IJBM

International Journal of

BIOMEDICINE

ISSN 2158-0510

Available online at
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International Journal of Biomedicine (ISSN 2158-0510) is published four times a year by International Medical Research and Development Corp. (IMRDC), 6308, 12 Avenue, Brooklyn, NY 11219 USA

Customer Service: International Journal of Biomedicine, 6308, 12 Avenue, Brooklyn, NY 11219, USA; Phone number: 1-917-740-3053; E-mail: editor@ijbm.org

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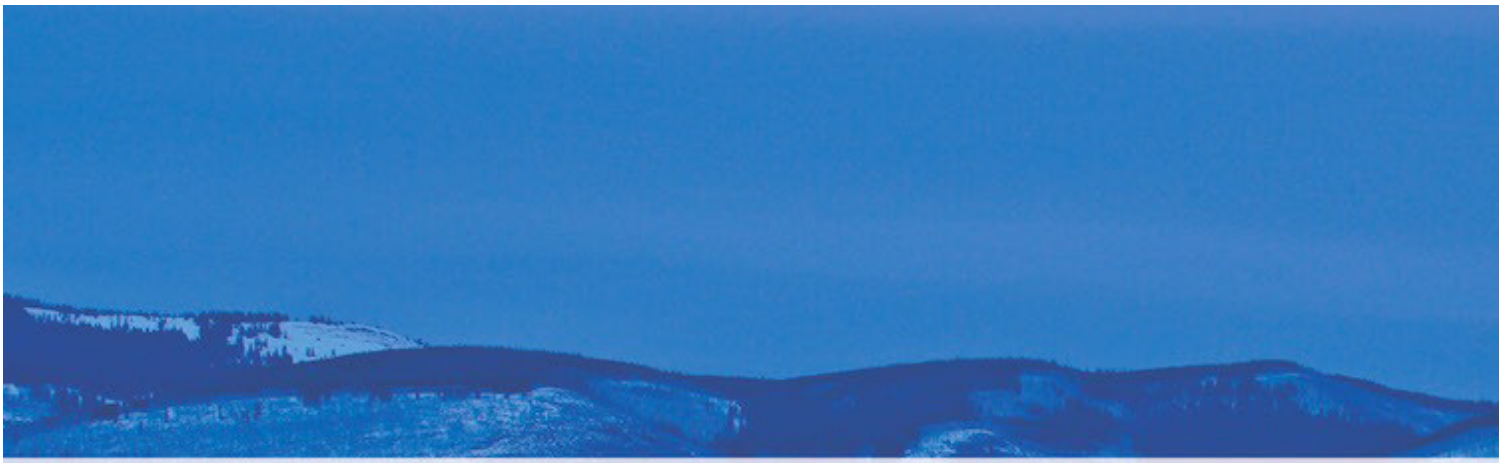
Volume 7 Issue 2 June 2017

CONTENTS

CONTENTS
CONTINUED



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International Journal of Biomedicine 7(2) (2017) 85-90
doi: 10.21103/Article7(2)_RA1

REVIEW

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Nonpsychotic Psychiatric Disorders in Juvenile Myoclonic Epilepsy

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Abstract

The association of epilepsy with mental illness has been described in recent years. Scientists are trying to relate certain epilepsies, such as juvenile myoclonic epilepsy (JME), with certain personality traits marked by emotional instability. The goal of this review is to evaluate the scientific literature about nonpsychotic psychiatric disorders in JME patients, the most common form of idiopathic generalized epilepsy (IGE). Data in this review were collected through an extensive literature search of available full-text publications in PubMed, Springer, Clinical Keys and eLIBRARY.RU databases. Comorbid personality and nonpsychotic psychiatric disorders are a common and interdisciplinary problem in JME management. The disorders in patients with JME often go undiagnosed and hence untreated. Therefore, this problem requires further and extensive investigation. **(International Journal of Biomedicine. 2017;7(2):85-90.)**

Key Words: juvenile myoclonic epilepsy • personality disorders • nonpsychotic psychiatric disorders • antiepileptic drugs

Abbreviations

ADCME, autosomal dominant cortical tremor, myoclonus, and epilepsy; **AEDs**, antiepileptic drugs; **BIS**, Barratt impulsiveness scale; **DSM**, Diagnostic and Statistical Manual of Mental Disorders; **IGE**, idiopathic generalized epilepsy; **JME**, juvenile myoclonic epilepsy; **PDs,** personality disorders; **SAS**, social adjustment scale; **TLE**, temporal lobe epilepsy.

Introduction

JME is one of the most widespread forms of IGE in adolescents and young adults. JME is characterized by the classical Herpin-Janz triad: frequent myoclonic jerks, most often of the upper limbs; possible generalized tonic-clonic seizures; and, less commonly, absences.[1] Seizures are often preceded by stress and a disturbance in the circadian rhythm of sleep with a predominance of seizures after awakening (83% of patients); a frequent cause of these seizures is sleep deprivation (77%).[2] A decrease in the frequency and severity of seizures depends not only on the doctor, who needs to choose the therapy correctly, but also on the patient, who in turn adheres to the sleep-wakefulness regime, who prevents menstrual irregularities, and who avoids strong emotional shakes, and other behaviors that might cause stress.

Despite recognizing the above factors, which cause more frequent seizures and worsening of the general condition, many patients cannot cope with their emotions singly; they note a decrease in stress resistance, and therefore complain about the difficulties of falling asleep, resulting in an undesirable lack of sleep.

Janz and Christian, who first distinguished JME as a syndrome in 1957,[3] in their classic characterization of JME described the distinctive personality features of such patients as unsteadiness, lack of discipline, hedonism, and an indifference to their disease.[4,5] The authors also identified lack of drive and endurance, unstable self-concept, and rapidly changing affect and mood states, that, according to them, often complicated treatment of JME.[4,6] People with personality disorders (PDs) often experience other mental health problems, especially depression and substance misuse.

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Executive functions refer to processes involved in the elaboration of cognitive and behavioral responses and strategies for the attainment of immediate or future goals.[7] JME patients often demonstrate non-compliance with the doctor's prescriptions. Even if they say that they are doing everything, in fact they forget to attend control visits and to take their medication regularly.[8] Such inadequate behavior (aggravation of executive and intellectual functions) may be associated with long-term anxiety. Thus, the harmful effects on cognition of an early onset of seizure and longer duration of the disease have been well demonstrated.[9]

The cause of such disorders may be concomitant nonpsychotic psychiatric disorders, often described with JME. These include various types of anxiety and mood disorders: generalized anxiety disorder, phobias, depression, dysthymia, and psychosomatic disorders.[10] In addition, the presence of seizures in social circumstances reduces social interaction and compromises the quality of interpersonal relationships.[11] The prevalence of psychiatric disorders in nearly one-third of JME patients is similar to that reported in patients with temporal lobe epilepsy, where psychiatric aspects have been more commonly studied.[12] Such disorders further aggravate the patient's condition, closing the vicious circle.

The association of epilepsy with mental illness has been recently described. Scientists are trying to relate certain epilepsies, such as JME, with certain personality traits marked by emotional instability.[13] Furthermore, comorbid personality and nonpsychotic psychiatric disorders in JME, which have been increasingly appearing in the literature recently, are also associated with higher seizure frequency and less seizure control.[14]

The goal of this review is to evaluate the scientific literature about nonpsychotic psychiatric disorders in JME patients.

Methods

Data in this review were collected through an extensive literature search of available full-text publications in PubMed, Springer, Clinical Keys and eLIBRARY.RU databases. We considered studies published from 2010 to 2017 and several earlier works referred to us by other authors. "Juvenile myoclonic epilepsy," "psychiatric disorders," "anxiety," and "impulsivity" were used as keywords. In general, for a specified period we identified 34 publications dealing with the problem of anxiety and depression with JME in the foreign databases. Only 16 of these publications have been included in the review based on search criteria.

Results

PDs become distinct during adolescence or earlier and continue throughout life. The peak of the JME onset comes at the age of 14 to 16 years, with a range of 8-26 years.[2] Thus, the time periods of the beginning and development of these manifestations are the same, and we can assume that their occurrence is interrelated. Patients with JME inevitably face difficulties in life and employment, such as family problems, reduced social interactions, decreased job opportunities, and low self-esteem. Therefore, doctors often find them anxious and depressed, even with controlled seizures.[11]

Studies available in the literature can be divided into two different approaches to PDs in JME: clinical observation, and categorical classification according to the DSM. The first method, clinical observation, is subjective, depends on the doctor's personal perception of the patient and reveals the characteristic personality traits. The second method is not directed at personality traits; rather, it categorizes PDs, distinguishing 10 different subtypes of PDs as well as depressive PD and passive aggressive PD, which are grouped into three clusters based on their descriptive similarities.[6,15]

Personality traits in JME

Impulsivity is a multidimensional and complex personality trait that influences the pathogenesis of the course and clinical severity of several disorders. A pathological increase in impulsivity is the reason for impulse control disorders and hence impulsive PDs.[16]

Patients with JME, compared to people without the disorder, have a higher expression of impulsive personality traits that demand early recognition to avoid further consequences and facilitate social insertion, consequently avoiding future stigma.[6]

S. Moschetta et al. made the first study addressing the social functioning of 42 patients with JME using SAS (a standardized instrument using objective questions about the subject's life) and demonstrated that JME patients had worse performance than controls, considering overall social adjustment, work, and family relationships. They demonstrated that higher seizure frequency and impulsive traits, but not cognitive performance, were correlated with worse social adjustment, unlike TLE, in which cognitive deficits are very relevant (attention and verbal memory dysfunction were correlated with social adjustment), therefore recognizing the impact of higher seizure frequency on social adjustment.[11]

P. Rzezak et al., taking into consideration the necessity of different neuropsychological approaches to distinct epileptic syndromes, studied impulsivity with the help of BIS-11. They compared 20 patients with JME, 20 with TLE, and 26 healthy controls and showed higher BIS scores in all domains of impulsivity (i.e., motor, attentional, and lack of planning) in JME patients.[17]

Impulsivity is a temperament feature that results from a combination of low harm avoidance and high novelty seeking, hence a high risk-seeking predisposition in subjects with a low level of temperamental inhibition leads to an impulsive temperament (which is a highly heritable personality trait). [6, 18])

E. Congton et al., in recent study, highlighted the heritability of impulsivity—familial transmission of impulsivity—within and outside the DSM-IV categories of mental disorders, which emphasizes the idea that the impulsivity trait may not be diagnosable by standard psychiatric investigation, as it may not represent a PD.[6,19]

The deficit of executive functions and decision-making behavior is also not always determined in conventional behavioral measures, but neuropsychological deficits were

detected in the imaging studies and more challenging frontal lobe tasks. Br. Wandschneider et al., explaining the risk-taking impulsive behavior of 21 patients with JME, suggested frontal lobe dysfunction. This was corroborated by reports on impaired working memory and executive functions, functional and microstructural changes within the medial and dorsolateral prefrontal cortex.[1] They characterized decision-making behavior in JME and its neuronal correlates using functional magnetic resonance imaging and concluded that there is a greater proportion of patients with seizures than seizure-free patients having difficulties making advantageous decisions, but no difference in performance between seizure-free patients and controls. Such results add further evidence that continuous seizure activity affects daily life decision making (sign of impulsivity).[20]

Poor impulse control results in inadequate behaviors and predicts greater difficulties in solving social problems and acquiring social skills. It has been shown that better impulse control is significantly associated with higher social functioning in JME patients.[11]

Nonpsychotic psychiatric disorders in JME

The 10 official PD subtypes, which are identified in the DSM-IV, are grouped into 3 clusters based on their descriptive similarities.[6] The patients with JME most often exhibit cluster B disorders—histrionic, narcissistic, antisocial, borderline—and are characterized by emotional instability, immaturity, lack of discipline, and rapid mood changes. Patients with these characteristics have been associated with a worse seizure outcome.[21]

The problem of the categorical method is that many patients who meet criteria for one PD may also meet criteria for other PDs in different clusters. Also, the method does not take into account the manifestations of impulsivity—the most frequent personality traits in JME.[19] However, the presence of personality traits may indicate a risk of developing personality disorders, since a combination of traits often leads to the manifestation of such disorders.

The patients with PDs cluster B often demonstrated the executive dysfunctions. J. Walsh et al., trying to find out why JME patients did not observe doctor's prescriptions, demonstrated that abnormal personality traits and psychiatric disorders are associated with worse intellectual and executive functioning, because half of the cohort of their patients exhibited moderate to severe anxiety symptoms. People with JME and higher neuroticism scores reported more anxiety symptoms and more concentration and motor difficulties compared to those with lower scores. Therefore, they concluded that specific patterns of executive dysfunction are related to maladaptive behavior in JME, the cause of which is anxiety.[5]

A. Coppola et al. studied psychiatric comorbidities in patients with ADCME and JME. They analyzed both state anxiety (reflecting various subjective and somatic manifestations of anxiety at a given moment) and trait anxiety (stable individual differences in anxiety proneness, a personality trait). State anxiety was more prevalent in ADCME patients compared to JME patients and healthy controls. However, the anxiety trait was prevalent in both patients with ADCME and patients with JME, compared to healthy controls, suggesting that it might be a common trait of patients with epilepsy. The myoclonus score positively correlated with both state and trait anxiety, which can be explained by the chronic excitation of the cerebello-thalamo-cortical circuits involving the basal ganglia. In addition, the correlation with the duration of the disease and not with the number of seizures confirmed the assumption. Therefore, the authors conclude that anxiety represents a consequence of cortical hyperexcitability.[22]

Devrimsel Harika Ertem et al. also compared JME with other types of epilepsy. They found that 37% of JME patients had comorbid psychiatric disorders, such as anxiety disorders (17%), mood disorders (13%) and psychotic disorders (7%). However, their results showed that there were no statistical differences in terms of type of the psychiatric disorders between JME and TLE, which suggested that psychiatric disorders might not be specific to the type of epileptic syndromes; however, such disorders often occurred and affected the quality of life, especially significantly attention and concentration problems in JME patients with a mood disorder.[23]

Sh. Somayajula et al. studied the association between clinical factors and the two major PDs (anxiety disorders and depressive disorders) in 165 JME patients and the implications for their psychosocial and socioprofessional integration. The 77(46.6%) patients were diagnosed with PDs; while 50 were categorized as having anxiety disorders, 27 patients had depressive disorders: depression is more common in the elderly, and anxiety is more common in young people. They concluded that lower emotional well-being was associated with anxiety disorders. It was shown that JME patients with depressive disorders have lower social functioning scores, especially patients with marital dissatisfaction and negative marital quality.[14]

Increased doses of AEDs, rapid initiation, and polytherapy entail an increased risk. In general, the negative effects of AEDs are less than the sum total of other factors and are usually reversible.[24] R. Thomas et al. examined 60 patients with drug-refractory JME and concluded that the patients were profoundly impaired across the range of tests evaluating intellectual function, language and naming, executive function, the impact of epilepsy, and AED side effects. Eighty-three percent of patients exhibited frank executive dysfunction, which was moderate to severe in 66%. A high prevalence of neurotoxicity symptoms, such as fatigue and poorer functioning across intellectual and memory tests, was also identified.[25]

Discussion

Persons with epilepsy and associated brain lesions have greater psychiatric comorbidity as compared to those without brain lesions.[26] However, JME is commonly associated with psychiatric manifestations, like depression, anxiety, attention deficit hyperkinetic disorder, conduct disorder and aggressive behavior.[12,27,28]

Depression in JME can be explained on the basis of

the bio-psycho-social model: biological (endocrine related effects of seizure; metabolic effects of seizure; adverse effects of antiepileptic drugs); psychological (personality factors, individual's perception and attitude towards epilepsy and its treatment); social (stigma attached to epilepsy. psychosocial support burden of treatment, employment related issues, compromised quality of life due to epilepsy).[29]

The cause of anxiety disorders in JME can be summarized as of neurobiological origin, psychosocial origin, iatrogenic origin (drug induced), or a combination of the above factors.[30,31] The results obtained in the study of O'Muircheartaigh et al. provide convincing evidence for abnormalities in a specific thalamo-cortical circuit, with reduced structural and task-induced functional connectivity, which may underlie the functional abnormalities in JME.[1] Dysfunction in these areas results in deficits of concept formation, abstract reasoning, planning and self-regulation of behavior, and control of impulsivity and emotions.[31] T. Chachua et al.[32] found a highly significant dominance trait (aggression) in *Brd2+/-* haploinsufficient mice compared with the wild type, more pronounced in females. *Brd2+/-* mice of either sex did not differ from wild-type mice in spatial learning and memory tests. Compared with wild-type littermates, it was found that there were decreased numbers of GABA neurons in the basolateral amygdala, which is consistent with the increase in aggressive behavior. *Brd2+/-* haploinsufficient mice showed no cognitive impairment but had behavioral traits similar to those found in JME patients (recklessness, aggression. [9, 32] It should be noted that Mendelian JME genes and non-Mendelian risk alleles have not been detected in over 90% of affected patients.[33,34]

In several patients with JME, AEDs contribute to the psychiatric manifestations; however, it does not mean that antiepileptic drugs need to be stopped. The focus should be on proper selection of AEDs after assessing the risks and benefits.[35]

Comorbid anxiety and depressive symptoms in patients with epilepsy adversely affects the quality of life.[36] Timely identification and appropriate intervention improves the quality of life of patients with nonpsychotic psychiatric disorder in epilepsy.[37]

Personality and nonpsychotic psychiatric disorder may be mild, moderate or severe, and patients with JME may have periods of "remission" where they function well. Mild disorders that do not seriously interfere with a person's ability to function socially are common. Severe disorders are rare in JME. However, whenever there is persistently impaired social functioning in conditions in which it would normally not be expected, the evidence suggests that this is more likely to be created by personality abnormality than by other clinical variables.[38] It is very important to understand this, because these disorders can significantly disrupt the lives of both the affected patient with JME and those who care about that person. The disorders may cause problems with relationships, work or school, and can lead to social isolation, alcohol or drug abuse, or seizure aggravation.

The management and treatment of personality and nonpsychotic psychiatric disorders can be a challenging and controversial area, for by definition the difficulties have been enduring and affect multiple areas of functioning in JME patients.[39,40]

This often involves interpersonal issues, and there can be difficulties in seeking and obtaining help from organizations in the first place, as well as with establishing and maintaining a specific therapeutic relationship. Unfortunately, there are substantial social stigma and discrimination related to the diagnosis of personality and nonpsychotic psychiatric disorder in JME patients. There is always a need to maintain appropriate professional-personal boundaries, while allowing for emotional expression and therapeutic relationships.

A comprehensive psychiatric evaluation should be offered at the time of diagnosis to detect these comorbidities and to treat them.[22] Different types of psychological therapies have been shown to help patients with JME and comorbidity PDs. There is no single approach that suits everyone; treatment should be tailored to the individual. Along with the professional treatment plan, we should discuss lifestyle and self-care strategies of patients with JME. Coping skills needed to adapt effectively to epilepsy are often lacking in many patients; this too needs to be improved in order to improve the quality of life.[41]

Competing interests

The authors declare that they have no competing interests.

Conclusion

Comorbid personality and nonpsychotic psychiatric disorders are a common and interdisciplinary problem in JME management. The disorders in patients with JME often go undiagnosed and hence untreated. Therefore, this problem requires further and extensive investigation. The neurologist–epileptologist should be able to identify the comorbid disorders and understand that liaising with mental health professionals is beneficial.

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International Journal of Biomedicine 7(2) (2017) 91-95
http://dx.doi.org/10.21103/Article7(2)_OA1

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Relationship Between Within-Visit Blood Pressure Variability and Kidney Function in Patients with Arterial Hypertension

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Abstract

The main purpose of this study was to investigate the features of within-visit blood pressure variability (BPV) in patients with arterial hypertension (AH) and to assess the relationship of those features to impaired renal filtration.

Material and Methods: The study included 120 patients with AH Stages I and II (ESH/ESC, 2013): 58(48.3%) men and 62(51.7%) women aged from 22 to 73 years (mean age 58.7±15 years). Average duration of AH was 15.0±6.7 years. BP was measured three times at 2-minute intervals during one visit. Within-visit BPV was evaluated by the presence of an absolute difference (AD) between any two readings of three SBP measurements of more than 5 mmHg. All patients underwent a comprehensive examination, which included blood and urine tests, kidney ultrasound, assessment of blood levels of creatinine and TnT, and calculation of GFR.

Results: Depending on the response of BP to repeated measurements, three types of BPV were identified. A prognostically unfavorable type of BPV, which is characterized by SBP-AD > 5 mmHg between the third and first measurements, was identified. Patients of this group had the lowest eGFR value that indicates more pronounced renal damage, and, as a consequence, worse prognosis. Also in these patients, there was an increase in TnT level, which is a predictor of the development of adverse cardiovascular complications. **(International Journal of Biomedicine. 2017;7(2):91-95.)**

Key Words: arterial hypertension • blood pressure variability • glomerular filtration rate • chronic kidney disease

Abbreviations

AH, arterial hypertension; **BP**, blood pressure; **BPV**, BP variability; **CKD**, chronic kidney disease; **Cr**, creatinine; **GFR**, glomerular filtration rate; **eGFR**, estimated GFR; **SBP**, systolic BP; **SBP-AD**, absolute difference in SBP; **TnT**, troponin T.

Introduction

AH is an important global health challenge because of its high prevalence and resulting cardiovascular disease and CKD.[1] Epidemiological studies have shown that in Russia AH is observed in 40.8% of the adult population (more than 42 million people).[2] In recent years, researchers have found that cardiovascular (CV) complications of hypertension depend not only on the absolute values of BP, but also on the degree of BPV.[3,4] Short-term BPV within a 24-hour period is increasingly recognized as both a marker and a risk factor for cardiovascular disease.[5-9] However, few studies have examined the importance of BPV measured during a single clinic visit (i.e., very-short-term BPV). Recent studies have demonstrated that within-visit BPV is associated with target organ damage (left ventricular hypertrophy and albuminuria).[10]

CKD, since the earliest stages, has been associated with a high risk of premature CV events.[11-13] Only a few studies have

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explored the relationship between short-term BPV/within-visit BPV and markers of early renal damage, with conflicting results.[10,14-19] It could be hypothesized that an enhanced within-visit BPV in patients with early renal abnormalities may help to explain in part their increased CV risk.

Currently, the most sensitive indicator of myocardial damage is the blood level of troponin. The troponin complex consists of three subunits: troponin T (tropomyosin binding), troponin I (inhibitory), and troponin C (calcium binding). TnT has the highest specificity for myocardial damage.[20] In several studies, it was noted that a stable increase in TnT level is often observed among patients with decreased renal function. Reduced renal clearance is probably not the primary mechanism of persistently elevated troponin levels in patients with CKD, although this issue is controversial.[21-24] Elevated troponin levels in patients with CKD may be explained by cardiac injury associated with chronic structural heart disease (such as coronary artery disease or heart failure) rather than acute ischemia, especially when levels do not change rapidly over time.[25] A recent study showed that an increased TnT level is associated with an increase in signs of heart failure.[26] In this case, even a slight increase in TnT level can be considered a negative sign in predicting adverse CV complications at the subclinical level.

The main purpose of this study was to investigate the features of within-visit BPV in AH patients and to assess the relationship of those features to impaired renal filtration.

