life period increased the glial activation and subsequent rise of pro-inflammatory levels in the brain. It is believed that this initiate the epileptogenesis. From the moment of this precipitating event until appearance of spontaneous reccurent seizures exist latent period. We suppose that applied in this critical period, levetiracetam could exert antiepileptogenic and disease modifying effect. Our results support the role of neuroinflammation in the pathogenesis of epilepsy and delineate new strategy in antiepileptic therapy.

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Medicine today - strong systemization, zero empathy. Thoughts on necessary changes.

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Медицината днес – силна на систематизация, но бедна на емпатия. Предложения за необходими промени.

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РЕЗЮМЕ

Медицината съществува за благото на човешкия вид. Неговото обслужване изисква грижа както за физическите, така и за психичните нужди на тялото. В момента обаче се наблюдава растящ недостиг на финансови и човешки ресурси. Той налага негативни промени в начина, по който се осъществява оздравителния процес. От лекарите се очаква бързото и ефективно разрешаване на клинични случаи; емоционалните потребности на пациента често остават на заден план. Така, болните се чувстват обезличени, а живота им - лишен от стойност. Коригирането на тази тенденция налага институционални промени на три нива – **международно** (нов клон на медицинско право със Световната Здравна Организация като основополагателен и върховен отсъждащ орган; медицински съдилища; лекари-прависти), **национално** (реформа в медицинското образование; своевременно проследяване, насочване и коригиране на индивидуалното развитие на всеки студент-медик) и **местно** (индивидуално медицинско обслужване на пациенти основано на електронно сътрудничество между лекари и медицински сестри).

Ключови думи: медицина, емпатия, систематизиране, дехуманизация на медицината, законодателство

ABSTRACT

Medicine's main *raison d'être* is securing the uninterrupted existence of the human species. In this quest, catering for the mental as well as the physical needs of the human body is of paramount importance. An ever growing strain on financial and human resources, however, diverts from the emotional aspects of the healing process. Instead, it directs physicians' efforts towards the efficient solution of clinical cases. As a result, patients feel objectified and dehumanized. To correct this trend would mean institutionalizing changes at three levels - the international (new body of medical law enforced by the WHO, medical courts and lawyers-physicians), the national (medical education reform, monitoring and correcting the individual development of each student) and the local (digitized physician – nurse collaboration to provide customized medical service).

Key words: medicine, empathy, systemization, dehumanization of medicine, policy making

INTRODUCTION

Medicine is an investigative field of science and healing stemming from humanity's desire to ensure the continued evolution of its species, despite the vagaries of nature. Center-stage in it is occupied by the human and the unique needs of his/her body. And these needs are not trivial. Every second, the human body is ridden with mechanical and bio-chemical processes informing cellular life, reproduction and death. Medicine aims at sustaining the dynamic relationship between these processes within limits allowing for an uninterrupted physical existence of ever increasing duration.

THE ISSUE

In the absence of telepathic capabilities, the correct determination of life-sustaining boundaries based on patient's description alone is problematic. More often than not, humans possess limited knowledge on the internal workings of their bodies and the normal parameters of their function. The interface of bio-matter and related chemical processes involving the brain onsets intangible constructs in the form of feelings and emotions. Patient's symptoms are often masked by these constructs, the strongest of them being fear and insecurity. In such conditions, diagnosing an illness is difficult.

Medical practitioners face three options: **a/ address patient's issues directly** - based on physician's individual ability to relate to a patient's suffering (in other words, to empathize), **b/ negate the "human element"** and act systemically, resolving a medical case, or **c/ meet both approaches half-way through**.

Most physicians favor the second option. The reason, some argue, is that medical practitioners are overworked, underpaid and having personal problems of their own.[8,14] It is therefore easier to identify patients with a particular type of illness rather than becoming emotionally involved.

As a result however, more and more patients feel detached from the healing process. Instead of occupying center-stage in it, they see themselves as the subject of medical discussion, the only outcome of which is chemical substance- and machine-supported treatment. Empathy towards one's pain and suffering is altogether missing. [2,4,17] The understanding is that when ill, one needs to endure what one must in order to get physically better. Crying or complaining about it is futile. Such an act would only burden the physician unnecessarily, slowing him/ her down in catering for other patients. The phrase "the dehumanization of medicine" has made its debut. [9,13]

A FAULTY HEALTH SYSTEM

Amidst patients' discontent and physicians' indignation, an important fact gets omitted. Twenty-first century's medical education requires physicians to have:

- good memory to absorb a great amount of factual data
- patience in listening to patient complaints in order to diagnose illnesses correctly
- informed decision-making skills in consultation with narrower medical specialists
- tenacity to fight for the life of a patient with every scientific tool available
- the courage to accept the transient nature of human life – at present, no one is immortal.

With this specification in mind, physicians are bred to be cool-headed, calculating objectifiers. Their result-oriented attitude allows for the uninterrupted "conveyor belt" medicine the national Health System seeks. Policy-makers seem to support the opinion that an ever growing and ageing population calls for efficiency rather than person-centered medical care.

Instead of just healers, physicians are also expected to be managers of the patient flow. Such a shift in responsibilities means strict schedules, streamlined procedures and a staunched pursuit for cost-effectiveness. Empathy provision is viewed as counter-productive. It requires time, mental effort and personal profile building for each patient. Providing it may be feasible for a GP operating in a small

town, but not for a MD working in a mid- or a big-sized city hospital.