Materials and methods

The study included 120 patients with AH Stages I and II. (ESH/ESC, 2013)[4]: 58(48.3%) men and 62(51.7%) women aged from 22 to 73 years (mean age 58.7±15 years). 20(16.67%) people were cigarette smokers. Average duration of AH was 15.0±6.7 years. Type 2 diabetes was identified in 32(26.67%) patients. 58 (48.34%) patients had myocardial infarction in anamnesis.

The diagnosis of AH was based on 2013 ESH/ESC Guidelines for the management of AH.[13] All patients were checked on office BP using Korotkov's method after a 5-min rest in the seated position with back support. BP was measured three times on the left arm at 2-minute intervals with the use of a validated semi-automated electronic device (UA-787). All measurements were carried out on the same time between 9:00 and 10:00. All patients underwent a comprehensive examination, which included blood and urine tests, kidney ultrasound, and assessment of blood levels of creatinine (Cr) and TnT. Blood samples were collected within 4-48 hours after admission.

Determination of serum TnT was performed on the Elecsys-2010 automatic analyzer Roche Diagnostics GmbH, Germany) using Elecsys Troponin T Stat Assay. The limit of detection (LOD) for this assay is 0.01 ng/ml. A cut-off of 0.1 ng/ml was used in assessing the prognostic significance of TnT.

GFR was estimated using the CKD-EPI (eGFR(CKD-EPI)) equation according to the recommendations of the National Kidney Foundation.[27,28] The CKD-EPI equation reclassifies people at lower risk of CKD and death into higher eGFR categories, suggesting more accurate categorization. [29]

The study was approved by the local ethics committee. Written informed consent was obtained from each patient. Statistical processing of data was carried out using the program SPSS 10.0 software. Correlation analysis was carried out using the non-parametric Spearmen criterion. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Within-visit BPV was evaluated by the presence of an absolute difference (AD) between any two readings of three SBP measurements of more than 5 mmHg. According to this criterion, we have identified several options for BPV. In 37(30.84%) patients, there was a systematic increase in BP by more than 5 mmHg in successive measurement (Response A). Reduction of BP during repeated measurements >5 mmHg was noted in 23(19.17%) patients (Response B). No difference > 5 mmHg between the measurements was observed in 24(20%) patients (Response C). In 36(30%) patients, multidirectional BP fluctuations were noted between the measurements (Response D) (Fig.1).

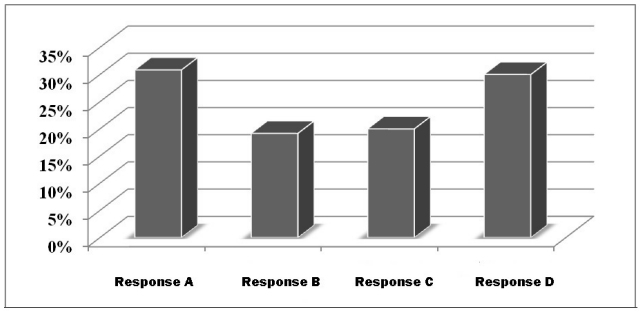

Fig. 1. *Frequency of the different BP responses during repeated measurements.*

Depending on the response of BP to repeated measurements, we identified three types of BPV. Type 1: SBP-AD is > 5 mmHg between the third and the first measurements; Type 2: SBP-AD is > 5 mmHg between the first and the third measurements. Type 3: SBP-AD is < 5 mmHg during repeated measurements. Analysis of BPV types allowed us to better analyze the body's response to repeated measurements and to evaluate the relationship with other indicators.

Different authors distinguished several types of short-term BPV;[3,30,31] however, we have not previously encountered in the literature an approach such as ours to the evaluation of the types of BP response to repeated measurements, which indicates the relevance and insufficient knowledge of this problem.

After GFR calculation, we determined CKD stages in our patients. Most frequent (46.7%) was Stage 2, next was Stage 3A (22.5%), then 3B (20%), Stage 1 (10%), and finally Stage 4 (0.83%) (Fig.2).

We also analyzed the distribution of CKD stages depending on the BPV type (Fig.3). CKD Stages 3A and 3B were more often observed in patients with BPV Type 1 (25%

and 33.34% of cases, respectively). Stage 2 had almost the same frequency distribution by BPV type. Stage 1 did not exceed 5 cases in each type; Stage 4 was registered in one patient with BPV Type 1.

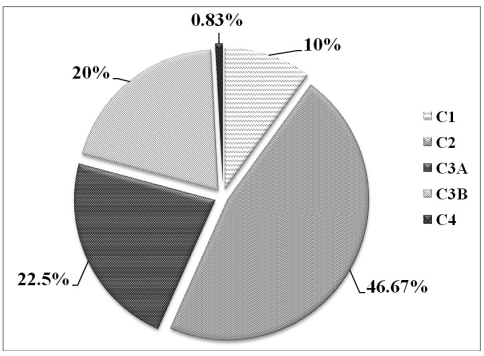

Fig. 2. *CKD stages in studied patients.*

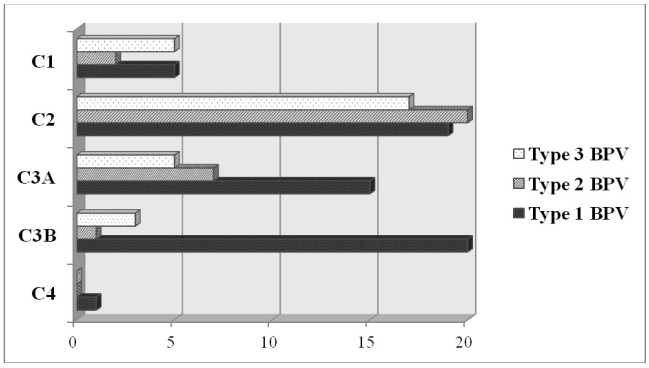

Fig. 3. *Distribution of CKD stages depending on the BPV type*

To establish the relationship between BPV and a number of parameters, we conducted a correlation analysis (Table 1). According to the data obtained, the greatest prognostic value belonged to BPV Type 1. This type was associated with decreased eGFR - up to Stage 3B.

Table 1.

Correlations between BPV types and clinical characteristics of patients.

BPV Type	Gender	Age	BMI	Cr	eGFR	Diabetes	TnT
1	-0.015 P=0.12	-0.09 P=0.21	0.36 P=0.03	0.62 P=0.001	-0.58 P=0.001	-0.14 P=0.27	0.48 P=0.0021
2	-0.019 P=0.21	-0.078 P=0.31	0.096 P=0.27	0.22 P=0.012	-0.19 P=0.021	-0.23 P=0.17	0.21 P=0.07
3	-0.024 P=0.28	-0.1 P=0.18	0.16 P=0.11	0.2 P=0.025	-0.14 P=0.023	-0.1 P=0.24	0.09 P=0.15

In the literature, there are few reports on the connection between the decrease in eGFR and BPV. One opinion is that this phenomenon can be associated with dysfunction of the endothelium.[32] As GFR decreases, the blood accumulates the inflammatory cytokines, lipid peroxidation products, and other vasoactive metabolites.[33,34] According to M. Zwolinska, in this condition, the synthesis of VCAM-1 is enhanced, triggering several reactions that cause endothelial dysfunction.[35]

When measuring serum TnT, we found that in patients with BPV Type 1, TnT level ranged between 0.1 ng/ml and 0.3 ng/ml (0.23±0.12 ng/ml), whereas in patients with other types of BPV, TnT level did not exceed the upper reference limit. This fact indicates a more significant lesion of the myocardium as a target organ in AH patients with BPV Type 1, which is associated with an unfavorable cardiovascular prognosis. During analysis, we found correlations between the increase in SBP during the first and third measurements, a decrease in eGFR, and an increase in TnT level in AH patients.

Conclusion

Thus, our results show that within-visit BPV may be one of the important criteria for assessing cardiovascular complications in hypertension. We have identified a prognostically unfavorable type of BPV, which is characterized by SBP-AD > 5 mmHg between the third and first measurements. Patients of this group had the lowest eGFR value that indicates more pronounced renal damage, and, as a consequence, worse prognosis. Also in these patients, there was an increase in TnT level, which is a predictor of the development of adverse cardiovascular complications.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 96-103
http://dx.doi.org/10.21103/Article7(2)_OA2

ORIGINAL ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Association Between IL1B and SCN1A Polymorphism and Febrile Seizures in Children in Siberia

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Abstract

Background: Febrile seizures (FS) are a benign, age-dependent, genetically determined state, in which the child's brain is susceptible to epileptic seizures occurring in response to hyperthermia. We assessed whether polymorphisms of *IL1B* and *SCN1A* genes, encoding the proinflammatory cytokine IL1B and SCN1A, respectively, could help to predict FS development and find a new way to treat FS.

Methods: We examined 121 children with FS and 30 children with hyperthermia syndrome (HTS) aged from 3 to 36 months. SNPs rs1143634 and rs16944 of *IL1B* gene, and rs3812718 and rs16851603 of *SCN1A* gene were determined by quantitative real-time PCR.

Results: The analysis for rs1143634 revealed an association between the *CC* genotype and increased risk of FS development (OR 6.56; P=0.0008) against the background of acute respiratory viral infection. The same result was obtained for rs16944 (OR 3.13; P=0.04) and an association of two homozygous genotypes *CC*/*CC*. For rs3812718, the carriage of heterozygous genotype *CT* demonstrated a direct relationship with FS development (OR 44.95; P=0.000).

Conclusion: Children with high FS risk need preventive treatment and joint observation of a pediatrician, pediatric infectionist, and a neurologist-epileptologist. **(International Journal of Biomedicine. 2017;7(2):96-103.)**

Key Words: febrile seizures • *IL1B gene* • *SCN1A gene* • single nucleotide polymorphisms

Abbreviations

CNS, central neural system; **GEFS+**, generalized epilepsy with febrile seizures plus; **EDTA**, ethylenediaminetetraacetic acid**; GABA**, gamma aminobutyric acid; **HWE**, Hardy-Weinberg equilibrium; **HHV-6**, human herpes virus type 6; **HSV**, herpes simplex virus; **HTS**, hyperthermia syndrome; **IL1RA,** interleukin1 receptor antagonist; **IL1B,** interleukin-1 beta; **PCR-RT**, polymerase chain reaction real time; **SCN1A**, sodium voltage-gated channel alpha subunit 1; **SNPs**, single nucleotide polymorphisms.

Introduction

Febrile seizures (FS) are a benign, age-dependent, genetically determined state, in which the child's brain is susceptible to epileptic seizures occurring in response to hyperthermia. FS are the most common variant of paroxysmal states in pediatric practice. FS are a transient condition but may be considered as the debut of the different epileptic syndromes. FS prevalence in the pediatric population is 2%-5% but there is an increased rate in some of geographic regions where it reaches 14%.[(1)]

Despite the widespread occurrence of FS in childhood, the reasons for their development are still subject to debate.

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Many authors believe that along with such factors as perinatal CNS damage and infectious diseases, the development of FS occurs as a result of the instability and immaturity of neuronal membranes. Indeed, in childhood the membrane of the CNS neuron becomes easily susceptible to the pathological effect of elevated temperature and, thus, reacts by violating the structure and functional properties, which can contribute to epileptogenesis.[2] On the other hand, the development of FS can be genetically determined.[3]

The family history is one of the important risk factors in the development of FS—the more relatives have suffered or are suffering from a given state, the higher is the risk of developing FS in the proband and siblings. Earlier research confirms the relationship between family susceptibility and a high risk of developing FS. As shown, a positive family history of FS can be found in 25%-40% of patients.[4]

FS are a multifactorial disease whose implementation depends on other genes (modifying the course of FS) as well as environmental factors. Recent studies show that the differences in the genes that control defensive reactions of the organism can affect the level of production of the encoded proteins and, thereby, the character of immune response.[5] In this regard, a functional polymorphism of cytokine genes is of great interest, since these proteins contribute most to the regulation of immunity, in epilepsy as well.[6-8] The most frequent causes of differences in gene structure are point mutations, tandem repeats of parts of the gene, and deletions of nucleotides or small gene fragments. The mutant fragments of one or several genes, located very close to each other, can be inherited together (coupling) as the result of selective advantages or, more typically, because of the irregularities of meiotic recombination in this chromosomal region.[5-7] Multiple animal studies have shown that the components of the immune response can play some role in FS pathogenesis.

On the other hand, the analysis of data found in the literature has shown that the development of generalized forms of epilepsy, both with and without FS—including the formation of pharmacoresistant forms and adverse drug reactions related to the administration of sodium channel blockers, as well as the development of medial temporal sclerosis—can be associated with polymorphism rs3812718 (*166909544C>T*) of *SCN1A* gene encoding SCN1A. Nevertheless, we have not found studies of the association of this polymorphism with FS development in young children in the available literature, although there are reports of the possible association of this polymorphism with the risk of GEFS+ development.[3,9]

Based on these findings, one can suppose that polymorphisms of *IL1B* and *SCN1A* genes, encoding the proinflammatory cytokine IL1B and SCN1A, respectively, are one of the factors that can help determine FS development and further help in finding a new way of FS treatment.[10] **The aim** of the study was the investigation of frequency for high-producing alleles of the IL1B- and SCN1A genes polymorphisms in children with FS.

Material and Methods

Patients

Our investigation was observational, continued (retrospective, prospective), and case-control designed. The study was approved by the Ethics Committee of V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University (Protocol №52/2013 from 27.11.2013). All patients were included in the present study after a voluntary informed consent was signed by legal representatives of a child (parents or guardians). Patients were enrolled in accordance with methods of stratified randomization using inclusion and exclusion criteria. The inclusion criteria were typical FS against the background of acute respiratory infection; both genders; age 3-36 months; Caucasian race; place of residence – Krasnoyarsk. The exclusion criteria were age under 3 months and older 3 years, acute neuroinfection, atypical FS, epilepsy or epileptical syndromes, and congenital brain malformations or cerebral palsy.

A total of 151 children were examined. The main group consisted of 121 children (73 males and 48 females) between the ages of 3 and 36 months. All children were hospitalized in the Krasnoyarsk inter-district children's hospital #1 with acute respiratory viral infection (ARVI), complicated by FS, for the period from October 2013 to September 2014. The control group consisted of 30 children (10 males and 20 females) with HTS against the background of ARVI without FS in anamnesis.

The study of etiological structure of FS

Along with routine methods of laboratory diagnostics, we detected for all patients the markers of influenza virus, adenovirus, parainfluenza virus, and for randomly selected 62 patients from the main group, the markers of herpesvirus infection (HSV types 1 and 2, CMV, and HHV-6). Specific classes of IgG and IgM in blood serum with determination of the avidity index were revealed by immunosorbent assay (ELISA; "Vector-best", Russia) using a chemistry analyzer STAT FAX 3300 (Awareness Technology, USA). DNA of listed viruses was detected in blood, urine, and nasopharyngeal mucus by PCR-RT.

Detection of IL1B and SCN1A genes polymorphisms by PCR-RT

From each patient, 2mL of peripheral blood were drawn into an EDTA tube. Genomic DNA was extracted from 0.15mL samples by using a DNA-sorb-B kit (K1-2-100-CE, AmpliSens), according to the manufacturer's instructions. Two SNPs for *IL1B* gene rs1143634 (*c.3954C>T*) and rs16944 (*c.-511C>T*) and two SNPs for *SCN1A* gene rs3812718 (*166909544C>T*) and rs16851603 (*166991436C>T*) were determined by quantitative real-time PCR using "Rotor-Gene 6000" (Corbet Life Science, Australia). Genotyping was performed using TaqMan allele discrimination technology and commercially available TaqMan probes (Applied Biosystems, USA). PCR master mix contained 2.5x reaction mix for PCR-RT, 25 mM $MgCl_2$, ddH_2O (M-428; Syntol). PCR-RT conditions were as following: 95°C – 10 min; 92°C – 15 c, 60°C – 90 c (40 cycles). To designate the genotype variants, the following designations were taken: homozygous low-producing genotype - *TT* (thymine/thymine), heterozygous genotype for high-producing allele - *CT* (cytosine/thymine),

homozygous genotype for high-producing allele - *CC* (cytosine/cytosine).

Statistical analysis was performed using STATISTICA 7.0 (StatSoft, USA) and SPSS 22.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. The genotype frequency distribution for each variant was separately tested for Hardy-Weinberg equilibrium (HWE) with a chi-square test in the patient and control groups. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results

Description of population

The main group of patients (mean age, 21.69±10.32 months) with FS consisted of 60.3% boys and 39.7% girls. The control group (mean age, 14.63±8.24 months) consisted of 66.7% girls and 33.3% boys. For the main group, the first FS episode was determined in 57.9% of examined patients, whereas in 42.1% of children there was a recurrent course of FS. Boys were predominant over girls among patients with recurrent FS. Interestingly, the greatest number of FS patients (80.2%) was registered at the age of more than 12 months, and in 13.2% of cases, the debut of FS development occurred at the age of 36 months. Studying the family history, we have found that 31.4% of FS children had a genetic predisposition. Thus, FS at the age up to 3 years were observed in proband's parents in 28.9% of cases, and a family history of epilepsy in 2.5% of the children. However, in view of the lack of information about hereditary anamnesis, since the vast majority of parents (98.3%) could not clarify the data of the family history of FS in the pedigree of more than the second generation, one can only assume that the contribution of genetic predisposition to FS development in examined children was significantly higher. Studying the history, we did not discover any statistically significant differences in the influence of perinatal factors between the comparison groups. For the main group, the frequency of ARVI reached 4-5 or more episodes per year in 68.6% of children, whereas in the control group the number of ARVI episodes did not exceed 4-5 per year ($P<0.05$).

The study of etiological structure of FS

All patients from the main and control groups were examined using serological tests to identify the etiology of the disease. ARVI was revealed in 86.8% of patients from the main group, and only 13.2% of children had no markers of viral infection according to available diagnostic methods. Etiological structure of ARVI in the main group was represented by the following pathogens with a statistically insignificant difference within the group ($p>0.05$): influenza A (H3N2) virus was found in 37.2% (45/121) of patients, human orthopneumovirus was registered in 23.1% (28/121) of cases, adenovirus was determined in 12.4% (15/121), and parainfluenza virus type 2 caused the disease in 14.0% (17/121) of children with FS.

In the control group, respiratory virus markers were determined in 83.3% (25/30) of cases, particularly, human orthopneumovirus was identified in 46.7% (14/30) of children, adenovirus and parainfluenza virus type 2 were revealed in 30.0% (9/30) and 6.7% (2/30) of patients, respectively, and in 16.7% (5/30) of cases, the markers of a viral infection were not detected.

In addition, 62(51.2%) randomly selected children from the main group (54.8% (34/62) of children with the first FS episode and 45.2%(28/62) of patients with recurrent course of FS) were tested for the presence of herpesvirus markers. Markers of one, two, and more representatives of herpes viruses were revealed in 48.4%, 24.2%, and 6.5% of examined children, respectively.

High titers of IgG antibodies to HHV-6 were identified in 38.7% (24/62) of children having herpesviruses markers, and the optical density of samples was up to 1.07±0.38 (critical OD=0.333). IgG antibodies against HHV-6 were determined in 46.4% (13/28) of examined patients with recurrent FS, more often than antibodies against other investigated herpes viruses.

Serum IgG antibodies against HSV-1 and 2 were found in high titers in 17.7% (11/62) of herpesvirus carriers. The avidity index of antibodies against HSV-1 and 2 was 87.25±14.76%, indicating the prolonged persistence of the virus in the child's organism. At the same time, DNA of HSV-1 and 2 was detected in nasopharyngeal mucus in only 3.2% (2/62) of FS patients. However, the absence of DNA of HSV-1 and 2 in nasopharyngeal mucus cannot be taken to mean that the virus is absent in the organism. It is also important to note, that the persistence of HSV-1 and 2 was observed in 35.7% (10/28) of examined children with recurrent course of FS.

The IgG antibodies against CMV were detected in high titers in serum of 46.8% (29/62) of FS patients. Anti-CMV-IgG avidity index was 83.2±7.61% that indicated a prolong persistence of the pathogen. The exacerbation of CMV-infection was determined in 10.3% (3/29) of children with CMV markers, while the avidity index turned out to be equal to 81.1±3.2%. Only 3.4% (1/29) of patients had low-avidity antibodies, confirming early and acute courses of CMV-infection. The CMV DNA was detected in the urine of 24.2% (15/62) of examined children, and in 4.8% (3/62) of them the IgM antibodies were found in serum together with CMV DNA, indicating an acute CMV-infection. Among all CMV carriers, the first FS episode was observed in 58.6% (17/29) of cases, other patients (41.4%; 12/29) had a recurrent course of FS.

IL1B gene polymorphisms distribution

Studying SNPs markers rs16944 (*-511C>T*) and rs1143634 (*3954C>T*) of *IL1B* gene, we have found the prevalence of homozygous and heterozygous genotypes over high-producing allele *C* in both comparison groups, and the frequency of the heterozygous genotype was statistically significantly higher in the control group (children with HTR) ($P<0.05$) (Table 1).

Analysis of allele frequency for rs16944 (*-511C>T*) has shown that in the main group the frequency of allele *C* (wild type) was statistically significantly higher: 69.8% compared to 55.0% in the control group (OR=1.89, 95% CI: 1.06–3.38;

P=0.03). At the same time, the frequency of allele *T* in the main group was statistically lower: 30.2% versus 45.0% in the control group (OR=0.53, 95% CI: 0.30–0.94; P=0.03).

For allele frequency of rs1143634 (*3954C>T*), a statistically significant prevalence of high-producing allele *C* (70.2% versus 50.0%) was found in the main group (OR=2.36, 95% CI: 1.33–4.20; P=0.003). On the contrary, the frequency of low-producing allele *T* was significantly higher in the control group compared to the main one (50.0% versus 29.8%; OR=0.42, 95% CI: 0.24–0.75; P=0.003). Based on these findings, the carriage of allele *C* can be considered as a risk factor for FS formation for both SNPs (rs16944 and rs1143634).

Table 1.

Gender-specific genotype distribution of IL1B gene polymorphisms in the main and control groups.

SNP	Genotype	Main group			Control group		
		Gender, n (%)		Total n (%)	Gender, n (%)		Total n (%)
		Males n=73	Females n=48		Males n=10	Females n=20	
rs16944 (-511C>T)	CC*	32 (43.8)	27 (56.2)	59 (48.8)	2 (20.0)	5 (25.0)	7 (23.3)
	CT*	37 (50.7)	14 (29.2)	51 (42.1)	6 (60.0)	13 (65.0)	19 (63.3)
	TT*	4 (5.5)	7 (14.6)	11 (9.1)	2 (20.0)	2 (10.0)	4 (13.3)
rs1143634 (3954C>T)	CC*	33 (45.2)	18 (37.5)	51 (42.1)	1 (10.0)	2 (10.0)	3 (10.0)
	CT*	39 (53.4)	29 (60.4)	68 (56.2)	8 (80.0)	16 (80.0)	24 (80.0)
	TT*	1 (1.4)	1 (2.1)	2 (1.7)	1 (10.0)	2 (10.0)	3 (10.0)

** - P<0.05 for total genotype frequency between the main and control groups*

Analyzing the genotype distribution for SNP rs16944 (*-511C>T*), we have shown statistically significant differences between the main and control groups, according to the multiple inheritance model (χ^2=6.32, P=0.04). Thus, the carriage of homozygous genotype *CC* demonstrated a direct relationship with the probability of an outcome, which in our study was FS development (OR=3.13, 95% CI: 1.25–7.83; P=0.04). On the contrary, the carriage of heterozygous genotype *CT* can be considered as a protective factor (OR=0.42, 95% CI: 0.18–0.96; P=0.04). The influence of the carriage of homozygous genotype *TT* to FS development was not statistically significant (OR=0.65, 95% CI: 0.19–2.21). In addition, the recessive inheritance model (*CC* vs. *CT+TT*) also showed significant differences in genotype distribution between comparison groups (χ^2=6.32, P=0.01).

For SNP rs1143634 (*3954C>T*), statistically significant differences between the main and control groups were determined according to the multiple inheritance model (χ^2=14.24, P=0.0008). The carriage of homozygous genotype *CC* was characterized by direct relationship with the high probability of FS development (OR=6.56, 95% CI: 1.89–22.80; P=0.0008), whereas the carriage of both heterozygous genotype *CT* and homozygous genotype *TT* can be considered as protective factors (OR=0.32, 95% CI: 0.12–0.84 for *CT*; OR=0.15, 95% CI: 0.02–0.95 for *TT*; P=0.0008).

We determined the HWE was stable in carriers of *IL1B* gene polymorphism (*c.-511C>T*) in the main group and moderate in the control one (χ^2=0.00, p>0.05 for the main group; χ^2=2.34, P>0.05 for the control group). At the same time, in the group of children with FS and the control group the unstable HWE of *IL1B* gene polymorphism (*c.3954C>T*) genotypes was indicated (χ^2=14.36, P<0.05 for the main group; χ^2=10.8, p<0.05 for the control group). Thus, if all the conditions of genetic equilibrium are kept, in subsequent generations it will be possible to observe the stable carrier state (*c.-511C>T*) of polymorphism genotypes and thus predict the outcome of the disease.

Interesting data were obtained by studying the associations of polymorphic allelic variants (*c.-511C>T*, *c.3954C>T*) for *IL1B* gene (Table 2). We have shown that 14.9% (18/121) of FS children were homozygous carriers of the association of genotypes on two high-producing *IL1B* gene allelic variants (*-511CC/3954CC*), and the frequency of this association was higher in boys (66.7%) than in girls (33.3%), but gender differences did not reach statistical significance (Table 2, Fig.1). In the control group, the association of homozygous variants was not detected. It is also noted that in the group of patients who were carriers of the *-511CC/3954CC* association, the recurrent course of FS was observed more frequently than the first episode of FS (61.1% (11/18) vs. 38.9% (7/18), P<0.05), and the disease proceeded against the background of exacerbation of the latent form of CMV-infection (mean avidity index was 70.8±2.5%).

Table 2.

The frequency of associations of polymorphic allelic variants for IL1B gene in the main and control groups.

Group	Association of polymorphic allelic variants for IL-1β gene rs16944 (c.-511C>T) / rs1143634 (c.3954C>T) n (%)								
	CC/CC	CC/CT	CC/TT	CT/CC	CT/CT	CT/TT	TT/CT	TT/CC	TT/TT
MG	18 (14.9)*	39 (32.2)	2 (1.6)	29 (24.0)*	20 (18.2)	0 (0.0)	0 (0.0)	4 (3.3)	7 (5.8)*
CG	0 (0.0)	7 (23.3)	0 (0.0)	3 (10.0)	11 (36.7)	0 (0.0)	5 (16.7)	4 (13.3)	0 (0.0)

*MG - Main group; CG - Control group; * - P<0.05 vs. the control group*

In the main group, the *-511CC/3954CT* association occurred in 32.2% of children, and the numbers of boys and girls were approximately equal. In the control group, the frequency of this association did not exceed 23.3% with statistically significant prevalence among girls (71.4%), P<0.05). The frequencies of *-511CT/3954CC* and *-511CT/3954CT* associations were up to 24.0% and 18.2%,

respectively, with a statistically significant predominance of boys in the group of patients with FS development. In the control group, there was a predominance of these associations among girls (P<0.05) (Table 2, Fig.1). At the same time, the frequency of homozygous carriers of the association of low-producing allelic variants (*-511TT/3954TT*) in children with FS was low (5.1%), and more frequent in girls than in boys; gender differences did not reach statistical significance. It is important to note, that in the control group the association of low-producing allelic variants (*-511TT/3954TT*) was not observed (Fig. 1).

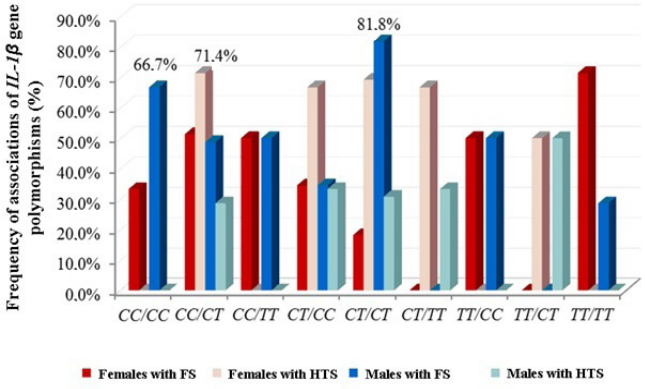

Fig. 1. *Gender-specific distribution of frequency of IL1B gene polymorphisms (rs16944 [c.-511C>T] and rs1143634 [c.3954C>T]) in two groups.*

In the study of the relationship between the persistence of herpes viruses and the presence of polymorphisms of the gene *IL1B* in young children with FS, it was found that the persistence of CMV-infection was most frequently revealed in the carriers of homozygous genotype for high-producing allelic variant *3954CC* (Table 3). In general, observed children with FS were characterized by a predominance of homozygous genotype on high-producing polymorphic allelic variants of the *IL1B* gene in association with herpesviruses persistence.

Table 3.

The frequency of herpes virus persistence and Il1B genotypes in children with FS (n=62)

Herpesvirus	rs1143634 (c.3954C>T) n (%)			rs16944 (c.-511C>T) n (%)		
	CC	CT	TT	CC	CT	TT
HSV-1 and 2	2 (3.2)	0 (0.0)	2 (3.2)	1 (1.6)	0 (0.0)	0 (0.0)
CMV	20 (32.3)	12 (19.4)	0 (0.0)	18 (29.0)	10 (16.1)	2 (3.2)
HHV-6	11 (17.7)	15 (24.2)	0 (0.0)	16 (25.8)	12 (19.4)	3 (4.8)
Mixt-persistence (IgG to 2 and more herpesviruses)	9 (14.5)	7 (11.3)	1 (1.6)	8 (12.9)	7 (11.3)	2 (3.2)

The results allowed us to form a group of those at risk of FS development that depends on the presence of polymorphic allelic variants of the *IL1B* gene (Table 4), which is important both for dispensary observation of children with FS and for treatment and prevention tactics. Thus, the management tactics for the children with FS, from the viewpoint of a personalized approach, should be different.

Table 4.

Recurrent FS risk groups depending on the IL1B gene allelic variants

Degree of risk	Group characteristics
Low-risk group	Children having association of homozygous genotypes on two low-producing allelic variants (-511TT/3954TT) of IL1β gene polymorphisms
Moderate-risk group	Children having association of heterozygous genotypes on one (-511CT or 3954CT) or two allelic variants (-511CT/3954CT) of IL1β gene polymorphisms
High-risk group	Children having association of homozygous genotypes on one (-511CC or 3954CC) or two high-producing allelic variants (-511CC/3954CC) of IL1β gene polymorphisms

SCN1A gene polymorphisms distribution

Analyzing the genotype frequency of *SCN1A* gene polymorphisms, we determined the prevalence of heterozygous genotypes with statistically significant differences between groups (P<0.05) (Table 5).

Table 5.

The genotype frequency of SCN1A gene polymorphisms in the main and control groups.

SNP	Genotype	Main group n (%)	Control group n (%)	P value
rs3812718	CC	2 (1.7)	15 (50.0)	P<0.05
	CT	118 (97.5)	14 (46.7)	
	TT	1 (0.8)	1 (3.3)	
rs16851603	CC	27 (22.3)	10 (33.3)	P>0.05
	CT	86 (71.1)	20 (66.4)	
	TT	8 (6.6)	0 (0.0)	

Analyzing the allele distribution for rs3812718, we have shown that in the main group the frequency of allele *C* (wild type) was statistically significantly lower and constituted to 50.4% compared to 73.3% in the control group (OR=0.37, 95% CI: 0.20–0.69; P=0.001). At the same time, the frequency of low-producing mutant allele *T* in the main group was statistically significantly higher and made up 49.6% versus 26.7% in the control group (OR=2.70, 95% CI: 1.45–5.05; P=0.001).

For allele frequency of rs16851603, insignificant prevalence of allele *C* (66.7% versus 57.9%) was found in the control group (OR=0.69, 95% CI: 0.38–1.24; P>0.05). The frequency of mutant allele *T* was insignificantly higher in the main group compared to the control one (42.1% versus 33.3%; OR=1.46, 95% CI: 0.80–2.64; P>0.05).

Analyzing the genotype distribution for SNP rs3812718, we have determined high statistically significant differences between the main and control groups according to the multiple inheritance model (χ^2=58.16, P=0.000). Thus, the carriage of heterozygous genotype *CT* demonstrated a direct relationship with the probability of an outcome, which in our study was FS development (OR=44.95, 95% CI: 11.63–173.73; P=0.000). On the contrary, the carriage of homozygous genotype *CC* can be considered as a protective factor (OR=0.02, 95% CI: 0.00 – 0.08; P=0.000). The influence of the carriage of homozygous genotype *TT* to FS development was not statistically significant (OR=0.24, 95% CI: 0.01 – 3.98).

For SNP rs16851603, we have not found statistically significant differences between the main and control groups according to the multiple inheritance model (χ^2=3.24, P=0.2). OR and 95% CI for the carriage of genotypes *CC, CT, and TT* were 0.57 and 0.24–1.37, 1.23 and 0.52–2.89, and 4.57 and 0.26–81.39, respectively.

The genotype distribution for SNP rs381271 (*166909544C>T*), located in the splice-donor site of *SCN1A* exon 5N, showed the stable HWE in the control group and unstable HWE in the main group (χ^2=109.33, P<0.05 for the main group; χ^2=1.12, P>0.05 for the control group).. We also detected the unstable HWE for genotype frequencies of SNP rs16851603 (*66991436C>T*), located in the intron of *SCN1A* gene, both in the main group (χ^2=25.32, P<0.05)and in the control group(χ^2=7.50, P<0.05). Thus, if all the conditions of genetic equilibrium are kept, in subsequent generations it will be possible to observe the stable carrier state of studied SNPs and thereby predict the outcome of the disease.

Analyzing associations of genotypes of studied *SCN1A* gene polymorphisms, we determined a statistically significant prevalence of association represented by homozygous genotypes over wild-type alleles (*166909544CC/166991436CC*) in the control group of 13.3% versus 1.7% in the main group (P<0.05). Moreover, in the main group this association was found only among girls (table 6). It was also noted that in the main group the frequency of association of homozygous genotypes by the *C* allele (*166909544CC*/*166991436CC*) was insignificantly higher among children with the first episode of FS (1.7%) compared to children with the recurrent FS course, and in children with a recurrent course of FS, the incidence of heterozygous genotypes was observed in more than a third of cases (38.8%). In the main group, the genotype association (*166909544CC*/*166991436CT*) was not found in any case, while in the control group the frequency of this association was 20.0% (P<0.05). The frequency of the *166909544CT*/*166991436CC* association among children with FS was 21.5%, and the association of heterozygous genotypes (*166909544CT*/*166991436CT*) was determined in 70.2% of cases with a statistically significant prevalence among boys compared to girls (68.1% vs. 31.9%, respectively, P<0.05) (Table 6). It should be noted that patients with the *166909544CT*/*166991436CT* association had the recurrent course of FS in 55.3% of cases. The association of homozygous genotypes by the *T* allele (*166909544TT*/ *166991436TT*) was not found in both comparison groups. However, the frequency of the *166909544TT*/*166991436CT* association was significantly higher in the control group compared to the main one (23.3% vs. 0.8%, respectively, P<0.05).

Table 6.

Gender-specific distribution of associations of polymorphic allelic variants for SCN1A gene in the main and control groups.

Gender	Association of polymorphic allelic variants of SCN1A gene rs3812718 (166909544C>T) / rs16851603 (66991436C>T) n (%)							
	CC/CC	CC/CT	CT/CC	CT/CT	CT/TT	TT/CC	TT/CT	TT/TT CC/TT
Main Group								
F	2 (1.7%)	0 (0.0%)	12 (9.9%)	27 (22.3%)	6 (5.0%)	0 (0.0%)	1 (0.8%)	0 (0%)
M	0 (0.0%)	0 (0.0%)	14 (11.6%)	58 (47.9%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0%)
Total	2 (1.7%)*	0 (0.0%)*	26 (21.5%)	85 (70.2%)*	7 (5.8%)	0 (0.0%)*	1 (0.8%)*	0 (0%)
Control group								
F	2 (6.7 %)	5 (16.7%)	2 (6.7%)	5 16.7%	0 (0.0%)	2 (6.7 %)	4 (13.3%)	0 (0%)
M	2 (6.7%)	1 (3.3%)	1 (3.3%)	2 (6.7%)	0 (0.0%)	1 (3.3%)	3 (10.0%)	0 (0%)
Total	4 (13.3%)	6 (20.0%)	3 (10.0%)	7 (23.3%)	0 (0.0%)	3 (10.0%)	7 (23.3%)	0 (0%)

*F- Females; M - Males; * - P<0.05 vs. the control group without taking into account the gender.*

Thus, the data analysis showed statistically significant differences between the frequency of both homozygous and heterozygous genotypes of studied SNPs (rs3812718 and rs16851603) in FS children, compared to children without FS who suffered from HTS (P<0.05).

For carriers of rs16851603, the risk of FS development in the main group was statistically significant higher and made up 1.14 vs. 0.28 in the control group, respectively (OR=4.12, 95% CI: 1.96–8.69; P<0.05). It was also found, that in the main group the risk of FS development was statistically significantly higher in heterozygous carriers of rs3812718 and constituted to 7.86 vs. 0.32 in the control group, respectively (OR=24.44, 95% CI: 9.46–63.14; P<0.05).

In view of the foregoing, it can be argued that polymorphisms rs16851603 and rs3812718 of the *SCN1A* gene are prognostically unfavorable biological risk factors (predictors) for FS development. At the same time, children carrying the polymorphic allele *166909544T* (rs3812718) are at greatest risk for FS development, which is consistent with previous studies showing the role of this locus in the GEFS+

development and other idiopathic epilepsies in a follow-up period.[9]

Discussion

The proinflammatory cytokine IL1B belongs to a large family of cytokines, including also proinflammatory cytokine IL1A and anti-inflammatory IL1RA. IL1B modulates cell proliferation, induces other cytokines,[10] and can serve as an endogenous pyrogen.[11] It is important to note that bacterial lipopolysaccharides stimulate production of IL1B, not only by macrophages on the periphery, but also by microglia, astrocytes and some neurons of the CNS. Increased IL1B level due to infection process leads to increased CNS hyperexcitability and excitotoxicity through Ca^{2+}, glutamatergic, and GABAergic mechanisms.[12] The *IL1B* gene, encoding proinflammatory cytokine IL1B, plays a role in the inflammatory response and fever development,[11] and is a genetic predictor of mesial temporal sclerosis and symptomatic temporal mediobasal epilepsy.[7,13,14] Our investigation of rs1143634 polymorphism of *IL1B gene*, demonstrating a high risk of FS development in carriers of high-producing allele *C*, is consistent with the data in the literature, according to which the *CT* genotype of rs1143634 leads to a higher IL1B, cerebrospinal, fluid/serum ratio and is associated with increased risk of developing posttraumatic epilepsy.[15] Moreover, the data shown by Diamond and coauthors, suggests that *TT* homozygotes are relatively protected from posttraumatic epilepsy,[15] which in our study corresponds to the low risk of FS development.

Some researchers hold the opinion that an imbalance in the levels of pro- and anti-inflammatory cytokines during the infectious process can contribute to FS development.[16] In our study, we paid special attention to revealing the etiological role of infectious pathogens, especially herpes viruses, in FS development. According to the literature, about a third of all FS in children are associated with the persistence of HHV-6.[17] We discovered IgG antibodies against HHV-6 in 46.4% (13/28) of examined patients with recurrent FS, more often than antibodies against other investigated herpes viruses. It is well known that maximum concentration of this virus is found in the most epileptogenic areas of CNS, particularly, in the temporal lobe and adjacent regions of the brain, where HHV-6 causes dysfunction of astroglia. Epileptogenic effect on sensitive neurons in the hippocampus ultimately leads to sclerosis of its structures with the subsequent development of mediobasal temporal epilepsy.[17] Thus, the prolonged persistence of HHV-6 in the child's organism can be considered as a trigger for the development of recurrent FS. We have shown that the persistence of CMV-infection was most frequently revealed in the carriers of a homozygous genotype for high-producing allelic variant *3954CC*. The mention of CMV in the development of convulsive states was noted in single articles, but generalized data on the role of this pathogen in FS development were not found.[18]

An important role in FS development is given to the change in the electrical membrane potential of neuronal, voltage-dependent ion channels. Some stuties have suggested that a moderate violation of the permeability of sodium channel NaV1.1, as a result of mutation or SNP of *SCN1A* gene, can predetermine FS development,[19] especially in family cases. Normally, RNA encoding NaV1.1, NaV1.2 and NaV1.3 channels undergoes a controlled change in the alternative splicing of exon 5, which has a striking effect on the activation of a potential-dependent sodium channel.[20] Regulation of this alternative splicing is interrupted due to SNP rs3812718, which is associated with a change in the response to antiepileptic drugs and the risk of FS development in European population. [21, 22] In our research into the rs3812718 allele and genotype distribution, the frequency of the low-producing mutant allele *T* in the main group was statistically significantly higher (P=0.001). At the same time, the carriage of heterozygous genotype *CT* demonstrated a direct relationship with FS development (OR=44.95; P=0.000). An interesting opinion was discussed by M.Hong that heterozygous associations for genetic variation for genes coding membrane receptors can occur and may result in multiple actions that affect the phenotype of interest (e.g., differential effects of membrane trafficking for receptor protein heterodimers vs. homodimers). [15]

In general, FS are a common multifactorial disease, and only the interaction of genetic and environmental factors can induce FS development.[23] Despite the fact that the issues of treatment and prevention of FS have been discussed for several decades, many aspects of genetics and preventive measures of FS recurrences and their transformation into afebrile epileptical seizures remain debatable.

Conclusion

Our research complements the previously published data on the problem of FS and confirms the feasibility of a personalized approach to the management of children with this pathology. This approach allows us to optimize the tactics (clinical examination, treatment, primary and secondary prevention) for managing young children with FS and to reduce the risk of symptomatic focal epilepsy in a follow-up period.[13] We propose that children with FS who are both carriers of the association of high-producing allelic variants of *IL1B* gene and the active herpesvirus mixed-infection need the preventive treatment for FS and joint observation by a pediatrician, a pediatric infectionist, and a neurologist-epileptologist. In addition, obtained data indicate the importance of detecting rs3812718 polymorphism in children with FS and the necessity of dispensary observation of them by a children's neurologist (or a neurologist-epileptologist in the presence of indications), along with conducting video electroencephalography monitoring for timely diagnosis of the debut of afebrile seizures and personalized selection of antiepileptic drugs with the rejection of sodium channel blockers due to the previously shown pharmacoresistance to this group of drugs in the carriers of allele *166909544T* (rs3812718) and high risk of AED-induced seizure aggravation.

Competing interests

The authors declare that they have no competing interests.

Sources of funding

This study was funded by the Krasnoyarsk Regional Fund for Support of Scientific and Scientific-technical Activity" (additional agreement №03/15 from 12.05.2015).

Acknowledgements

We would like to thank A.V. Muravyova, Department of Medical Genetics at V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University, for technical assistance.

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International Journal of Biomedicine 7(2) (2017) 104-107
http://dx.doi.org/10.21103/Article7(2)_OA3

ORIGINAL ARTICLE

Pulmonology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Efficacy of Oxidative Stress Correction During Asthma Treatment

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Abstract

Background: The improvement of asthma treatment is still one of the urgent issues of modern medicine. The objective of this study was to evaluate the dynamics of lipid peroxidation parameters in patients with asthma receiving complex therapy with ceruloplasmin (Cp).

Methods and Results: The study included 92 patients with severe uncontrolled asthma. Patients were divided into 2 groups. The case group consisted of 45 patients, whose treatment was conventional therapy combined with Cp produced by "MICROGEN" (Russia). Cp 100 mg was administered intravenously once daily for 7 days. The control group consisted of 47 patients who received conventional treatment: inhaled and systemic corticosteroids, bronchodilators, and, if necessary, antibiotics and oxygen therapy. All participants underwent a comprehensive examination, including clinical investigation, chest radiography, assessment of spirometry parameters (VC, FVC, FEV1, FEV1/FVC ratio, PEFR, FEF 25-75), and determination of MDA, metHB, HbCO, total sulfhydryl groups, and SOD activity in blood plasma. Patients of the case group showed a statistically significant decrease in elevated rates of MDA and HbCO, an increase in SOD activity and the content of sulfhydryl groups.

Conclusion: Cp in complex treatment of patients with asthma allows eliminating the imbalance in the prooxidant-antioxidant system and providing an obviously positive clinical effect. **(International Journal of Biomedicine. 2017;7(2):104-107.)**

Key Words: asthma • lipid peroxidation • oxidative stress • ceruloplasmin

Abbreviations

FEV1, forced expiratory volume in one second; **FVC**, forced vital capacity; **FEF**, forced expiratory flow; **HbCO**, carboxyhemoglobin; **LPO**, lipid peroxidation; **MDA**, malondialdehyde; **metHB**, methemoglobin; **OS**, oxidative stress; **PEFR**, peak expiratory flow rate; **RNS**, reactive nitrogen species; **ROS**, reactive oxygen species; **SAD**, superoxide dismutase.

Introduction

Asthma is a global health problem affecting 1%–18% of the population in different countries.[1] An estimated 300 million people worldwide suffer from asthma, with 250,000 annual deaths attributed to the disease.[2] The improvement of asthma treatment is still one of the urgent issues of modern medicine.[3-5] The long-term goals of asthma management are to achieve good symptom control, and to minimize future risk of exacerbations, fixed airflow limitation and side-effects of treatment.[1]

There is strong evidence that asthma is associated with a strong oxidant stress (OS), which is a result of both increased oxidant forces and decreased antioxidant capacity.[6,7] Endogenous and exogenous ROS and RNS play a major role in airway inflammation and are determinants of asthma severity.[6] One key component of the oxidant-antioxidant hypothesis centers on the huge burden of oxidants derived from inflammatory cell infiltration into the lung.[8] Lung tissue contains large amounts of unsaturated fatty acids, substrates of lipid peroxidation (LPO). Alveolar macrophages and other phagocytic cells are activated in inflammation and produce ROS, triggering peroxidation.[9] In addition, ROS and RNS

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contribute to an imbalance in the proteolysis-antiproteolysis system. Oxidative damage to proteins and glycoproteins results in enzyme inactivation and modification of receptor activity. It has been established that the damages caused by ROS are localized mainly in areas with transition metal ions, primarily iron and copper ions.[10] Karmen et al.[11] described the structural and functional changes in erythrocyte membranes associated with peroxidation hyperactivation in asthma. Disturbances caused by an imbalance in the oxidant-antioxidant system—which are manifested by bronchial hyperresponsiveness, lowered lung volume, and vaso- and broncho- constriction—are revealed in all structures of the respiratory tract.[12]

Anti-inflammatory therapy using corticosteroids remains to be the mainstay of treatment and is emphasized in all guidelines.[13,14] The efficacy of current asthma therapy in preventing the effects of oxidative stress is not yet clear and sometimes controversial.[15-17] Sartorelli et al.[18] suggested that imbalance in the oxidant-antioxidant system is associated with the resistance to corticosteroid therapy. The use of antioxidants or other pharmacological agents to boost the endogenous antioxidant system could be used to redress the imbalance in the oxidant-antioxidant system. There is enough evidence to support the conclusion that antioxidants can play a positive role in complex therapy of diseases associated with LPO, including asthma.[19]

The main extracellular antioxidant in the body is ceruloplasmin (Cp), which inhibits LPO by 50% due to the superoxide radical inactivation. The known functions of Cp include copper transport, iron metabolism, antioxidant defense, and involvement in angiogenesis and coagulation.[20,21] The antioxidant activity of Cp has been reported in several studies, and there are reasons to believe that this is one of the most important functions of Cp during inflammatory and acute-phase reactions.[20]

The objective of this study was to evaluate the dynamics of lipid peroxidation parameters in patients with asthma receiving complex therapy with Cp.