Historically, empathy was never part of the profession anyway. The issue of its supposed absence was first raised by the post-WWII human rights movements demanding equal access and "quality of life" medical service. [3,10,16] In other words, the pressure on medicine to become more person-centered is an outcome of political pressure from bellow, not a natural evolution in humanity's way of thinking.

THE ROAD AHEAD

Is there a way forward? Following the maxim "if there is a will, there is a way," the international, national and local levels of medical care provision would need to be addressed.

The international fora is in the direst need of institutional and judicial change. It has been too long since (at least, the 17th century European Judicial Renaissance) any significant law-formulation has come into being. The steady concentration of power from a plethora of kingdoms, fiefdoms and municipalities into a larger nation-state has subtracted significantly from the ability of law-makers to support strong legal traditions and produce original law. In a nationalized and then a globalized world, there is a push to simplify, standardize and unify. As a result, instead of issue-targeted law, today we witness predominantly precedence-based case law. [6, 11] Case law however is a "shifty" law. It is not based on sound principles, but on judges' interpretation of related previous occurrences.

The creation of medicine-specific body of national and international law would break this streak. At present, the term "medical law" describes civil court cases of supposed malpractice with fatal or long-lasting negative effects on a patient's life. It is exercised in regular civil courts using mainstream legal provisions supplemented by "independent" medical testimony. The objective is to prove professional incompetence or mal intent resulting in a crime against human nature. [5, 15]

The proposed new law would be a branch of the constitutionally enforced national and WHO-regulated international law. It would codify clearly defined humanistic principles. First amongst them should be the idea that the guardship of human life regardless of race, gender or nationality stands above monetary matters. Following this precept should be an understanding that besides patients, sick people are also emotional beings in need of empathy and psychological support as part of the healing process. One should also delineate the right of patients and their relatives to full disclosure as to all procedures and medications applied to them on a daily basis.

Under this new medical law each state would have to create and independently manage a national database of its citizens' medical records. Administered by a specialized branch of the national Ministry of Health, this database would grant case-restricted access to established medical specialists. Whenever a legal dispute arises, they would be randomly-selected by the national and international medical databases (according to the magnitude of the case and the availability of a specialist pool) to provide an objective peer-review.

The new judicial system would also have to employ national and international lawyers as well as judges with medical background (i.e. MD graduates), thus creating a new field of medical specialization. Individuals capable of exercising both professions in unison can best defend physicians' and patients' interests alike.

Outside the judicial changes, world's medical community must agree on a standard codification of specialized medical fields based on their degree and nature of interaction with the patient. It is true that some medical fields are more technical than others (e.g. surgery, anesthesiology, orthopedics versus cardiology, gynecology, physiotherapy, psychiatry). Their technical nature however does not exclude contact with the patient. Every physician must possess not only systemization skills, but also a certain level of empathy, good communication abilities

and inter-personal diplomacy. A system reflecting the different profiles of medical specializations would lead to the development of specific requirements for each field's practice to ensure quality of service and positive patient feedback.

In conjunction to international innovation and standardization, a shift in the way medical education itself is conducted is long overdue. It must include, but not remain limited to:

- **profiling the personality type of the physician and the efficiency level of his/ her social skills** – a beginning-of-studies' profile for each medical student would allow for a timely correction of any negative tendencies and ensure proper instruction based on the individual needs of each future physician.
- **follow-up on physician's personal development throughout their studies** - besides knowledge grades, medical universities need to continuously assess and foster individual talents and skills (e.g. associative reasoning in diagnosis or machine-smart attitude).
- **practical instruction on empathy provision outside the curriculum - before entry and throughout medical studies** (e.g. in institutions for the elderly, orphanages, centers for the homeless and the destitute, funerary homes). Exposure to suffering and death would increase student awareness of their future patient's needs, work on the philosophical study of the "life-illness-death" relationship and, hopefully, stimulate personal growth and maturation.
- **empathy-based clinical practice as an integral part of student grading on a par with theoretical knowledge** - here, the guiding role of medical psychology and role-model guidance (e.g. study of famous physicians in history or tutelage by established, in-house medical practitioners) would prove valuable.
- **directed instruction of students who wish to specialize in a particular medical field, based on the above-mentioned WHO medical fields' codification (if implemented)** - students choosing a theoretical field, for example Anatomy or Biochemistry, would not need so much empathy instruction. When clinical fields such as surgery or internal medicine are involved however, higher systemization level versus lower empathy level is advisable. In cases where gynecology, gerontology or pediatrics are the choice - the reverse - "lower systemization/ higher empathy."

At the local level, one should entertain the idea of a better symbiosis between physicians and other medical personnel, particularly nurses. The latter are the first point of contact with the patient. They are the greeting party at hospital receptions, the implementers of patient's daily treatments, the ones to call when pain strikes the body. [1] Easy to approach, patients feel free to share with them personal stories. The latter often include information deemed „insignificant" to one's condition or „improper" (even "shameful" if the subject is sex or social taboos) to communicate to the resident physician. [7, 12]

A system could be devised whereby nurses add such details to a patient's electronic file. As such a new job requirement would put an extra toll on an already over-stretched nursing staff, nurses-in-training could come in handy. As part of their study, they can accompany resident nurses on their rounds, take instruction and in-put electronically all the relevant data. Physicians with access can then use this information on a par with patient's medical history to provide better diagnosis and more individual-centered service.

CONCLUSION

The proposed changes are not difficult to accomplish. With an increasing pressure from below (patients) and within the health system (the state, hospital management, physicians, other medical professionals), policy-makers need to ask themselves – *cui bono*? The answer is – each and every one of us.

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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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