Materials and Methods

The study included 92 patients with severe uncontrolled asthma: 42(45.7%) men and 50(54.3%) women aged from 18 to 65 years. The diagnosis was made according to the criteria of Global Initiative for Asthma.[1] The inclusion criteria were the variability of PEFR>30% and FEV1<60% of predicted. Exclusion criteria were malignant tumors, pneumonia, anaphylactic reactions in medical history, and associated chronic diseases in exacerbation state.

The study was approved by local ethics committee. Written informed consent was obtained from each patient. Patients were divided into 2 groups. The case group consisted of 45 patients, whose treatment was conventional therapy combined with Cp produced by "MICROGEN" (Russia). Cp 100 mg was administered intravenously once daily for 7 days. The control group consisted of 47 patients who received conventional treatment: inhaled and systemic corticosteroids, bronchodilators, and, if necessary, antibiotics and oxygen therapy.

All participants underwent a comprehensive examination, including clinical investigation, chest radiography, assessment of spirometry parameters (VC, FVC, FEV1, FEV1/FVC ratio, PEFR, FEF 25-75), and determination of MDA, metHB, HbCO, total sulfhydryl groups, and SOD activity in blood plasma.

Statistical processing of the data was performed with STATGRAPHICS Plus 5.1. The mean (M) and standard error of the mean (SEM) were calculated. Student's unpaired and paired t-tests were used to compare average values for data with normal distribution. A probability value of $P<0.05$ was considered statistically significant.

Results

Patients of the case group demonstrated faster improvement in basic clinical and laboratory parameters compared to patients of the control group. After complex treatment with Cp, 40(88.9%) patients showed controlled asthma signs: nighttime awakenings disappeared, day symptoms reduced to less than 2 times a week, the drug use for the symptoms' management reduced to less than 2 times a week, and no activity limitations for 4 weeks. Dyspnea and cough significantly decreased. In 5(11.1%) patients of this group, nocturnal symptoms/awakening (partially controlled asthma) persisted. Patients of the case group also showed a statistically significant improvement in the spirometry parameters. With only conventional therapy, the dynamics of these indicators was less pronounced; 45(95.7%) patients of the control group continued showing nocturnal symptoms/awakening or daily episodes more than 2 times a week(partly controlled asthma), 2 patients (4.3 %) had uncontrolled asthma (Table 1).

Table 1.

Spirometry parameters in patients with asthma during therapy

Variable	Case group		Control group	
	Before	After	Before	After
VC	62.39±1.7	82.4±1.73*	60.62±1.76	66.84±1.55*#
FVC	56.98±1.59	82.1±1.79*	54.39±1.63	58.97±1.81#
FEV1	41.27±0.91	74.86±1.1*	40.82±0.95	50.88±1.15*#
FEV1/FVC, %	74.64±2.3	90.2±1.33*	76.64±1.89	86.3±1.6*
PEFR	42.01±1.57	72.53±1.58*	41.18±1.42	50.08±1.37#
FEF25	41.48±0.78	73.97±1.05*	40.4±0.83	44.64±0.89#
FEF50	39.58±0.8	71.61±0.98*	38.18±1.82	41.59±0.86#
FEF75	37.96±0.76	64.49±1.01*	35.18±1.85	39.04±1.79#

** - P<0.05 – intragroup differences before and after treatment;*
 # - P<0.05 – differences between groups after treatment
VC, FVC, FEV1, PEFR, FEF25-75 - in % of predicted

Patients of the case group showed a statistically significant decrease in elevated rates of MDA from 20.67±0.63 nmol/ml to 12.25±0.48 nmol/ml and HbCO from 11.96±0.24% to 9.43±0.22%, an increase in SOD activity from 0.75±0.02 AU/ml to 1.07±0.04 AU/ml and the content of sulfhydryl

groups from 98.27±2.2mg% to 110.56±1.67mg%. There was no statistically significant reduction in metHb level. However, in the control group, lipid peroxidation indicators remained high, while antioxidant protection indicators were low (Table 2).

Table 2.

Parameters of the oxidant-antioxidant system in patients with asthma during therapy

Variable	Case group		Control group	
	Before	After	Before	After
MDA, nmol/ml	20.67±0.63	12.25±0.48*	20.18±0.65	16.76±1.66#
metHB, %	1.64±0.12	1.14±0.07	1.57±0.09	1.49±0.08#
HbCO, %	11.96±0.24	9.43±0.22*	11.63±0.28	10.6±0.6
SOD, AU/ml	0.75±0.02	1.07±0.04*	0.76±0.01	0.82±0.03#
SG, mg %	98.27±2.2	110.56±1.67*	97.62±2.26	99.26±2.47#

** - P<0.05 – intragroup differences before and after treatment*
\# – P<0.05 – differences between groups after treatment
SG- sulfhydryl groups

Discussion

Asthma is an inflammatory lung disease that is characterized by systemic and chronic localized inflammation and OS.[7,8] Sources of oxidative stress arise from the increased burden of inhaled oxidants, as well as elevated amounts of reactive oxygen species (ROS) released from inflammatory cells.[8,22] Environmental antigens stimulate ROS overproduction and abnormal function of DNA, proteins and lipids, which lead to hyperreactivity and inflammation in airways.[23] Oxidants decrease the activity of the surfactant and damage fibroblasts. They also cause an increase of epithelial permeability and stimulate the production of thromboxane, which provoke inflammatory changes in the lungs.[24] It is established that OS leads to dysfunction, cytolysis and apoptosis of bronchial epithelial cells.[25] A number of studies have shown that OS is involved in the development of asthma exacerbation and persistence of inflammation in the bronchi that plays an important role in repeated episodes of airway obstruction.[26,27] It is noted that treatment aimed at the recovery of redox processes is a potential strategy to reduce airway inflammation induced by OS. The use of antioxidants in the treatment of asthma contributes to the elimination of imbalance in the oxidant-antioxidant system and improves the clinical course of the disease.[23] Cp is a major protein that circulates in the plasma. Cp has either antioxidant or prooxidant effects, depending on the particular environment.[20,28] Purified human Cp inhibits the oxidation of tissue lipid extracts, lisosomal membranes, polyunsaturated fatty acids, and phospholipids.[29] Several mechanism have been hypothesized for the antioxidant activity of Cp, including a mechanism which acts through sequestration of free copper ions and $O_2^{-\bullet}$ scavengers.[29] Most of the experimental proofs seem to indicate the ferroxidase activity of Cp as the mechanism underlying its antioxidant effects. The conversion of Fe^{2+} into Fe^{3+} can decrease oxidation by blocking the Fenton reaction through a decrease in the quantity of oxidant Fe^{2+} or sequestration of iron by apotransferrin.[30] In our study, Cp reduced the imbalance in the oxidant-antioxidant system and increased the efficiency of the treatment.

Conclusion

Thus, the data obtained have proved OS presence in asthma, which is consistent with the findings of other studies.[6-8,11,18,31] Cp in complex treatment of patients with asthma allows eliminating the imbalance in the prooxidant-antioxidant system and providing an obviously positive clinical effect. In this regard, it is useful and justified to use antioxidants, including Cp, in the complex treatment of patients with asthma.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 108-110
http://dx.doi.org/10.21103/Article7(2)_OA4

ORIGINAL ARTICLE

Pulmonology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Melatonin in Treatment of Sleep Disorders in Elderly Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) may frequently be complicated by sleep disorders, which worse quality of life and lead to other adverse consequences. The objective of the study was to analyze clinical course of the disease and quality of life in elderly patients with both COPD and sleep disorders.

Methods and Results: The study included 50 patients with moderate and severe COPD in age from 60 to 75 years (mean age, 66.12±0.76 years). Patients were divided into 2 groups randomly. For patients in Group 1, 3 mg of melatonin was prescribed orally 30–40 minutes before bedtime for 14 days on the background of standard COPD therapy. Patients of Group 2 received only standard COPD therapy. After 12 months of follow-up, the number of exacerbations and hospitalizations for COPD significantly decreased in Group 1: from 3.74±0.19 to 1.91±0.20 times a year (P=0.0000) and from 2.08±0.18 to 1.35±0.18 (P=0.0046). In Group 2, the dynamics of these parameters was not statistically significant. In addition, along with the improvement of sleep quality in COPD patients during treatment with melatonin, both state and trait anxiety scores and depression level improved. In Group 1, SF-36 scores (PF, RP, BP, and GH) have also significantly improved.

Conclusion: Correction of sleep disorders by melatonin in elderly patients with COPD improved the effectiveness of COPD treatment, and reduced the frequency and duration of exacerbations and the number of outpatient visits and hospitalizations. **(International Journal of Biomedicine. 2017;7(2):108-110.)**

Key Words: chronic obstructive pulmonary disease • melatonin • quality of life • sleep disorder

Abbreviations

BP, bodily pain; **COPD**, chronic obstructive pulmonary disease; **FEV1,** forced expiratory volume in one second; **FVC,** forced vital capacity; **FEF,** forced expiratory flow; **GH**, general health; **MH**, mental health; **PF**, physical functioning; **RP**, role-physical; **RE**, role-emotional; **SF**, social functioning.

Introduction

Chronic obstructive pulmonary disease (COPD), being one of the most common chronic lung diseases, is a very important medical and social problem. COPD is currently the fourth leading cause of death in the world [1] but is projected to be the 3rd leading cause of death by 2020.[2-6] Pharmacologic therapy for COPD is used to reduce symptoms, reduce the frequency and severity of exacerbations, and improve exercise tolerance and health status.[2] To date, there is no conclusive clinical trial evidence that any existing medications for COPD modify the long-term decline in lung function.[2,7-11]

COPD may frequently be complicated by sleep disorders, which worse quality of life and lead to other adverse consequences.

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The goals of treatment for COPD exacerbations are to minimize the negative impact of the current exacerbation and prevent the development of subsequent events.[12] At the same time, sleep disorders that occur in every second or third patient in the older age group are not corrected by a standard therapy and, therefore, are one of the reasons that lead to worsening in a patient's condition and quality of life.[2, 13-15] Thus, it is especially important to develop algorithms for planning drug therapy in COPD patients with sleep disorders, which would lead to a correction of sleep disorders and improve quality of life in these patients.

In recent years, the use of melatonin (the hormone of the pineal gland) as a hypnotic agent has attracted a lot of attention. Melatonin plays a major role in control of circadian rhythm and sleep regulation and has other effects on the immune system, neuroprotection, and oxidant/anti-oxidant activity.[16] The available experimental and clinical data allow use of melatonin in order to correct sleep disorders and as an adaptogen in disorders of the circadian rhythm. The undoubted advantage is the safety of melatonin and its antioxidant and light antidepressive effects.[17-21] Thereby it is relevant to evaluate the clinical course of disease in elderly patients with COPD and sleep disorders during melatonin treatment and according to this newly acquired information to develop algorithms for treating such patients in order to get better results and improve their condition.

The objective of the study was to analyze clinical course of the disease and quality of life in elderly patients with both COPD and sleep disorders.

Materials and Methods

The study included 50 patients with moderate and severe COPD in age from 60 to 75 years (mean age, 66.12±0.76 years). The study was approved by local ethics committee. Written informed consent was obtained from each patient.

Patients were divided into 2 groups randomly: Group 1 (16 men and 4 women) and Group 2 (20 men and 10 women). The diagnosis of COPD was based on a) clinical symptoms (cough, sputum production, shortness of breath), b) a history of exposure to risk factors, and c) signs of airflow limitation on spirometry: a post-bronchodilator FEV1/FVC ratio < 70%.[2] For patients in Group 1, 3 mg of melatonin was prescribed orally 30–40 minutes before bedtime for 14 days on the background of standard COPD therapy. Patients of Group 2 received only standard COPD therapy. Standard COPD therapy was conducted in accordance with the GOLD guidelines.[2]

We analyzed the frequency of COPD exacerbations that required a visit to a general practitioner and the frequency of hospitalizations. Psychological status evaluation was performed using the Spielberger State-Trait Anxiety Inventory and Zung Self-Rating Depression Scale. Sleep disorders were studied using sleep quality questionnaires. (PSQI). Quality of life was evaluated by an SF-36 questionnaire.[22,23] Examinations were conducted at baseline and 12-month follow-up.

The statistical analysis was performed using the statistical software «STATISTICA 7» and STATGRAPHICS Plus 5.1. Data are shown as mean+SEM. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The number of exacerbations and hospitalizations for COPD in patients of Groups 1 and 2 at baseline of the study was comparable. After 12 months of follow-up, the number of exacerbations and hospitalizations for COPD significantly decreased in Group 1: from 3.74±0.19 to 1.91±0.20 times a year (P=0.0000) and from 2.08±0.18 to 1.35±0.18 (P=0.0046) (Table 1). In Group 2, the dynamics of these parameters was not statistically significant.

Table 1.

Frequency of exacerbations and hospitalizations before and after treatment in COPD patients

Variable	Group 1		Group 2	
	Before	After	Before	After
Exacerbations	3.74±0.19	1.91±0.20*	3.60±0.20	3.45±0.29
Hospitalizations	2.08±0.18	1.35±0.18*	1.95±0.13	1.75±0.13

** - P< 0.05*

In addition, along with the improvement of sleep quality in COPD patients during treatment with melatonin, both state and trait anxiety scores and depression level improved, suggesting that the general mechanisms are the same in the development of anxiety, depression and sleep disorders in elderly patients with COPD (Table 2).

Table 2.

Psychological status of COPD patients before and after treatment

Variable (in points)	Group 1		Group 2	
	Before	After	Before	After
State anxiety	43.12±0.59	37.51±0.45*	44.11±0.71	42.48±0.66
Trait anxiety	44.87±0.42	38.13±0.65*	43.91±0.62	42.18±0.45
Depression	57.18±0.76	49.42±0.81*	58.48±0.71	56.53±0.78
Sleep quality index	28.12±0.43	17.21±0.51*	29.12±0.43	27.93±0.58

** - P< 0.05*

In Group 1, SF-36 scores (PF, RP, BP, and GH) have also significantly improved. At the same time, there were not any significant improvements in such domains of SF-36 scales as SF, RE, and MH (Table 3).

Clinically, melatonin has been studied and used for a wide variety of sleep disorders.[16,24-28] Anxiolytic and antidepressant effects of melatonin are associated with its biorythmological and normalizing effects on sleep and on the level of endogenous peptides. Melatonin is not only a unique synchronizer of endogenous biological rhythms, but also to a certain extent is a tranquilizer.

Table 3.

Dynamics of SF-36 scores in COPD patients during treatment

Variable	Group 1		Group 2	
	Before	After	Before	After
RF	54.6±3.32	64.1±2.71*	53.2±3.45	52.2±2.32
RP	28.9±5.66	48.3±3.90*	29.4±5.09	27.8±4.21
BP	49.7±2.40	60.6±2.89*	48.1±2.65	47.2±2.11
GH	43.3±2.46	51.4±3.02*	44.2±2.72	42.2±3.14
VT	50.4±2.62	56.1±2.60	50.4±2.62	49.1±2.90
SF	65.2±3.69	69.3±2.80	65.2±3.69	63.1±2.98
RE	36.9±7.25	41.8±6.29	36.9±7.25	35.1±5.76
MH	52.5±3.36	56.9±3.24	51.2±3.16	50.9±3.54

** - P< 0.05*

In conclusion, correction of sleep disorders by melatonin in elderly patients with COPD improved the effectiveness of COPD treatment, and reduced the frequency and duration of exacerbations and the number of outpatient visits and hospitalizations. The algorithm of treatment for COPD patients should include use of sleep quality questionnaires, which would allow increasing the efficiency of therapeutic and preventive measures and quality of life of patients in primary care.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 111-114
http://dx.doi.org/10.21103/Article7(2)_OA5

ORIGINAL ARTICLE

Dermatology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Are Incisional and Excisional Skin Tension Lines Biomechanically Different? Understanding the Interplay between Elastin and Collagen during Surgical Procedures

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Abstract

Background: Since Langer's first foray into studying cutaneous lines, although people have studied skin lines across the body, there has not been a study that elucidates changes to skin structure of elastin and collagen at different load levels. This study set out to look at whether incisional lines and excisional lines have different biodynamics and have to be considered differently.

Materials and Methods: For this study, we used a two-photon microscopic camera using optimal wavelengths to detect collagen and elastin. Measurements were taken in 5 patients at the center of the excisional wound (high-load) and at the ends of the wound (tapered end of the ellipse) where effectively the wound is an incisional wound.

Results: Wounds were observed after they were surgically closed. When incisional wounds were observed, where there was minimal tension (<1.5N force) we found that, in each case, elastin stretched and collagen buckled, revealing mostly elastin. Where larger defects were created after excisions (as in the figure where a skin cancer had been removed, where forces to close wounds were typically greater than 2N), we noted that the image revealed mostly collagen, suggesting that the reverse had occurred (i.e. collagen stretched and elastin buckled).

Conclusion: This difference between tension loads on skin and the interplay between collagen and elastin has never before been elucidated for incisional and excisional wounds, and in the author's view has great research interest for a cutaneous surgeon seeking to identify the best skin lines to utilize to minimize scarring. **(International Journal of Biomedicine. 2017;7(2):111-114.)**

Key Words: skin lines • collagen • elastin • skin tension • surgery • excisions • incisions • keloid • scarring

Introduction

Elastin, as the name indicates, makes skin elastic – providing the organ with the ability to stretch and recoil.[1] Collagen in skin responds to mechanical forces by altering its molecular structure and generates biochemical signals to influence wound healing and tissue remodeling.[2] Levels of collagen and elastin change with the depth of the dermis and age – and in the lower dermis a significant difference between young and old has been noted for elastin with varying collagen/elastin ratios.[3] Since Langer's seminal work in 1861, his 'cleavage lines of skin' ended up de facto surgical lines, even for most surgical excisions of skin lesions.[4,5] It has already been suggested by others that in the trunk and limbs, Langer's lines predominantly align with elastin fibers.[6]

After Langer marked out cleavage lines by using a round-tipped cutting instrument and noting the direction the clefts elongated, Kocher, in 1892, suggested that these lines be used for surgical procedures.[7] It has been noted in recent times that incisions placed at right angles to the direction of skin cleavage lines had a higher risk of hematoma and tension, and thereby a higher risk of hypertrophic scarring.[8] But the mechanisms of wound tension, especially when defects are created due to removal of skin lesions, have been poorly understood. Biomechanical studies have shown that skin behaves elastically only at low-load levels. For example, on the feet, due to weight-bearing tissues, where the load increases skin reveals increased viscoelastic behavior (i.e. strain becomes a function of load and time).[9] While it is well

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known that skin is anisotropic, it also exhibits orthotropy (i.e. a degree of symmetry with regard to two normal planes, especially in regions of the body close to bone).[10] One of the theories has been that this is due to a preferential orientation of collagen fibers.[11]

Incisions made along the long-axis of Langer's skin cleavage lines are essentially lines in the direction of minimum skin extensibility, and in areas like the calf 76% of elastin fibers aligned themselves along Langer's lines.[12] Yet, we also know from other studies that in the superficial dermis, collagen fibers are not oriented along cleavage lines and it is the reticular dermis that determines skin anisotropy.[13] However, the precise roles of elastin and collagen during skin stretch or load for excisional surgery are unknown. In this article, the author studies differences between incisional and excisional skin wounds to try and understand whether the roles of elastin and collagen under low- and high-tension loads differ.

Materials and Methods

For this study, we used a two-photon microscopic camera using optimal wavelengths to detect collagen and elastin. Previous studies have detected collagen and elastin in skin using fluorescence imaging using specific emission wavelengths: for collagen (lambda c) at 380nm, and for elastin 450 nm, using excitation wavelengths (lambda e) of 340 nm, and 380 nm, respectively.[14] Therefore, these parameters were used as a guide in planning for this study. The study was undertaken in 5 patients (age range 25–74) who were undergoing cutaneous surgery for skin cancer. Measurements were taken at the center of the excisional wound (high-load) and at the ends of the wound (tapered end of the ellipse) where effectively the wound is an incisional wound. Using a two-photon microscopic camera (developed in-house in conjunction with Shenzhen Do3think Technology Co., Ltd), measurements were taken of incisional and excisional sites in each case (Fig.1).

Under a spectroscope, elastin is similar to collagen with an absorption peak around 320 nm and an emission peak near 400 nm. Collagen can similarly be viewed, using a SHG microscopic camera, where two photons combine from the laser field to produce a scattered photon of exactly half the wavelength.[15] The study showed that second-harmonic generation signals derived from collagen can be spectrally isolated from elastin two-photon fluorescence. Two-photon fluorescence signals can be further characterized by emission maxima at 495 nm and 520 nm, corresponding to elastin and cellular contributions, respectively. This method may not be strictly fluorescence, but is very useful to visualize collagen and elastin separately. Others have also noted that second-harmonic generation microscopy has emerged as a powerful modality for imaging fibrillar collagen in a diverse range of tissues, including skin.[16]

Alongside confocal microscopy, two-photon microscopy is now also being used to detect skin cancer, and some authors have successfully used this method to monitor collagen remodeling in vivo after micro-ablative fractional laser resurfacing.[17]

Results

Wounds were observed after they were surgically closed, as shown in the image. When incisional wounds were observed, where there was minimal tension (<1.5N force) we found that, in each case, elastin stretched and collagen buckled, revealing mostly elastin. Where larger defects were created after excisions (as in the figure where a skin cancer had been removed, where forces to close wounds were typically greater than 2N), we noted that the image revealed mostly collagen, suggesting that the reverse had occurred (i.e. collagen stretched and elastin buckled).

Multi-photon microscopy has found favor as a technique to elucidate elastin and collagen in tissues.[18]

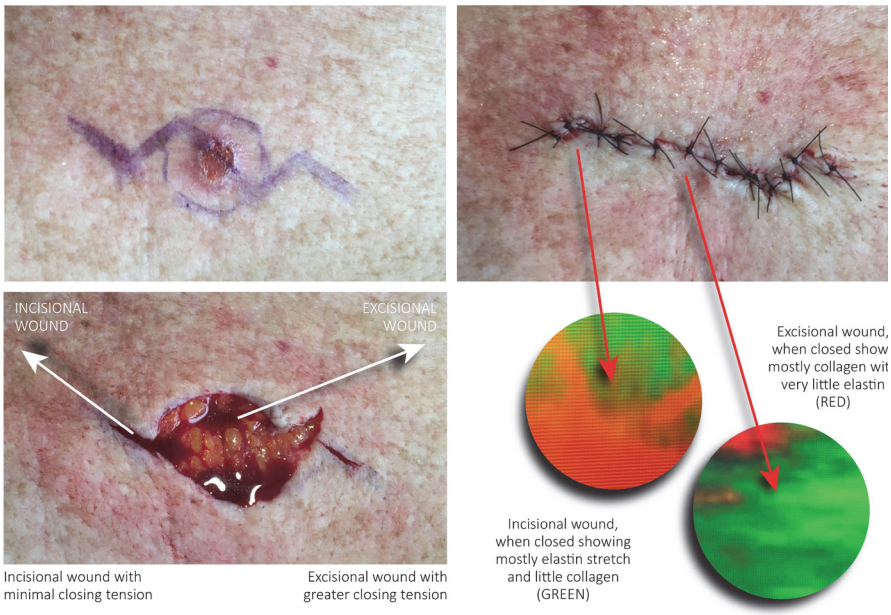

Fig. 1. *Interplay between collagen and elastin in surgical wounds.*

The main advantage of multi-photon microscopy is based on two-photon excited fluorescence (TPEF) and second-harmonic generation (SHG) imaging, allowing us to observe non-fixed, unstained tissue samples. Because collagen in the skin dermis can easily produce an SHG signal and elastin is more effective in generating TPEF, multi-photon microscopy has found widespread applications in dermatology for studying and differentiating cutaneous collagen and elastin – in general, using this technique the collagen images are color-coded in green and elastin images are color-coded in red to increase the contrast. [18,19]

The findings were similar and in fact virtually identical in all cases. The images demonstrating a typical finding (Fig.1) show that for incisional lines (low tension wounds) elastin stretches and collagen buckles, and for excisional wounds (after removal of skin lesions) it is predominantly collagen at play with very little elastin involvement. This not only confirms our contention regarding biodynamic excisional skin tension (BEST) lines[20] that we must view skin lines differently for incisions and excisions, but we see this as the starting point for more research into wound, scar and skin dynamics after excisional surgery. This is why this study set out to look at the roles of elastin and collagen in surgical wounds when closing incisions (inherently low-load) or excisions (higher-loads due to larger defects).

Our finding that incisional (low-load) wound closures are primarily under the influence of elastin, and excisional wound (higher-load) closures depend on collagen has implications for research into both wound healing and scar formation.

Discussion

This difference between tension loads on skin and the interplay between collagen and elastin has never before been elucidated between incisional and excisional wounds, and in the author's view has great research interest for a cutaneous surgeon seeking to identify the best skin lines to utilize to minimize scarring. However, until now there has been no attempt to differentiate incisional and excisional skin lines.

We contend that therefore we need to view incisional and excisional wounds differently and has been mapping best excisional skin tension (BEST) lines.[20] Human skin, when viewed as a mere physical membrane ends up with skin lines and wrinkles because a keratinocyte-stiffened epidermis drapes a softer and thicker dermis. Of course, anatomical sites like knees and elbows have wrinkles that can be considered 'tension' wrinkles (two-dimensional, due to geometry, pre-tension and joint action) and in other areas like the forehead, muscle action causes 'compression' wrinkles (one-dimensional due to muscle action only), but in our view, BEST lines for surgical excisions may not be along these lines.[21] Add to this, other's findings that in keloid scars the increase of both elastin and collagen occurs in deep dermis, whereas a sharp decrease of elastin is found in the upper dermis of keloid,[22] and we have the beginnings of new insights and research into cutaneous surgery and wound healing.

Keloids are unique to humans with no comparable animal models.[3] Researchers have found significant differences in the morphology and content of collagen and elastin in the upper dermis and deep dermis of keloid tissue. In the upper dermis, elastin is not very visible and in the lower dermis, elastin is abundant. Given the findings in this study that incisional wounds are full of elastin and excisional wounds are filled with collagen, further avenues for research into skin lines used during surgery and the resultant scars beckon. We also know from studies into aging that changes as a function of the depth of dermis are significant only for elastin for both young and old.[3] The lower dermis is less rich in elastin, and shows significant diminution between young and older age groups.[3] This also has implications for suture placement in incisional and excisional wounds. This study also demonstrates the use of multi-photon microscopy for assessing the morphology and quantity variations of collagen and elastin in incisional and excisional wounds, a technique used by others to study keloid scarring,[18] and this study is a starting point for further research to understand the basic science behind surgical wounds we create, and resultant scar formation – as ultimately, it is the latter that patients worry about the most.

Conflicts of interest

There are no commercial interests or conflicts of interest to declare.

Acknowledgement

This paper resulted from my PhD research project at the University of Queensland's School of Medicine and I would like to acknowledge my supervisors, Assoc. Professor Cliff Rosendahl, University of Queensland, and Professor John Windsor, University of Auckland. I would also like to thank Ryan Butler, Auckland University of Technology, for his help with illustrations.

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International Journal of Biomedicine 7(2) (2017) 115-119
http://dx.doi.org/10.21103/Article7(2)_OA6

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Perinatal Outcomes in Women with Extragenital Diseases

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Abstract

An increase in the incidence of extragenital diseases (EGDs) in the population against the background of the rise in the birth rate actualizes the problems of pregnancy management in women with EGD. Pregnancy-induced physiological changes in the body lead to a worsening of the course of diseases that were at the stage of unsustainable compensation before pregnancy. The purpose of our study was to determine the effectiveness of hyperbaric oxygenation (HBO) in the prevention of obstetric complications in pregnant women with EGD and neonatal morbidity. The inclusion of HBO in a complex of therapeutic and prophylactic measures in pregnant women with high perinatal risk contributed to a significant reduction in premature birth and a statistically significant improvement in neonatal morbidity parameters. **(International Journal of Biomedicine. 2017;7(2):115-119.)**

Key Words: extragenital diseases • hyperbaric oxygenation • neonatal morbidity • perinatal risk

Abbreviations

EGDs; extragenital diseases; **HBO**, hyperbaric oxygenation; **HIBI**, hypoxic–ischemic brain injury; **LBWN**, low-birth-weight newborns; **PL**, premature labor; **PB**, premature birth; **SMs,** spontaneous miscarriages.

Introduction

Over the past 20 years there has been an increase in the prevalence of EGD in pregnant women; the same can be noted for complications of pregnancy, including chronic fetal hypoxia.[1] According to the Federal State Statistics Service,[2] during the period from 1990 to 2014, the anemia rate increased from 12.1% to 32.0%, urinary system diseases - from 5.9% to 17.0%, arterial hypertension (AH) - from 5.1% to 9.0%. EGDs significantly increase the risk of developing various obstetric complications.[3] In recent years, EGDs have occupied a leading position (28%) in the structure of causes of maternal mortality in developed countries and Russia (23%).[2]

The onset and development of pregnancy against the background of such EGDs as anemia, AH, and chronic pyelonephritis (ChP), occurs in conditions of angiopathy, primarily of the uterus vessels.[4] Angiopathy contributes to the unavoidable development of placental insufficiency during all EGDs.[1]

The literature data indicate that an excessive "medication" of the gestational process leads to a decrease in adaptive resources of the mother and fetus and affects the health of children.[1] Currently, the search for safe means and technologies, including non-medicinal products that will enable a woman to go through pregnancy and childbirth without difficulties, is especially important. Issues related to the prevention of obstetric and perinatal complications in pregnant women with EGDs are still open. Therefore, there is a need for an in-depth study of the possibilities of using non-medicament means of influencing the mother-placenta-fetus system, including HBO, in order to reduce reproductive losses.

The purpose of our study was to determine the effectiveness of HBO courses in the prevention of obstetric complications in pregnant women with EGDs and neonatal morbidity.

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Material and Methods

The study was performed in Municipal clinical hospital #29 named after N.E. Bauman. The study was conducted in accordance with ethical principles of the Declaration of Helsinki.

A total of 235 pregnant women were examined prospectively at 5 to 40 weeks of gestation. Depending on the presence of EGD, the women were divided into 2 groups. The main group included 191 pregnant women with EGD; the control group included 44 women with physiological pregnancy without EGD.

Depending on the nature of the EGD, the main group was divided into three subgroups: subgroup Ia comprised 88 pregnant women with anemia (code ICD-X: O99.0 - anemia complicating pregnancy, childbirth and the puerperium), subgroup Ib comprised 50 pregnant women with AH (code ICD-X: O10 - pre-existing hypertension complicating pregnancy, childbirth and the puerperium), and subgroup Ic comprised 53 pregnant women with ChP (code ICD-X: O23.0 - kidney infection in pregnancy). In turn, the three subgroups were divided into sub-subgroups, depending on the nature of the therapy: standard therapy (ST) or ST+HBO. All examined women with EGD belonged to a group with high perinatal risk (PR). We identified the degree of PR based on the scale developed by O.G. Frolova and E.I. Nikolaeva (1981) and modified in 2003 by V.E. Radzinsky et al.[5] According to this modified scale, there is three level of PR: low risk (<15 points), moderate risk (from 15 to 20 points), and high risk (≥25 points).

Inclusion criteria were singleton pregnancy, high PR, and voluntary informed consent to HBO. Exclusion criteria were cancer, multiple pregnancies, and the contraindications to the use of HBO.

Statistical analysis was performed using the SPSS for Windows. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean (M) and standard error of the mean (SEM) for continuous variables. Group comparisons with respect to categorical variables are performed using chi-square tests. Multiple comparisons were performed with one-way ANOVA. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

98 women of the main group received, besides ST for EGD, the HBO sessions (excess air pressure 1.3-1.5 atm. in a pressure chamber). We used a single-system OKA-MT (Russia), equipped with air-conditioning 54–58 A and designed to conduct sessions in a high-pressure oxygen environment. The mode is one excess atmosphere.[6] The course includes 5–7 daily sessions lasting 40 minutes each. The first course was carried out in 6–8 weeks, the second in 16–18 weeks, and the third in 22–24 weeks of pregnancy.[7]

The inclusion of HBO in a complex of therapeutic and prophylactic measures contributed to a significant reduction in PL ($P<0.05$). Thus, the PL incidence decreased 3-fold in subgroup Ia, 2.3-fold in subgroup Ic, and 1.7-fold in subgroup Ib. Terms delivery are presented in Table 1. The type of therapy influenced the choice of the delivery method only in subgroup Ic (Table 2). In subgroup Ic, on the background of ST+HBO, the rate of programmed births was significantly higher compared to ST: 7.7% and 17.4% ($P<0.05$).

Table 1.

Terms delivery depending on the nature of the therapy.

Group/ Subgroup			Timely delivery	Premature labor	Delay delivery
Main group	Anemia	ST (n=42)	31 (73.8%)	11 (26.2%)*	0
		ST+HBO (n=46)	42 (91.3%)	4 (8.7%)**	0
	AH	ST (n=25)	20 (80.0%)	5 (20.0%)*	0
		ST+HBO (n=25)	22 (78.0%)	3 (12.0%)	0
	ChP	ST (n=26)	15 (57.7%)	11 (42.3%)*	0
		ST+HBO (n=27)	22 (81.5%)	5 (18.5%)*	0
Control group (n=44)			40 (90.9%)	1 (2.3%)	3 (6.8%)
Total (n=235)			192 (81.7%)	40 (17.0%)	3 (1.2%)

** - P<0.05 vs. the control group; ** P<0.05 between anemia subgroups.*

Table 2.

The choice of the delivery method depending on the nature of the therapy.

Group/ Subgroup			Spontaneous labor	Cesarean delivery	Programmed births
Main group	Anemia	ST (n=42)	3 (7.1%)*	33 (78.6%)*	6 (14.3%)*
		ST+HBO (n=46)	5 (10.9%)*	33 (71.7%)*	8 (17.4%)*
	AH	ST (n=25)	2 (8.0%)*	21 (42.0%)*	4 (16.0%)
		ST+HBO (n=25)	2 (8.0%)*	21 (42.0%)*	4 (16.0%)
	ChP	ST (n=26)	7 (26.9%)*	17 (65.4%)*	2 (7.7%)
		ST+HBO (n=27)	5 (18.5%)*	17 (62.9%)*	5 (18.5%)*
Control group (n=44)			42 (95.4%)	1 (2.3%)	1 (2.3%)
Total (n=235)			64 (27.2%)	143 (60.8%)	30 (12.7%)

** - P<0.05 vs. the control group*

Maternal and perinatal mortality has not been documented in any of the subgroups. A total of 235 newborns were born. Parameters of body height and weight of newborns

are presented in Table 3. Using ANOVA, we found differences among the subgroups in terms of weight, height, and circumference of the head and chest. In the comparison of subgroups, the Dunnett criterion was used.

Table 3.

Anthropometric indicators of newborns.

Group/ Subgroup			Weight, g	Height, sm	Circumference of the head , sm	Circumference of the chest, sm
Main group	Anemia	ST (n=42)	2707±337.5 *	47.3±2.3 *	32.9±1.8 *	30.4±2.0 *
		ST+HBO (n=46)	3255±211.7 **	51.7±1.7 **	34.9±0.8 **	32.4±0.6 **
	AH	ST (n=25)	2920±370.5 *	47.3±2.3 *	32.9±1.8 *	30.4±2.0 *
		ST+HBO (n=25)	3250±210.2 **	51.4±1.7 **	34.5±0.8 **	32.0±0.8 **
	ChP	ST (n=26)	2507±556.7 *	46.3±2.5 *	32.5±1.8 *	30.1±2.0 *
		ST+HBO (n=27)	3105±210.1 *,**	49.7±1.7 **	34.0±0.8 **	32.4±0.5 **
Control group(n=44)			3457±504.6	51.1±2.0	34.1±0.9	32.1±0.9
Total(n=235)			3028±343.0	42.1±2.0	33.6±1.0	31.4±1.2

** - P<0.05 vs. the control group; ** P<0.05 - between subgroups depending on on the nature of the therapy*

Thus, newborns born to mothers of subgroup Ia who received only ST were significantly less in weight, height, and circumference of the head and chest when compared with newborns born to mothers who received ST+HBO (P<0.05) and newborns in the control group. The data obtained were quite expected, since a significantly higher incidence of PB and fetal growth retardation (P<0.05)was noted in women of subgroup Ia, who received ST for anemia during pregnancy.

The average weight of the newborns of the subgroup Ic mothers against the background of ST was 2507±556.7g compared to 3105±210.1g (P<0.05) against the background of ST+HBO. In pregnant women who received ST+HBO, the body weight, height, and circumference of the head and chest of the newborns were significantly higher when compared to those measurements in newborns born to mothers who received only ST (P<0.05). However, the analyzed anthropometric indices in both subgroups significantly lagged behind those of the control group (P<0.05).

Newborns born to mothers of subgroup Ib, who received ST+HBO, did not significantly differ in anthropometric parameters from children born to mothers of the control group . Moreover, in newborns born to mothers who received only ST, weight, height, and circumference of the head and chest were significantly less compared to the control group and newborns whose mothers received ST+HBO (P<0.05) during pregnancy.

First- and fifth-minute Apgar scores (AS) revealed statistically significant differences in subgroups (P<0.05) (Table 4). It was established that first-minute ASs in all newborns born to mothers of the main group were significantly lower compared to the control group (P<0.05). A similar situation was observed when assessing fifth-minute ASs. There was no statistically significant effect of HBO on AS in newborns born to mothers of subgroups Ia and Ib, only a tendency for AS to increase. Statistical differences were observed in newborns born to mothers of subgroup Ic compared to subgroups Ia and Ib. Thus, in newborns born to mothers who received only ST, first- and fifth-minute ASs were significantly lower compared to newborns born to mothers who received ST+HBO (6.7±1 and 7.2±0,8 points versus 7.5±0.7 and 7.9±0.5 points, respectively).

Table 4.

Apgar scores in newborns.

Group/ Subgroup			AS	
			First-minute	Fifth-minute
Main group	Anemia	ST (n=42)	7.2+0.6*	7.4+0.4*
		ST+HBO (n=46)	7.3±0.7*	8.0±0.5
	AH	ST (n=25)	7.1±0.7*	7.7±0.5*
		ST+HBO (n=25)	7.5±0.7*	7.7±0.5*
	ChP	ST (n=26)	6.7±1.0*	7.2±0.8*
		ST+HBO (n=27)	7.5±0.7*/**	7.9±0.5**
Control group (n=44)			8.2±0.7	8.8±0.5
Total (n=235)			7.3±0.7	7.8±0.7

** - P<0.05 vs. the control group; ** P<0.05 - between subgroups depending on on the nature of the therapy*

Analysis of neonatal morbidity showed that this indicator was 654.4$^{0}/_{00}$ in newborns born to mothers with EGDs, while in cases of the control group it was 68$^{0}/_{00}$ (Fig.1).

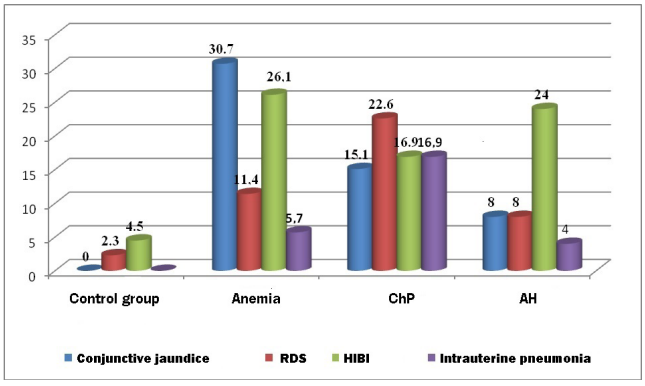

Fig. 1. *Neonatal morbidity (%) in studied groups.*

Conjunctional jaundice was diagnosed in 30.7% of newborns of the subgroup Ia, which was significantly more

frequent compared to the control (0) and subgroups Ic (15.1%) and Ib (8%).

RDS was significantly more often (22.6%) diagnosed in neoborns of subgroup Ic (22.6%) compared to the control and subgroups Ia (11.4%) and Ib (8%), P<0.05 in all cases. Congenital pneumonia was recorded in 16.9% of newborns born to mothers of subgroup IC, which was significantly more often than in subgroups Ia (5.7%) and Ib (4%), P<0.05 in all cases.

HIBI was significantly higher among all newborns in the main group compared to the newborns born to mothers of the control group (P<0.05). HIBI frequency in subgroups Ia and Ib was 26.1% and 24%, respectively; it was 16.9% in subgroup Ic, which was significantly more often compared to the control group (P<0.05), but significantly less often compared to the subgroup Ia (P<0.05). HIBI was diagnosed in every third newborn born to mothers of subgroups Ia and Ib who received only ST (35.7% and 32%, respectively), and in 23.1% of newborns born to mothers of subgroup Ic who received only ST. The inclusion of HBO in complex therapy contributed to a 2-fold (P<0.05) decrease in HIBI frequency.

In order to assess the effectiveness of HBO in a complex of therapeutic and prophylactic measures in pregnant women with high PR, a comparative analysis of neonatal morbidity was performed (Table 5).

Table 5.

Neonatal morbidity depending on the nature of the therapy.

Group/ Subgroup			Conjunctive jaundice	HIBI	RDS	Intrauterine pneumonia
Main group	Anemia	ST (n=42)	18(42.8%) *	15(35.7%) *	8(19.0%) *	4(9.5%)
		ST+HBO (n=46)	9(19.6%) */**	8(17.4%) */**	2(4.3%) **	1(2.2%)
	AH	ST (n=25)	3(12.0%)	8(32.0%) *	3(12.0%)	3(12.0%)*
		ST+HBO (n=25)	1(4.0%)	4(16.0%)	1(4.0%)	1(4.0%)**
	ChP	ST (n=26)	6(23.1%) *	6(23.1%) *	9(34.6%) */**	7(26.9%)*
		ST+HBO (n=27)	2(7.4%) **	3(11.1%) **	3(11.1%)	2(7.4%)**
Control group (n=44)			0	2(4.5%)	1(2.3%)	0
Total (n=235)			39(16.5%)	46(19.5%)	27(11.4%)	18 (7.6%)

** - P<0.05 vs. the control group; ** P<0.05 - between subgroups depending on on the nature of the therapy*

Data obtained showed that conjunctive jaundice was significantly less frequent in newborns whose mothers received HBO: 2.2 times for subgroup Ia, 3 times for subgroup Ib, and 3.1 times for subgroup Ic. Intrauterine pneumonia was more often diagnosed in newborns of the subgroup Ic mothers: 26.9% against ST, and 7.4% against ST+HBO (a 3.6-fold decrease) (P<0.05). The inclusion of HBO in complex therapy also contributed to a significant decrease (P<0.05) in RDS frequency: 4.4 times for subgroup IA, 3.1 times for subgroup IC, and 3 times for subgroup IB.

Obviously, a decrease in the frequency of PL in the subgroups of pregnant women in the main group who received ST+HBO promoted a statistically significant reduction in neonatal morbidity.

Conclusion

The inclusion of HBO in a complex of therapeutic and prophylactic measures in pregnant women with high PR contributed to a significant decrease in all subgroups:

In subgroup Ia:

- Threatened late SMs from 19.0% to 8.7%;
- Threatened PL from 33.3% to 19.6%;
- Preeclampsia from 30.9% to 17.4%;
- Preterm birth from 26.2% to 8.7%;
- LBWN from 26.2% to 13.0%.

In subgroup Ib:

- Threatened late SMs from 20.0% to 12.0%;
- Threatened PL from 32.0% to 16.0%;
- Preeclampsia from 48.0% to 28.0%;
- Preterm birth from 20.0% to 12.0%;
- LBWN from 20.0% to 12.0%.

In subgroup Ic:

- Threatened late SMs from 34.6% to 14.8%;
- Threatened PL from 69.2% to 37.0%;
- Preterm birth from 42.3% to 18.5%;
- LBWN from 23.1% to 11.1%.

A significant decrease in the incidence of LBWN in pregnant women with EGD who received ST+HBO contributed to a statistically significant improvement in neonatal morbidity parameters. The rate of neonatal morbidity significantly decreased in patients with anemia, GhP, and AH from 1071‰ to 435‰, from 1077‰ to 370.4 ‰, and from 1080‰ to 280‰, respectively.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 120-125
http://dx.doi.org/10.21103/Article7(2)_OA7

ORIGINAL ARTICLE

Women's Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Anthropometrical Reference Data for Uzbek Women: Results of the Population Research in the Republic of Uzbekistan

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Abstract

The aim of this study was to develop anthropometrical reference data for women of Uzbek nationality. In total, 1413 women of Uzbek nationality aged ≥35 years were studied. BMI was calculated for 411 women. The reference values of BMI for Uzbek women vary from 19.4 to 24.8 kg/m^2; BMI 22.7 kg/m^2 corresponds to the 50th percentile. These results correspond to the WHO criteria for European populations. The reference values of WC vary from 65.0 to 90.0 cm. The conventional cut-off for normal WC (80 cm) corresponded to the 75th percentile of the analysis among women of Uzbek nationality without any carbohydrate metabolism disorders and with normal BMI. WC equal to 76 cm corresponds to the 50th percentile. Thus, the cut-off of WC for Uzbek women is 90.0 cm, which is a mismatch to the European criteria. Average value of WHR was 0.80±0.07. Reference values of WHR vary of 0.69 to 0.93. WHR 0.8 corresponds to the 80th percentile. The obtained reference ranges are recommended to reveal the prevalence of MS and its components at the stage of revealing of risk groups in Uzbek women. **(International Journal of Biomedicine. 2017;7(2):120-125.)**

Key Words: body mass index • waist circumference • waist-to-hip ratio • hips circumference

Abbreviations

BMI, body mass index; **BP**, blood pressure; **CVD**, cardiovascular diseases; **DBP**, diastolic BP; **FFA,** free fatty acids; **FPG**, fasting plasma glucose; **HC**, hips circumference; **IGT**, impaired glucose tolerance; **IFG**, impaired fasting glucose; **OGTT**, oral glucose tolerance test; **SBP**, systolic BP; **T2D**, type 2 diabetes; **WC**, waist circumference; **WHR**, waist-to-hip ratio.

Introduction

Many studies are devoted to metabolic syndrome (MS) as all its factors lead to development of CVD and T2D. Abdominal obesity and insulin resistance are supposed to be the background of MS. [1-4]

As the prevalence of MS is high – 20% to 25% [5] – it is clear that the optimal criterion for its diagnosis will be the one that does not demand extra expenses and is not labor-consuming. Therefore, the basic criterion for diagnosis of MS is WC.[1,6-8] According to updated IDF consensus,[8] to be diagnosed with MS, one needs to have central obesity defined as WC ≥ 94 cm for Caucasian men and ≥ 80 cm for Caucasian women, plus any two of the below mentioned factors for diagnosis of MS:

- triglyceride level ≥1.7 mmol/l or specific treatment;
- HDL level <1.03 mmol/l for men and <1.29 mmol/l for women or specific treatment;
- SBP≥130mmHg and DBP≥85 mmHg or antihypertensive treatment;
- FPG≥5.6 mmol/l or earlier diagnosed diabetes.

Thus, the reservation is made that WC criteria should be specific to each ethnic group. (Tables 1,2)

Active revealing of T2D has a great value, as it has been proved that at the stage of prediabetes (IFG, IGT), patients already may have complications specific to diabetes.[1-3,9] For early diagnosis of T2D, WHO recommends a screening at least every 5 years in risk groups, which, in turn, are defined through use of a questionnaire, in particular FindRisk. Besides WC, in the given questionnaire, BMI, age, heredity, hypertension, and

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lifestyle are also considered. Thus, anthropometry indicators are a simple and effective way to obtain an early and mass estimation of risk of developing CVD and T2D.

WC is measured using a centimetric tape on an exhalation, without any expanding or contracting of the belly.[10]

Table 1.

Reference data for WC according to IDF recommendations[8]

Country/Ethnic group		WC
Europids	Male	≥94 cm
	Female	≥ 80 cm
South Asians	Male	≥90 cm
	Female	≥ 80 cm
Chinese	Male	≥90 cm
	Female	≥ 80 cm
Japanese	Male	≥90 cm
	Female	≥ 80 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available	
Sub-Saharan Africans	Use European data until more specific data are available	
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available	

Table 2.

Additional metabolic criteria for research (IDF) [8]

Abnormal body fat distribution	DEXA, CT/MRI, leptin, adiponectin, liver fat content
Atherogenic dyslipidemia	ApoB, small LDL particles
Dysglycemia	OGTT
Insulin resistance	Fasting insulin/proinsulin levels, HOMA-IR and other IR indices, clamp method, FFA
Vascular dysregulation	Endothelial dysfunction, microalbuminuria
Proinflammatory state	C-reactive protein, inflammatory cytokines, decreased adiponectin level
Prothrombotic state	Fibrinolytic factors, clotting factors
Hormonal factors	Pituitary-adrenal axis

WHR [WC(cm)/HC(cm)] is the indicator characterizing type of fat distribution. For Caucasian women, normal WHR is <0.85, for men - <0.9.

There are three types of fat distribution, depending on WHR value (Table 3). The android type of fat distribution (so-called "apple-type") is characterized by fat deposition in the area of the waist and abdomen and bears the greatest risk of developing CVD (atherosclerosis, ischemic heart disease, and stroke), T2D, and dyslipidemia.

BMI is calculated using Quetelet's formula: BMI = body weight(kg)/height(cm)^2

WHO developed criteria [4,11] and detailed techniques for measuring and estimating such indicators as BMI and waist and hips circumference, but at the same time underlined the necessity of working out national anthropometrical references for each population. Therefore, for example, the cut-off of normal BMI for the European population is 25 kg/m^2, for the Asian populations - 23 kg/m^2 (and a value above 25 kg/m^2 is assessed as obesity). The validity of such recommendations has been shown, in particular by C. Wen and co-authors [5] in a prospective cohort study, that applying European threshold BMI values (25 kg/m^2) instead of Asian ones (23 kg/m^2) leads to underestimating risk of lethal outcomes in 8.6% of cases and of cardiovascular death in 21.1%.

Table 3.

Types of fat distribution depending on WHR value.

WHR	Type of fat distribution
Men: 0.8-0.9	Intermediate
Women: 0.8-0.85	Intermediate
Women: < 0.8	Hynoid
Men: > 0.9	Android
Women: > 0.85	Android

On the diagram (Fig. 1), the authors clearly show [6] how widely the prevalence of excess weight and weight deficiency varies depending on the country; therefore, it is not a fact that different populations with identical distribution of BMI will have identical relative risk of morbidity and mortality associated with different degrees of excess weight or its deficit.

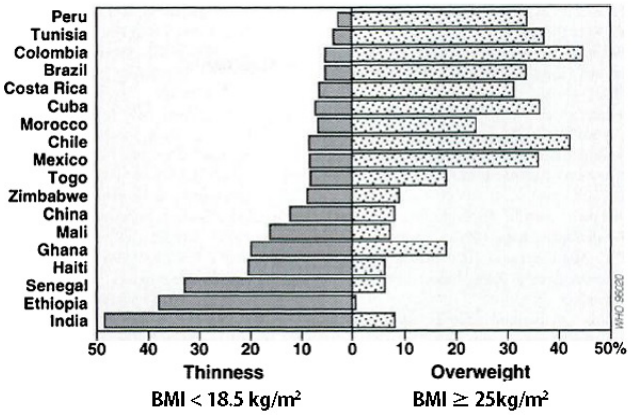

Fig. 1. *BMI distribution of various adult populations worldwide*

There are a large number of detailed, cited studies in the literature aimed at working out references of anthropometrical indicators for children;[12,13] however, similar studies of adults are lacking for a range of populations. A study was performed on the Uzbek population that worked out percentile tables of WC and BMI for children and teenagers,[14] but there were no similar studies of the adult population.

In 1996, the WHO Expert Committee decided that there is no necessity for reference data on BMI for adults, and interpretation should be performed based on pragmatic

threshold values.[6] However, subsequent studies have proved the need to develop separate criteria for results of anthropometrical measurements for different populations. Thus, Haldar et al.[15] studied the difference in the amount and distribution of fat between East Asians, South Asians, and Europeans. The authors showed that Asians, having higher BMI than Europeans, are more predisposed to obesity. Additionally, given the same amount of fat tissue, cardiometabolic risk is higher in Asians than in Europeans.

Other authors[16] assessed the possibility of applying European references for BMI and WC to estimate metabolic disorders associated with obesity in Canadians of East Asian and South Asian origin. The authors showed much lower reference values of BMI (23.2 kg/m^2) and WC (79.6 cm) for the East Asian Canadians than for the South Asian (26.1 kg/m^2 and 90.3 cm) and European Canadians (26.5 kg/m^2 and 89.3 cm)

Another research study performed in a multiethnic population of Canada[17] showed that the threshold value for the definition of obesity is approximately 6 kg/m^2 lower for non-European ethnic groups than for European groups(South Asians, Chinese and Canadian natives were studied). Earlier, these authors[18] showed that at the same BMI value in the abovementioned ethnic groups, HbA1c level was higher than in representatives of European races.

Similar results are shown for the Indian population, for which authors recommend lower threshold values for normal BMI. Deurenberg-Yap et al.[19] recommends 27 kg/m^2 as the threshold value of BMI for a diagnosis of obesity in Chinese and Malaysians and 26 kg/m^2 for Hindus.

As for WC, the Canadian authors[16] showed that cut-off values of WC for increased risk of metabolic disorders were lower for the East Asian men (83.3 to 85.2 cm) and women (74.1 to 76.7 cm) than for the South Asian men (98.8 cm) and women(90.1 to 93.5 cm), and also for men (91.6-95.2 cm)and women (82.8-88.3 cm) of European origin. In the same study, the authors provide cut-off values of BMI for estimation of increased risk of metabolic disorders: 23.1-24.4 kg/m^2 for East Asians, 26.6-26.8 kg/m^2 for South Asians, and 26.3-28.2 kg/m^2 for Canadians of the European origin.

In the study in population of Taiwan,[20] following cut-offs of anthropometrical data for obesity and excess weight were received: BMI 23.6 and 22.1 kg/m^2, WC 80.5 and 71.5 cm, WHR 0.85 and 0.76, accordingly, for men and women.

Thus, based on the abovementioned results, ***the aim*** of our study was to develop anthropometrical reference data for women of Uzbek nationality.

Material and Methods

This study was performed by a group of endocrinologists in the Tashkent, Qashqadarya, and Khorezm regions of Uzbekistan among women of Uzbek nationality aged 35 years and older. The sample was formed by the method of random numbers, and the design of sample formation was weighed cluster.

Inclusion criteria were female sex, age ≥35 years, absence of components of MS, and cardiovascular events in anamnesis. Exclusion criteria were high risk of T2D development, diabetes, prediabetes, BP>130/85 mmHg at the moment of measurement, receiving hypotensive medications, or having episodes of BP increase in the anamnesis.

In total, 1413 women of Uzbek nationality aged ≥35 years were studied; of them, 411 had no obesity, excess body weight (according to WHO criteria for European population), carbohydrate metabolism disorders, or hypertension. The study included filling out a questionnaire based on the FinnishDiabetesRiskScore with assessment of T2D risk.[3,7] Body weight was measured using on-floor scales (in kg); growth – using auxanometer (in cm), WC - using centimetric tape at the navel level on a horizontal line (in cm), HC- using centimetric tape on the most prominent points at the level of the hips (in cm). BMI was calculated according to the formula: BMI (kg/m^2)=(weight, kg)/(growth, cm)^2 [21,10] WHR ratio was calculated as WC/HC.[21,10]

All patients were checked on office BP using Korotkov's method after a 5-min rest in the seated position with back support. BP was measured two times on the left and right arms at 2-minute intervals with the use of a validated semi-automated electronic device. Blood pressure was measured using Korotkov's method on two hands twice.

All women were tested for FPG and a 2-hour OGTT. Prediabetes and diabetes were diagnosed according to the international recommendations.[22]

Statistical analysis was performed using the statistical software STATISTICA 6.0. The mean (M) and standard deviation (SD) were calculated. For normally distributed data, the percentile values were calculated. The references ranges were those between the 5th and 95th percentile values.[12,14]

Results

BMI in women of Uzbek nationality

BMI was calculated for 411 women. The average BMI value was 22.49±1.72 kg/m^2. Considering recommendations to use a BMI value of 23 kg/m^2 as the cut-off in Asians, we divided the women into two subgroups, with BMI 18-23 kg/m^2 and BMI 23-25 kg/m^2, and found that the number of women in the two subgroups was nearly identical (54% and 46%, accordingly); therefore, if one decreases the BMI reference from 25 to 23 kg/m^2, half of the women with "normal" BMI, according to European criteria, will automatically become members of the group with excess body weight. Therefore, calculating a reference BMI for the Uzbek female population, we considered it sensible to follow the European criteria recommended by WHO/IDF. We calculated percentiles for BMI in women to reveal their reference values (Table 4). Thus, the reference values of BMI for Uzbek women vary from 19.4 to 24.8 kg/m^2; BMI 22.7 kg/m^2 corresponds to the 50th percentile. These results correspond to the WHO criteria for European populations.[6]

WC in women of Uzbek nationality

Average WC was 76.06±7.98 cm. The reference range of WC varied from 65.0 to 90.0 cm. The conventional cut-off for normal WC (80 cm) corresponded to the 75th percentile of the analysis among women of Uzbek nationality without any carbohydrate metabolism disorders and with normal BMI.

WC equal to 76 cm corresponds to the 50th percentile (Table 4). Thus, the cut-off of WC for Uzbek women is 90.0 cm, which is a mismatch to the European criteria.

HC in women of Uzbek nationality

Concerning HC, this indicator itself is not informative when isolated from WC. However, it is important for a definition of the type of fat distribution in the presence of obesity or excess body weight. Average HC among the studied women was 95.73±6.93 cm. Reference values of HC vary from 84.0 to 107.0 cm. HC of 100cm corresponds to the 75th percentile. The 50th percentile is represented by HC 96.0 cm (Table 4). The reference values differ from the European and Asian data.

WHR in women of Uzbek nationality

Average value of WHR was 0.80±0.07. Reference values of WHR vary of 0.69 to 0.93. WHR 0.8 corresponds to the 80th percentile (Table 4).

Table 4.

Percentile values of BMI, WC, HC, and WHR for women of Uzbek nationality aged ≥ 35 years.

Percentile	BMI, kg/m^2	WC, cm	HC, cm	WHR
Percentile 2.5	18.8	63.8	82.0	0.68
Percentile 5	19.4	65.0	84.0	0.69
Percentile 10	20.0	67.3	86.0	0.71
Percentile 25	21.2	70.3	91.0	0.75
Median 50	22.7	76.0	96.0	0.80
Percentile 75	24.0	80.0	100.0	0.84
Percentile 90	24.6	87.7	104.0	0.88
Percentile 95	24.8	90.0	107.0	0.93
Percentile 97.5	24.9	93.0	110.0	0.95

Discussion

In 1996, the WHO Expert Committee published recommendations not to develop any reference values of BMI for adults[6] and to use in practice the threshold values of BMI, as the prevalence of excess weight and obesity varies widely in different countries. The Committee explained that different populations with identical distribution of BMI have different relative risks of morbidity and mortality, and the prevalence of obesity depends on the social welfare of a population. Thus, experts believe that in the future, if enough data is gathered, reference values of BMI still may be developed. Such data should be gathered in populations without problems with nutrition, in which growth in childhood is not influenced by chronic infections, and youth in the population are basically healthy and do not smoke. In addition to the experts' recommendations, we have excluded from our study persons with abnormal weight, both excessive and insufficient (referring to non-pregnant persons with good nutritional status), persons with obvious diseases, and those who are on special diets to lose weight.

Threshold values of BMI >25 kg/m^2 for excess weight and >30 kg/m^2 for obesity were received from the European populations. However, the South Asian populations [5,19,23] have increased risk of T2D, hypertension and dislipidemia even with BMI <25 kg/m^2. A possible explanation is that there is a relatively lower muscular weight and higher fat mass among these populations compared to Europeans. For the Asian populations, two positions of consensus tried to define BMI interval. In WHO's position dated 2000,[4] BMI >23 kg/m^2 was recommended to consider as excess weight, and >25 kg/m^2 as obesity. Moreover, in 2004, WHO's position specified that there are many threshold BMI values for Asians for a definition of excess weight and obesity, and it is impossible to deduce any common value in these populations.[11]

The difference in the received average data depends on the study method - getting an average data in populations, carrying out the ROC-analysis, or the multivariate analysis of risk factors; and within the multivariate analysis, methods of revealing threshold values of anthropometrical indicators also differ essentially. So for example, F. Razak has received the following average indexes depending on demographic characteristics: BMI for Europeans - 27.5 kg/m^2 (27.0-28.0), for South Asians - 26.1 kg/m^2 (25.6-26.6), for Chinese - 23.7 kg/m^2 (23.2-24.2), and for native Canadians - 31.2 kg/m^2 (30.6-31.8); WC for European women - 84.7 cm (82.7-86.6), for Southern Asians - 85.7 cm (83.9-87.5), for Chinese women - 74.8 cm (73.6-76), for native Canadians - 97.0 cm (94.4-99.7).It is interesting that the HOMA-IR index also increases with the increase in BMI and WC:. for Europeans - 2.29(2.05-2.54), for South Asians - 3.18(2.93-3.43), for Chinese - 2.46(2.2-2.71) and for native Canadians - 4.62 (4.33-4.92).

F. Razak et al.[17] showed that South Asians, Chinese, and natives of Canada have a similar distribution of factors of carbohydrate metabolism (fasting and 2-hour glucose, fasting and 2-hour insulin, HbA1c, HOMA-IR) and lipid metabolism (LDL, HDL, fasting and 2-hour triglycerides, fasting and 2-hour free fatty acids) at much lower values of BMI compared to Europeans, and the use of European norms of BMI in the given populations will lead to underestimating risk. Therefore, to minimize development of cardiometabolic risk factors in some non-European populations, health professionals should use lower goal values of BMI. The data we received in our study correspond to Razak's conclusions. A study by W. Lin et al.[20] among Taiwanese population also has led to the conclusion that values of BMI and WC for the Taiwanese population should be lower than the values received for the Western countries. Thus, the top border of normal BMI for women is 22.1 kg/m^2, WC – 71.5 cm, and WHR – 0.76.

F. Razak et al. also showed the influence of ethnic features on the ratio of obesity and glucose metabolism disorders.[17,18] For example, 58.8% of South Asians with increased WC (for the European population) will have BMI<30 kg/m^2, which underlines the necessity, first, of an obligatory account of WC in an estimation of CV risk, and, second, of working out reference values of WC for the given population.

Dudeja et al.[24] summarized data on different BMI values received for different ethnic groups. Thus, the cut-off of normal BMI in women varies from 22.1 kg/m^2 in Singapore Chinese and mainland Chinese, to 22.5 kg/m^2 in East Asians, 27.0 kg/m^2 in South Asians and black inhabitants of Jamaica,

29.7 kg/m^2 in Polynesians, and 30.9 kg/m^2 in inhabitants of the USA. For Indian women of Northern regions of India authors have received BMI value of 23.3 kg/m^2.

However, the study by S. Oh[25] did not confirm the rationale for use of lower threshold values of BMI for revealing of risks. The authors recommended using BMI 25 kg/m^2 as the threshold to start actions on prevention of obesity.

The age of the observed people varies in the abovementioned studies. Therefore, we consider it reasonable to deduce a reference range for each age group as it has been specified in the WHO recommendations.[6] Usually, borders of reference values, in particular WC, are calculated as the 5th and 95th percentiles; however, ideally such criteria should be based on their association with risk factors, in particular with the HOMA index.

In a study by C. Wen et al.,[5] the authors recommend using BMI ≥25 kg/m^2 as the threshold value for obesity and 23-24.9 kg/m^2 for excess weight in Asians. The authors specify that following the European standards leads to underestimating the contribution of obesity to the reasons for lethal outcomes among Asians.

In many studies, data are provided together with an estimation of risk factors for diabetes, hypertension, and dyslipidemia. We plan to carry out a similar analysis on the threshold values of anthropometrical data we receive and to develop data adapted to the Uzbek population in a way that is easy to use in clinical practice the questionnaires for estimating risk of CVD development. In further studies, we will investigate whether the CV risk is higher in the population with BMI higher than the obtained reference values, but lower than the values received for Europeans.

In conclusion, for women of Uzbek nationality:

- the cut-off value of BMI is 24.8 kg/m^2;
- the cut-off value of WC is 90 cm;
- the cut-off value of HC is 107 cm;
- the cut-off value of WHR is 0.93.

We recommend using the reference ranges we have received during epidemiological studies in order to reveal the prevalence of MS and its components at the first stage of research, according to WHO - at the stage of revealing of risk groups.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors gratefully acknowledge the team of endocrinologists headed by prof. Zair S. Akbarov for their help in data collection.

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International Journal of Biomedicine 7(2) (2017) 126-130
http://dx.doi.org/10.21103/Article7(2)_OA8

ORIGINAL ARTICLE

Women's Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Circadian Rhythms of Melatonin Secretion in Peri- and Postmenopausal Women with Insomnia

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Abstract

The aim of this study was to assess complaints about sleep quality and to investigate circadian rhythms of melatonin secretion in peri- and postmenopausal women.

Material and Methods: A total of 146 climacteric women were examined. All patients were divided into 2 groups: Group1 included 72 perimenopausal women and Group 2 included 74 postmenopausal women. Women were surveyed with special questionnaires: PSQI, FFS, ESS, Daytime Feeling and Functioning Scale. Insomnia Severity Index (ISI) was calculated. Salivary melatonin content was determined (4 times a day) by immunoassay using Microplate Reader EL×808 (USA).

Results: Perimenopausal women often complained about difficulties falling asleep (more than 20 minutes from the moment the light was turned off) and difficulties awakening in the morning, while postmenopausal women often complained about snoring and frequent awakenings during sleep (≥2 times). ISI was 21.3±0.54 in Group 1 and 24.8±0.31 in Group 2, which corresponded to insomnia. Daily melatonin secretion in perimenopausal patients with insomnia was altered – the maximal level was registered in the morning hours. The circadian rhythms of melatonin secretion in the group of postmenopausal women did not correlate to the occurrence of insomnia.

Conclusion: We can recommend administration of melatonin in the evening time and light therapy in the early morning hours in the complex treatment of sleep disprders in perimenopausal women for normalizing and shifting the chronobiological rhythms of melatonin secretion, and specific therapy is aimed to eliminate snoring for postmenopausal women. **(International Journal of Biomedicine. 2017;7(2):126-130.)**

Key words: melatonin • circadian rhythms • perimenopause • postmenopause • insomnia

Abbreviations

BW, body weight; **BMI**, body mass index; **ESS**, Epworth Sleepiness Scale; **FFS**, Flinders Fatigue Scale; **ISI**, Insomnia Severity Index; **PSQI,** Pittsburgh Sleep Quality Index; **SDs**, sleep disorders.

Introduction

The climacteric is a transition period from the reproductive phase to its extinction and a cessation of menstrual and genital function: follicular reserve of ovaries depletion, decrease in ovarian activity, changes in relationships between hormones, and decrease in estrogen level.[1] Of menopausal women, 60%-80% have vegetovascular, mental and metabolic disorders. One of the main signs of neurovegetative changes in climacteric women is the presence of sleep disorders (SDs).[2,3] Some studies have shown that SDs are reported by 39%-47% of perimenopausal women and 35%-60% of postmenopausal women.[3] The role of the regulator of circadian rhythms has been assigned to the melatonin

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hormone.[4] The cells producing this hormone are found in the pineal gland, retina, tract, urogenital system, and thymus. In healthy people, the melatonin level begins to increase in the evening when the level of illumination decreases. The pineal gland receives information about external light through complex nervous pathways, with a relay in the suprachiasmatic nuclei of the hypothalamus. At night, in darkness, when the majority of neurons in suprachiasmatic nuclei are inactive, nerve terminals excrete norepinephrine, which activates melatonin-generating enzymes in pinealocytes.[5] The age-related decrease in melatonin secretion is a well-known fact; not only its level but also its production curve changes.[6-8] These changes could be the cause of various diseases, including SDs.[9]

Results of our pilot research show that there is a relationship between the chronobiological rhythms of melatonin secretion and SDs in women of perimenopausal age.[10] The studies on age-related changes in melatonin level in women during various phases of the climacteric, and the effect of those changes on the appearance of SDs, is at the present time of great interest. This area of investigation makes it possible to elaborate the main principles of pathogenic therapy for certain SDs in climacteric women and a range of medical and social measures to conserve health and life quality in these women.

The aim of this study was to assess complaints about sleep quality and to investigate circadian rhythms of melatonin secretion in peri- and postmenopausal women.

Material and Methods

The study was approved by the Scientific Center of Family Health Problems and Human Reproduction Ethics Committee. Written informed consent was obtained from each patient.

A total of 146 climacteric women were examined. All patients were divided into 2 groups: Group1 included 72 perimenopausal women and Group 2 included 74 postmenopausal women.

Inclusion criteria for Group 1 were age 45-55; oligomenorrhoea or amenorrhea during last 12 months; ultrasounds criteria: 1) endometrial dysfunction: mismatch of structure and thickness corresponding to the first and the second phases of the menstrual cycle; 2) ovarian follicle reserve depletion

Inclusion criteria for Group 2 were age 56-60; amenorrhea ≥ 12 months; FSH level >20 iU/ml, index LH/FSH < 1; ultrasounds criteria: 1) thin nonfunctional endometrium, endometrial echo thinner than 5 mm; 2) lack of ovarian follicle reserve.

Exclusion criteria for both groups were hormone replacement therapy; decompensation of cardiovascular, mental, neurological, and endocrine diseases; an exacerbation of chronic diseases; presence of chronic SDs in the past; hypnotics administration during the last two weeks; surgical menopause; work in shifts.

After questioning, the each group was divided into 2 subgroups: the main (insomnia+) and control (insomnia–). Women were surveyed with special questionnaires: PSQI,[11] FFS,[12] ESS,[13] Daytime Feeling and Functioning Scale.[14] Insomnia Severity Index (ISI)[15] was calculated.

Questioning showed that 54.2% of perimenopausal and 40.5% of postmenopausal women did not have SDs. They were assigned to the control group. Basic characteristics, including age, height, BW, BMI are shown in Table 1. There were no differences in the characteristics of the control and insomnia group in perimenopause. At the same time, during postmenopause BW and BMI increased significantly in the group with insomnia compared to the control.

Table 1.

Clinical characteristics of patients.

Variable	Perimenopause		Postmenopause		P-value
	Insomnia– (n=39)	Insomnia+ (n=33)	Insomnia– (n=30)	Insomnia+ (n=44)	
	1	2	3	4	
Age, y	50.36±3.05	49.85±3.03	56.25±3.97	56.7±3.61	p_{1-3}=0.000 p_{2-4}=0.000
Height, cm	164.19±5.31	163.14±2.92	163.41±5.47	162.20±3.90	-
BW, kg	70.12±11.72	73.38±13.54	74.66±13.64	85.25±12.83	p_{2-4}=0.000 p_{3-4}=0.001
BMI, kg/m^2	25.9±4.73	27.09±1.56	28.0±2.35	34.15±1.5	p_{1-3}=0.029 p_{2-4}=0.000 p_{3-4}=0.000
GD	1 (2.56%)	10 (30.3%)	13 (43.3%)	17 (38.6%)	p_{1-2}=0.008 p_{1-3}=0.002
USD	2 (5.12%)	3 (9.09%)	2 (6.67%)	13 (29.5%)	p_{2-4}=0.031 p_{3-4}=0.012
OP	12 (30.8%)	19 (57.6%)	10 (30.33%)	36 (81.8%)	p_{3-4}=0.049
CVD	-	7 (21.2%)	5 (16.7%)	10 (22.7%)	p_{1-2}=0.019 p_{1-3}=0.048
T2D	-	2 (6.06%)	1 (3.33%)	1 (2.27%)	-
TD	5 (12.8%)	13 (39.4%)	8 (26.7%)	13 (29.5%)	p_{1-2}=0.049
PCOS	-	1 (3.03%)	1 (3.33%)	-	-
UF	13 (3.33%)	20 (60.6%)	9 (30.0%)	35 (79.5%)	p_{3-4}=0.041
EM	4 (10.26%)	7 (21.21%)	2 (6.67%)	7 (15.9%)	-

PCOS- Polycystic Ovary Syndrome, T2D - Type 2 diabetes, GD - Gastrointestinal disease, USD - Urinary system disorders, CVD - Cardiovascular disease, EM - Endometriosis, TD- Thyroid disease, UF- Uterine fibroids

To determine melatonin level, non-stimulated saliva was collected strictly at a fixed time 4 times a day (6 a.m. – 7 a.m., 12 p.m. – 1 p.m., 6 p.m. – 7 p.m., 11 p.m. – 12 a.m.) into a special 2 ml capsule, immediately frozen, and stored at -20°C. Melatonin content was determined by immunoassay using Microplate Reader EL×808 (USA). Salivary melatonin levels were expressed in pg/mL.

Statistical analysis was performed using STATISTICA 6.1 software (Stat-Soft Inc., USA). For descriptive analysis, results are presented as mean±standard deviation (SD), median, interquartile range (IQR; 25th to 75th percentiles). For data with normal distribution, inter-group comparisons were

performed using Student's t-test. Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney *U*-test. Categorical variables were analyzed using the Chi square test. A probability value of $P<0.05$ was considered statistically significant.

Results

Perimenopausal women often complained about difficulties falling asleep (more than 20 minutes from the moment the light was turned off) and difficulties awakening in the morning, while postmenopausal women often complained about snoring and frequent awakenings during sleep(≥2 times) (Table 2). ISI was 21.3±0.54 in Group 1 and 24.8±0.31 in Group 2, which corresponded to insomnia.

Table 2.

Basic sleep problems in menopausal women (results of questioning)

Complaint	Perimenopausal women (n=33)	Postmenopausal women (n=44)	P-value
Falling asleep difficulties	31 (93.9%)	15(34.1%)	0.000
Frequent awakenings during sleep	12 (36.4%)	28(63.6%)	0.043
Morning awakenings difficulties	26 (78.8%)	13(29.5%)	0.000
Snoring (sleep apnea)	7 (21.2%)	20(45.5%)	0.049
Excessive daytime sleepiness	7 (21.2%)	20(45.5%)	0.049
Restless legs syndrome	3(9.1%)	6(13.6%)	0.796

Melatonin levels in climacteric women without insomnia are presented in Table 3. In subgroups without insomnia, postmenopausal women had significantly lower daytime and nighttime melatonin secretion than perimenopausal ones.

The highest melatonin level in perimenopausal women from the control group was during the night hours; the level decreased during the morning hours and then tended to increase in the daytime and in the evening (Table 3). Daily melatonin secretion in perimenopausal patients with insomnia was altered – the maximal level was registered in the morning hours. A comparison of the two groups showed that in women with insomnia, the melatonin level in the daytime, in the evening, and at night was below that of the control group by 2.2($P<0.05$), 2.3($P<0.05$) and 1.3 times, respectively. However, in the morning these women had a 1.5 times ($P<0.05$) higher melatonin level than the control group (Fig. 1).

The circadian rhythms of melatonin secretion in the group of postmenopausal women did not correlate to the occurrence of insomnia. Postmenopausal women from the control group had the peak of melatonin secretion during morning hours, while melatonin concentration during the daytime and in the evening decreased. A similar tendency in melatonin secretion was observed in postmenopausal patients with insomnia (Fig. 2).

Table 3.

Melatonin levels in peri- and postmenopausal women with or without insomnia

Variable	Perimenopause		Postmenopause		P-value
	Insomnia– (n=39)	Insomnia+ (n=33)	Insomnia– (n=30)	Insomnia+ (n=44)	
	(1)	(2)	(3)	(4)	
	Mean ± SD Median 25th – 75th percentile				
Melatonin 6 a.m.-7 a.m., pg/ml	6.86±4.42 6.86 2.79-8.46	10.52±6.20 10.07 6.02-11.25	11.02±8.16 9.80 3.88-16.86	9.54±8.49 7.12 3.33-9.83	p_{1-2}=0.011
Melatonin 12 p.m.-1 p.m., pg/ml	4.91±3.36 5.71 1.64-8.93	2.27±1.59 2.34 0.82-3.14	2.21±1.16 2.39 1.43-2.61	3.60±3.78 2.56 0.94-5.27	p_{1-2}=0.031 p_{1-3}=0.021
Melatonin 6 p.m.-7 p.m., pg/ml	4.94±5.20 4.92 1.35-5.44	2.12±2.21 1.32 0.52-2.40	1.42±1.30 1.22 0.43-2.20	2.02±1.79 1.38 0.88-2.45	p_{1-2}=0.009 p_{1-3}=0.000 p_{1-4}=0.022
Melatonin 11 p.m - 12 a.m., pg/ml	10.03±7.38 9.51 6.40-10.67	7.97±5.07 7.97 3.88-9.53	5.66±2.25 6.08 3.77-7.30	8.29±6.59 8.02 5.28-8.79	p_{1-3}=0.008

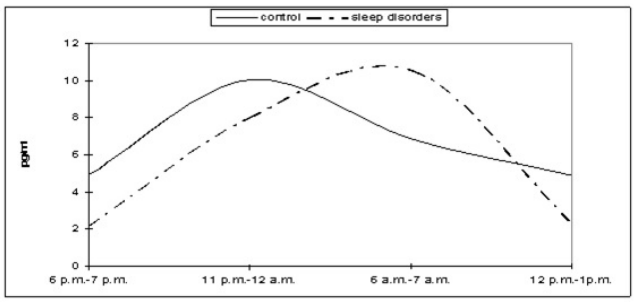

Fig. 1. *Circadian rhythms of melatonin secretion in perimenopausal women with or without insomnia*

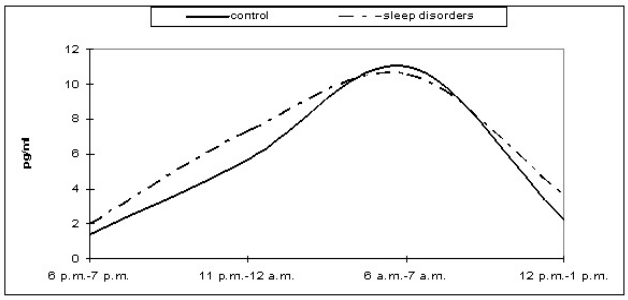

Fig. 2. *Circadian rhythms of melatonin secretion in postmenopausal women with or without insomnia*

Discussion

Some studies have shown an age-related decrease in the concentration of melatonin at the night peak.[6,16,17] This fact

indicates a decrease in those epiphysis functions that produce melatonin, which is a consequence of functional changes in the pineal gland and other links of the circadian system of the organism in the process of aging.[18] An age-related decrease in melatonin secretion is indicative of impaired pineal and pituitary control over ovarian rhythm and a progressive decline in fertility function.[19] It has been found that older females have significantly lower urinary 6-sulfatoxymelatonin than older males.[20] The results of some studies have demonstrated that melatonin and estrogens act on the feedback principle.[2]

LI Mal'tseva et al.[21] showed a decrease in melatonin level in most climacteric women that depended on menopause severity. However, changes in perimenopausal women were ambiguous and the melatonin level was increased in 13% of patients. Toffol et al.[22] examined how the serum melatonin concentrations in perimenopausal and postmenopausal women influence mood, sleep, vasomotor symptoms, and quality of life. It was shown that postmenopausal women had lower nighttime serum melatonin concentrations than perimenopausal women. The duration of melatonin secretion was shorter in postmenopausal women, whereas melatonin peak time did not differ.[22] The results of another study showed that nocturnal serum melatonin secretion in premenopausal women declined moderately from 17 to 45 years of age, and increased in the period from 46 to 50 years of age. Among postmenopausal women, a steep, age-related decline in nocturnal melatonin secretion was found for up to 15 years postmenopause, followed by an extremely gradual decline thereafter.[23] Waleca-Kapic et al.[24] found a negative correlation between urinary 6-sulfatoxymelatonin excretion and BMI in overweight postmenopausal women, which confirms the influence of melatonin on metabolism.

The relationship between melatonin secretion and SDs has been shown in many studies.[8,9,25,26] Thus, it was shown that in climacteric women insomnia was accompanied by a decreased melatonin level in blood serum.[26] Our data give some new insight into the role of melatonin in SDs of menopausal women. We found that the circadian rhythm of melatonin secretion in perimenopausal women with SDs is disrupted. Similar results were obtained by Parry et al., who studied the relationship between melatonin, menopausal depression, and sleep end time. According to the authors, the increased melatonin secretion that is phase delayed into the morning characterized menopausal depressed patients.[9] The authors suggested that higher melatonin levels or delayed offset in depressed patients might be caused by a long duration of sleep in women who may be trying to compensate for sleep disturbances.

In addition, we showed that peak melatonin secretion in postmenopausal women with and without SDs is registered in the early morning hours. Probably, changes in timing of the melatonin rhythm during postmenopause does not play a causal role in sleep disruption. Similar findings were suggested by Duffy et al.,[27] who investigated the relationship between sleep timing and melatonin circadian rhythm in young and older subjects without sleep complaints. The relationship between plasma melatonin rhythm and sleep time was such that the older subjects were sleeping and waking earlier relative to their nightly melatonin secretory episode. Consequently, the older subjects were waking at a time when they had higher relative melatonin levels, in contrast to younger subjects, whose melatonin levels were relatively lower by the time they woke up. However, the relationship between melatonin rhythms and sleep in subjects with sleep disorders was not investigated.

Conclusion

According to our results, nearly half of perimenopausal and more than half postmenopausal women have insomnia. Our findings suggest that insomnia in perimenopausal women is associated with altered 24h melatonin secretion that is characterized by a shift of the peak secretion from nighttime to the early morning hours, while there is a tendency for melatonin levels to decrease in postmenopausal women. Based on our study results, we can recommend administration of melatonin in the evening time and light therapy in the early morning hours in the complex treatment of SDs in perimenopausal women for normalizing and shifting the chronobiological rhythms of melatonin secretion, and specific therapy is aimed to eliminate snoring for postmenopausal women.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 131-134
http://dx.doi.org/10.21103/Article7(2)_OA9

ORIGINAL ARTICLE

Biotechnology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Prolongation of Anti-Inflammatory Activity of Glucocorticosteroids Encapsulated in Large Oligolamellar Liposomes in Treatment of Arthritis in Rabbits

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Abstract

Background: Liposomes have been shown to be an effective targeted drug delivery system used to decrease side effects of glucocorticosteroids in the treatment of rheumatoid arthritis.

Materials and Results: Experimental arthritis was induced in rabbits by a single intra-articular administration into the knee joint of poly-D-lysine (molecular weight, 175 kDa) and hyaluronic acid (7.5 mg per administration). To determine temperature readings over the joint a standard radiator was used with a temperature of 32^0C. Large oligolamellar liposomes from different phospholipids and and cholesterol containing hydrocortisone acetate in lipid phase and prednisolone hemisuccinate in water phase were used.

Conclusion: Intra-articular administration of the water-soluble prednisolone hemisuccinate (0.125 mg) and the lipid-soluble hydrocortisone acetate (0.125 mg) into the knee joint in the aqueous and lipid phases of large oligolamellar TSL (DPPC + 20 mole % cholesterol) prolongs the anti-inflammatory effect produced by glucocorticoids by 7–8 days compared to 1 day for free glucocorticosteroids at a total dose of 2.5 mg and 2 days for phosphatidylcholine-cholesterol liposomes at a total dose of 0.25 mg in rabbits with aseptic arthritis. **(International Journal of Biomedicine. 2017;7(2):131-134.)**

Key Words: aseptic arthritis • thermosensitive liposomes • glucocorticosteroids • anti-inflammatory activity

Abbreviations

DPPC, dipalmitoylphosphatidylcholine; **HA**, hydrocortisone acetate; **PH**, prednisolone hemisuccinate; **PhC,** phosphatidylcholine **TSL**, thermosensitive liposomes.

Introduction

Since common treatments for rheumatoid arthritis—such as nonsteroidal anti-inflammatory drugs, corticosteroids, disease modifying anti-rheumatic drugs and some biological agents—have proven to be unable to achieve drug-free remission,[1] a number of targeted drug delivery strategies have been developed in order to attenuate side effects to other tissues. These include microemulsions, microspheres, liposomes and others, of which liposomes have been shown to retain the drug in the synovial cavity effectively due to their chemical composition and size.[2,3] In our previous work we have demonstrated prolongation of the anti-inflammatory effect produced by HA encapsulated into the membrane of multilamellar TSL composed of DPPC and cholesterol in rabbits with aseptic arthritis.[4] However, this prolongation was limited to only about 5 days compared to 1 day in the case of intra-articular administration of free hydrocortisone acetate or 2 days for its liposomal form composed of egg lecithin and cholesterol. At the same time, no attempts have been done so far to study the effectiveness of administration of a combination of water-soluble and lipid-soluble glucocorticosteroids entrapped in the aqueous and lipid phases of 1 liposome formulation.

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The study was aimed at evaluating the potential for prolonging the anti-inflammatory activity of glucocorticosteroids encapsulated into TSL composed of DPPC and cholesterol, with the aqueous phase containing water-soluble PH and the lipid phase containing lipid soluble HA.

We compared glucocorticosteroids entrapped in liposomes with free glucocorticosteroids at a 10 times higher dose. Two types of liposomes were compared – egg-phosphatidylcholine (PhC) liposomes with a melting phase transition temperature (T_m) of $+10^0$C and DPPC liposomes with a T_m of 41.5^0C. Adding cholesterol to the liposome membrane decreases the amplitude and increases the T_m range. If liposomes are made from DPPC and 20% mole cholesterol, their membrane is in a metastable state over the temperature range from 37^0C to 47^0C, including the inflammation temperature range.[5]

Methods

Experimental arthritis was induced in rabbits by a single intra-articular administration into the knee joint of poly-D-lysine (molecular weight – 175 kDa) and hyaluronic acid (7.5 mg per administration).[6] There were four groups of rabbits containing 5 animals each. The first group received 0.5 ml saline, the second group received a 0.5 ml mixture of PH (1.25 mg) and HA (1.25 mg), the third group and the fourth received liposomal form containing both glucocorticosteroids at a 10 times lower dose (0.125 mg), the third group receiving egg-PhC liposomes and cholesterol and the fourth group receiving DPPC liposomes and cholesterol. In all groups, the injection was made on the third day after arthritis induction and at the very peak of the inflammatory reaction. Large oligolamellar liposomes were obtained using the reverse-phase technique.[7] Egg-PhC and DPPC purchased from Lipoid, Germany, and cholesterol purchased from Avanti Polar Lipids, Inc., USA, were taken at a ratio of 7:2 (molar), 21 µmol and 6 µmol correspondingly, and placed into a round-bottom flask. Then HA in chloroform and 1µCurie of ^{3}H-HA (specific activity of 48 µCurie/µmol, Izotop, St Petersburg, Russia) were added. The solvent was removed by a rotary evaporator to achieve dryness; the lipid film was cleared of residual chloroform under vacuum by an evaporator (Rotavapor R-114, Buche, Switzerland), dissolved in 3ml of freshly prepared diethyl ether and 9ml of saline containing 5 mg of PH and 1µCu of ^{51}Cr-EDTA (specific activity 100 µCu/ml, Izotop,Russia). The system was processed in a sonicator (UZD-H-1, Sumy, Ukraine) at a frequency of 22 kHz and a power 630 W of at $+4^0$C under argon protection. The obtained reverse emulsion (oil-in-water) was transformed into regular emulsion (water-in-oil) by eliminating the organic solvent using a rotary evaporator (Rotavapor R-114, Buche, Switzerland). Then the liposome emulsion was kept for two hours at 37^0C for egg-PhC and at 50^0C for DPPC and centrifuged for 1 hour at 10000×g using a Sigma 6K10 centrifuge, Germany, to spin down liposomes. Preliminary experiments have shown HA encapsulation into the liposome lipid phase to be 96%–98% as calculated from ^{3}H-HA inclusion into the liposome membrane, while PH encapsulation has been shown to amount to 30%–36% as calculated from ^{51}Cr-EDTA inclusion. The liposome residues were resuspended in the saline to reach HA and PH concentrations of 0.25 mg/ml each. The lipid concentration in samples amounted to 2mg/ml. The liposome samples were used within 1 week. Liposomal preparations were checked for sterility, and whenever possible argon protection was used. The inflammatory reaction in the joint was registered by a thermal camera. To determine temperature readings over the joint a standard radiator (Pergamed, Russia) was used with a temperature of 32^0C. Both hyperthermia severity and hyperthermic area, calculated from negative images using planimetric analysis, were taken into account. Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996)

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). The mean (M) and standard error of the mean (SEM) were calculated. The Wilcoxon criterion was used to compare the differences between the paired samples. Pearson's correlation coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P<0.05$ was considered statistically significant.

Results

A mixture of free HA and PH at a dose of 2.5 mg (1.25 mg per each glucocorticosteroid) caused a statistically significant ($P<0.001$) decrease in temperature over the joint 24 hours after the administration, which came back to baseline 1 day after the decrease (i.e. the anti-inflammatory effect lasted for a little over 1 day) (Fig.1). Glucocorticosteroids encapsulated in the aqueous and lipid phases of PhC/cholesterol liposomes (0.125 mg of each glucocorticosteroid amounting to 0.25 mg) also had an effect 1 day after administration (Fig.1), but their effect was statistically significantly ($P<0.01$) prolonged up to 2 days, the total dose being 10 times less than in free glucocorticosteroids.

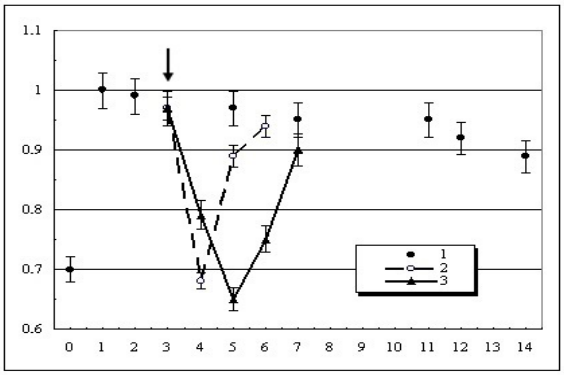

Fig. 1. Anti-inflammatory effect of mixed water-soluble and lipid-soluble free or encapsulated into lecithin-cholestrol liposomes glucocorticoids.

X-line: time after intra-articular administration of poly-D-lysine and hyaluronic acid (days),
Y-line: relative thermometrical index (relative units). The arrow indicates the time of drug administration.
(1) – no treatment; (2) – administration of mixed HA and PH (1.25 mg of each drug amounting to a total of 2.5 mg); (3) liposomes from PhC and 20% mole cholesterol containing 0.125 mg of HA and 0.125 mg of PH (0.5 mg of lipids in 0.25 ml of saline).

The anti-inflammatory effect calculated from both hyperthermia severity and inflammation area yields consistent results (Fig.2). The correlation test showed a strong positive correlation between these parameters: r=+0.88±0.180 (P<0.01). The structure and permeability of liposomes are known to change in the lipid melting phase-transition temperature (T_m) range.[8] This property of lipids is used to increase the rate of drug release from the aqueous phase of the vesicles due to hyperthermia. There are a number of reasons to believe that bringing closer the phase states of the vesicle membrane and the plasma membrane of the target cells, which are synovial cells in this case, may facilitate their interaction.[9]

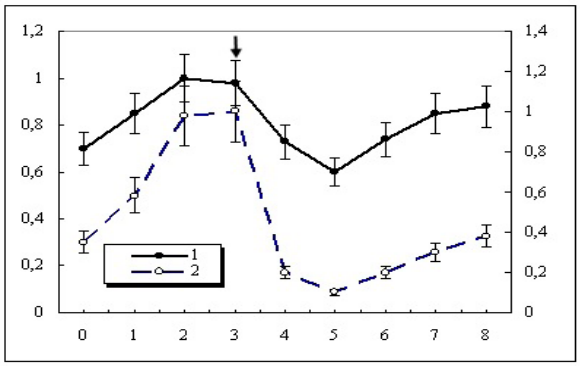

Fig. 2. Hyperthermia severity and hyperthermic area correlation.

X-line - time after arthritis induction (days).Y-line (left) - thermometric index; (right) - inflammation area index (relative units).
(1) - thermometric index after administration of mixed water-soluble and lipid-soluble encapsulated into egg-PhC and 20% mole cholesterol liposomes glucocorticoids; (2) - inflammation area index.

The duration of the anti-inflammatory effect produced by mixed glucocorticosteroids (HA and PH) entrapped in DPPC/cholesterol liposomes proved to be significantly longer, reaching 7–8 days after the drop in temperature (Fig.3).

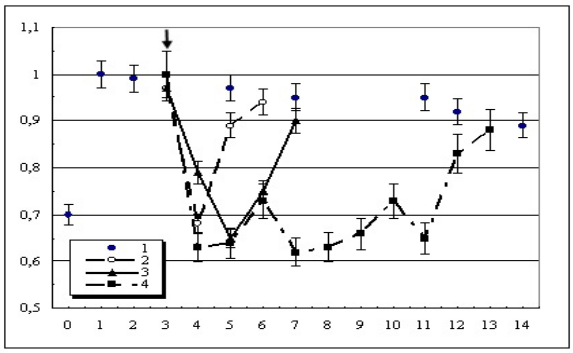

Fig. 3. Prolongation of the anti-inflammatory effect of HA and PH encapsulated in the liposomal membrane with various melting phase transition temperature**.**

X-line: time after intra-articular administration of poly-D-lysine and hyaluronic acid (days),
Y-line: relative thermometrical index (relative units). The arrow indicates the time of drug administration.
(1) - no treatment; (2) - administration of mixed HA and PH (1.25 mg of each drug amounting to a total of 2.5 mg); (3) - liposomes from PhC and 20% mole cholesterol containing 0.125 mg of HA and 0.125 mg of PH (0.5 mg of lipids in 0.25 ml of saline); (4) - liposomes from DPPC and 20% mole cholesterol containing 0.125 mg of HA and 0.125 mg of PH (0.5 mg of lipids in 0.25 ml of saline).

The temperature drop rate over the joint was the same for the both types of liposomes. However, when DPPC/cholesterol liposomes were used, the anti-inflammatory effect lasted for up to 7–8 days after administration.

Discussion

This shows that the prolonged effect of the anti-inflammatory action produced by mixed glucocorticosteroids (HA and PH) entrapped in DPPC/cholesterol liposomes is due to both HA present in the lipid phase and PH present in the aqueous phase of the liposomes. Since HA alone encapsulated in DPPC/cholesterol liposomes yielded a prolongation of only 5 days,[4] the greater prolongation effect can be attributed only to the fact that water soluble PH was also administered. It can also be assumed that when DPPC/cholesterol liposomes come into contact with inflammatory cells within the joint, the effectiveness of inflammatory cell (neutrophils, monocytes) membrane merger depends on the closeness of the phase state of liposome lipids and the inflammatory cells at inflammation temperature. Due to the hypothermic effect of the mixed glucocorticosteroids and the temperature decrease down to 32°C, the vesicles in the joint change to the solid crystalline state so that the period of their utilization increases as they stay there as a depot. However, in this state the water-soluble PH leaves the liposomes along a concentration gradient, which leads to an additional anti-inflammatory effect. The temperature rise peak during the third day after the first temperature drop following the administration of DPPC/cholesterol liposomes is of special importance. We believe that a slight increase of temperature followed by a temperature decrease over a short period of time indicates that the temperature increase in the joints transforms DPPC/cholesterol liposomes once again from the solid crystalline state into liquid crystalline state and HA once again shows an anti-inflammatory effect. This phenomenon clearly demonstrates that the activity of HA encapsulated in DPPC/cholesterol liposomes is temperature related.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We thank Evgenia Zvonareva for technical assistance and translation of the manuscript into English.

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International Journal of Biomedicine 7(2) (2017) 135-137
http://dx.doi.org/10.21103/Article7(2)_OA10

ORIGINAL ARTICLE

Biotechnology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Optical Barrier for Microbiological Control after a Sterilization Process

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Abstract

An optical barrier (OB) may eliminate the entrance of aerosol generated by clinical procedures in sterilization devices. The OB is a new alternative for sterilizing medical and dental instruments. The objective of the study was to evaluate the action of the OB on the entrance of bacteria into an autoclaving system and to correlate the time and distance of exposure. To test the configuration of the device, we used *Escherichia coli.* A lamp utilized in this instrument was low pressure with a wavelength around 254 nm. A homogenous distribution of light around the door was observed by the Inventor 2015 software. Microbiological tests showed efficient bacterial elimination at a distance of 8 cm from OB. The results show that the use of the OB radiation for 30 minutes guarantees the non-entry of microorganisms into the sterilized environment. The use of the OB may be recommended to maintain the surface of sterile materials for long periods of time. **(International Journal of Biomedicine. 2017;7(2):135-137.)**

Key Words: Optical barrier • bacteria • UV light • *Escherichia coli* • biomedical device

Introduction

Cross-contamination is a problem in the healthcare area which requires considerable attention, given that is necessary to promote the security of the patient during medical procedures and to eliminate the possibility of transmitting infectious diseases. Thus, a sterilization process is necessary, and one device used to kill microorganisms present in materials used in medical offices is the autoclave.[1]

Medical offices have a very high concentration of microorganisms in the air. Currently, the healthcare professional puts medical instruments into packages after applying the protocol to eliminate biological organisms (spores, bacteria, fungi, etc.). However, a lot of different concepts and methods for sterilizing materials can be found in the literature. One example present in the discussion is the use of ultraviolet (UV) light. Nevertheless, several factors need to be addressed, such as the procedure for cleaning equipment, existing and new materials to be used, and the development of new methods and instruments.[2-8]

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A common problem in dental autoclave devices is the increased probability of direct contact of microorganisms present in the environment with the interior of the autoclave after the door is opened, consequently decreasing the shelf-life of instruments already sterilized. The purpose of this study was to assess the effectiveness of a new adaptation of sterilization devices, an optical barrier UV to remove or inactivate microbial aerosol.

Materials and Methods

Lamp UV device system

The device contains one low-pressure mercury UV lamp (HNS-4W-OSRAM) with an emission wavelength of approximately 254 nm (Fig. 1), fixed by two supports and surrounded by a polymer curtain that focuses the UV rays towards the front slot of the autoclave (Fig. 2).

The autoclave is equipped with a sensor located on the side of the door such that the lamp system is switched on or off when the door is opened or closed, respectively.

Simulation of the curtain

A design of the prototype was made using Inventor 2015 software (Autodesk Inc.). In addition, an optical device

was developed with a light curtain designed to direct the UV light straight down. A simulation was done using TracePro 6.0 software (Lambda Research Corporation) in order to evaluate the distribution of the radiance in the front side of the autoclave.

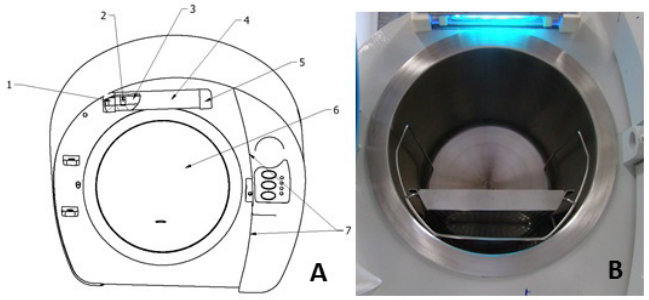

Fig. 1. A) *Front layout of the optical curtain: 1) Connector cap of UV lamp; 2) Reel for stabilizers a device on wall; 3) Quartz tube and a UV Lamp inside; 4) Polymer structure for direct a rays; 5) Protector of the cap; 6) Autoclave tank; 7) Sensor for control switches.* ***B)*** *Photograph of a tray device in the autoclave.*

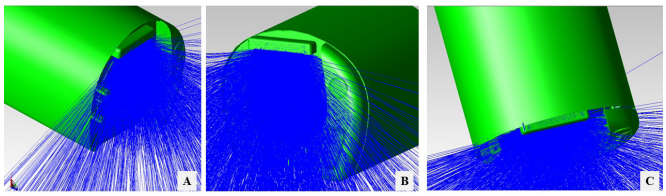

Fig. 2. *Curtain and distribution of light on the autoclave door in wavelength 254 nm with OSRAM UV lamp 4 W; A) left side view; B) Right side view; C) top view.*

Microbial strains and growth conditions

The microorganism used for the *in vitro* study was *Escherichia coli* (ATCC 25922). *E. coli* was cultivated in Brain Heart Infusion Broth at 37 ºC for 18 h. The microbial inoculum was centrifuged (1000x*g* for 15 min) and re-suspended in buffered phosphate saline. The initial inoculum was adjusted for 10^7cells/mL at 600 nm. For the analysis of the antimicrobial effect of optical barrier, plates containing *E. coli* (10^7 cells/mL) were positioned below the optical barrier and 5cm from the barrier.

Results and Discussion

We used an optical simulation in order to describe the behavior of light emitted from the UV lamp, and to characterize the device after mechanical construction. Figure 3 shows the radiance distribution in the area of the autoclave door. This model is suited for device simulation purposes of microbial decontamination in this study.

The different views in the figure above show the uniform distribution of the UV light around the opening of the tank. A second step in the simulation involves evaluating the intensity and distribution of the light in three distinct areas of the autoclave. The results are shown on Figure 4.

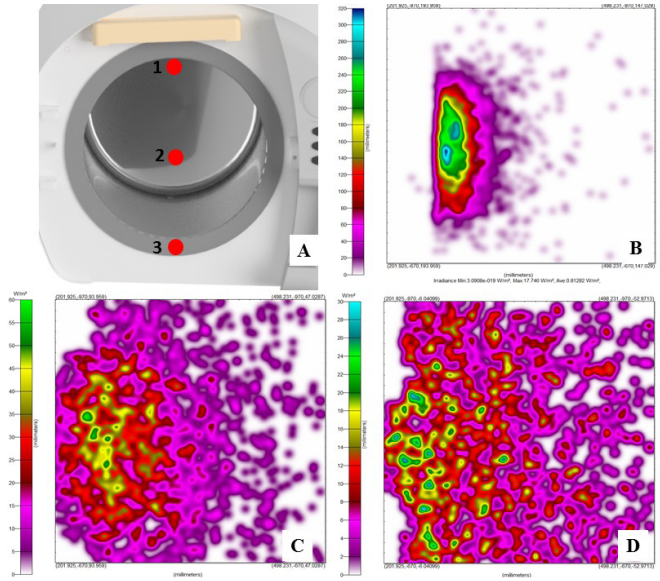

Fig. 3. *(A) Intensity measurement locations. (B) Intensity distribution on Location 1. (C) Intensity distribution on Location 2. (D) Intensity distribution on Location 3.*

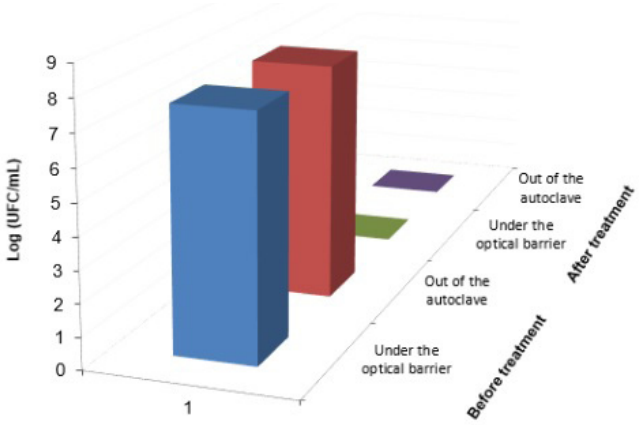

Fig. 4. *Antimicrobial response of UV barrier during 30 min. Plate with E. coli located on door area directly under UV light and plate in front of autoclave during 60 min of UV barrier.*

The results of the simulation show us that there is an even distribution of UV-C light in the front of the tank; however, microbiological experiments were performed for proof of concept. First, we present the results for plates with *E. coli* with initial concentration of 10^7CFU/mL.

The results show two different positions of the plates. Plate 1 is located directly under the light and Plate 2 is located out on the front of the autoclave. It is possible to verify that there is a reduction in the microbial agent in the plate directly exposed to light and within 5 cm on the front of the autoclave.

Another characterization performed consisted of measuring the time necessary for the microorganisms to increase in the autoclave with the light on and off. The results show the time necessary for recontamination of materials present in the autoclave after having been processed (Table 1).

Table 1.

Results of bacterial presence over time. The X denotes detection of colonies.

Condition	Time (min)				
	9	10	20	30	60
Light off	X	X	X	X	X
Light on				X	X

There is immediate detection of microorganisms with the UV light off; however, with the UV light on, the minimum time necessary for detection is 30 min.

UV light present at the front of the autoclave constitutes a practical method for establishing a barrier for microorganisms present in the environment and also for preventing recontamination of packages or instruments after the autoclaving process.

Our experimental setup shows that it is possible to adapt a barrier onto the autoclave. Future studies would consist of establishing different criteria for optimization of the device and testing different positions of the sensors. There are many UV light devices tested for viruses and bacteria by USEPA**,** the National Science Foundation, and WHO.[9]

UV radiation induces mutations in the pyrimidine dimers of DNA.[10] Distribution of light intensity shows that there is an even distribution of UV-C wavelengths in the front of the tank, which explains the oxidative damage in bacteria. Thus, it is possible that the air could be sterilized with total bacteria death within a margin of 15 cm of the optical barrier.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors acknowledge the support provided by FAPESP (São Paulo Research Foundation) – grant number: 2013 / 07276-1 (CEPOF – CEPID Program).

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International Journal of Biomedicine 7(2) (2017) 138-140
http://dx.doi.org/10.21103/Article7(2)_CR1

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Rare Presentation of Pulmonary Echinococcosis

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Abstract

In this paper, we present a rare case of pulmonary cyst echinococcosis, in which the patient presented with no symptoms and was misdiagnosed as having pulmonary tuberculosis. Our case is a prime example of why echinococcosis should be part of the differential when dealing with an immigrant population. **(International Journal of Biomedicine. 2017;7(2):138-140.)**

Key Words: pulmonary cyst echinococcosis • tuberculosis • fine needle aspiration • albendazole

Abbreviations

AFB, acid-fast bacillus; **CXR,** chest X-ray; **CT,** computed tomography; **FNA,** fine needle aspiration; **TB,** tuberculosis

Introduction

Pulmonary cyst echinococcosis (PCE) is a parasitic infection caused by the tapeworm genus *Echinococcus*. Infections in the United States are unusual and are mostly among immigrants from endemic countries. Initially PCE is asymptomatic and incidentally found on a routine X-ray. Symptoms start to develop when cyst volume increases and applies pressure on surrounding tissues. The most common symptoms are cough, chest pain, dyspnea, and hemoptysis.[1] Feared complications of cyst rupture are anaphylactic and septic shock, dissemination of cyst contents, along with empyema and pneumothorax.[1,2]

Diagnosis is usually established by a combination of the clinical and radiological presentation, where serology and cytomorphology are required for confirmation. In this paper, we present a rare case of PCE, in which the patient presented with no symptoms and was misdiagnosed as having pulmonary tuberculosis. After thorough evaluation in our hospital, patient was diagnosed with pulmonary cystic echinococcosis and received proper treatment.

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Case report

A 19-year-old male walked into emergency department; (ED) with a self-reported TB history and requested treatment. The patient presented with no complaint, denied any history of cough, sputum production, shortness of breath, night sweating, or known TB contact. He reported he had migrated from Ecuador 1 month prior to the ED visit and was initially held in a detention facility while awaiting immigration clearance. While detained, he was diagnosed with TB and started on treatment with 4 medications. After his papers were cleared, he was advised to seek medical attention in any hospital at his final destination.

On initial presentation, a physical exam was unremarkable, CXR was significant for right lower lung mass measuring 3.4 cm (Fig. 1), CT chest (Fig. 2) was positive for well circumscribed RLL mass with a small peripheral area of cavitation or air pocket and no mediastinal lymphadenopathy, and laboratory revealed elevated transminases. Patient was placed on airborne isolation. Quantiferon TB Gold and AFB sputum and cultures were sent and are pending. His detention facility was contacted for additional collateral tests. Their investigation revealed similar CXR findings and a positive purified protein derivate test; however, they reported 3 negative AFBs. At this that time, he was started on active

TB treatment. Our differential diagnosis remained broad, including hamartoma, granuloma, TB, and neoplastic process.

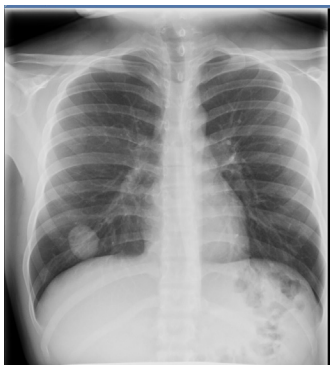

Fig. 1. *Chest X-ray*

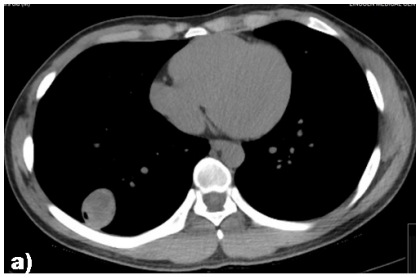

Fig. 2. *Chest CT scan*

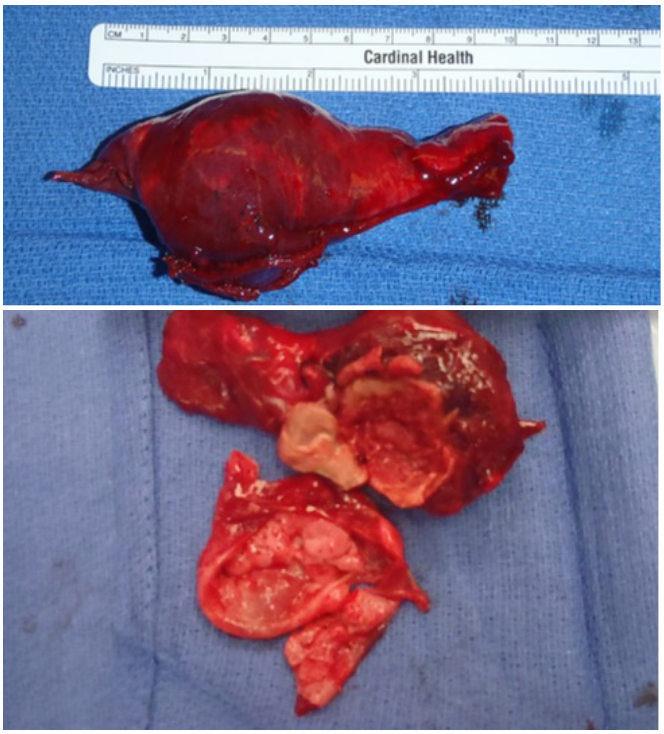

Fig. 3. *Hydatid cyst removed during the surgery*

Taking into account lack of symptoms, no history of TB contact, and low probability of TB on radiological images, active TB treatment was not re-initiated. By this time, the results of Quantiferon and 3 AFBs were negative. The decision was made to proceed with CT-guided FNA. An initial quick pathology read revealed particles of parasites, with a high suspicion of echinococcal cyst. Further work-up didn't find any features consistent with echinococcosis. No evidence of echinococcosis was found on CT abdomen or on brain. The final pathology report confirmed particles of echinococcal cysts. The patient underwent an open right thoracotomy with large wedge resection of the lung and was started on treatment with albendazole (Fig.3). Patient was discharged home in good condition and came for follow-up for suture removal, as well as parasite clinic for completion of albendazole therapy and had no complaints.

Case discussion

Echinococcus granulosus is a zoonotic parasitic infection caused by the larva of the dog tapeworm, and is known to cause PCE.[1] It is of particular concern in Asia, South America, the Middle East and Africa, where its prevalence is close to 15%.[3,4] Within the United States, PCE is quite unusual and mostly seen within immigrants from endemic countries. In an immigrant patient with a suspicious pulmonary lesion, PCE should be within the differential diagnosis. Raising awareness among physicians about PCE would decrease the associated disease burden.

Initially, PCE is asymptomatic and is often an incidental finding on routine X-rays. Symptoms begin to develop as the cyst enlarges and starts to apply pressure on surrounding structures. The most common symptoms are cough, chest pain, dyspnea, and hemoptysis.[1] PCE is most often diagnosed by a combination of clinical and radiological findings, which is then confirmed by serology and cytomorphology. Radiological diagnosis begins with CXR, which will show a typical well-defined cystic lesion. Unruptured cysts are often indistinguishable from a variety of other pulmonary lesions.[2,5] As the cyst enlarges, it will erode neighboring bronchioles producing air accumulation between cyst layers. This results in the characteristic "meniscus," "onion peel," or "water-lily" signs seen on radiographic imaging. While CT is not needed to establish diagnosis, CT will reveal a more detailed picture of the cyst and of surrounding structures. In addition, abdominal ultrasound should be part of the routine work-up as 20% of PCE cases have concomitant liver involvement.[2] Diagnostic percutaneous aspiration of the lung cyst is considered controversial due to possible cyst rupture, dissemination of cyst content, and anaphylaxis. Treatment of PCE is surgical removal with concurrent chemotherapy. The drug of choice is albendazole.

In our literature review of past publications, Echinococcosis is most often confused with lung malignancy.[6-8] There are several cases of an atypical presentation, in which radiographic features on CXR and CT are suggestive of lung malignancy. FNA and bronchoscopy were inconclusive and diagnosis of PCE was established at the time of surgical removal.

Conclusion

Diseases once rare within the United States are now found to be more prevalent due to the increase in immigration.

Because of this change, physicians should broaden their scope when considering their differential of infectious diseases. Our case is a prime example of why echinococcosis should be part of the differential when dealing with an immigrant population.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 141-143
http://dx.doi.org/10.21103/Article7(2)_CR2

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Disappearance of a Hepatic Mass in Anti-Synthetase Syndrome

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Abstract

Anti-Synthetase Syndrome (ASyS) is a rare chronic autoimmune disorder characterized by myositis, interstitial lung disease (ILD), polyarthralgia, "mechanic's hands" and Raynaud's phenomenon. Liver lesions are quite rare in ASyS. In our ASyS case, we will discuss a 58-year-old man presenting with muscle weakness, arthralgia, and ILD. He was positive for anti-Jo-1 antibodies, substantiating the diagnosis, and was started on treatment. This was followed by the appearance of a liver mass that disappeared when the patient achieved remission.**(International Journal of Biomedicine. 2017;7(2):141-143.)**

Key Words: Anti-Synthetase Syndrome • anti-Jo-1 antibody • nodular regenerative hyperplasia • treatment

Abbreviations

ASyS, Anti-Synthetase Syndrome; **CT,** computed tomography; **ILD**, interstitial lung disease; **ILM**, idiopathic inflammatory myopathies (IIM); **MSA**, myositis-specific autoantibodies; **NRH**, nodular regenerative hyperplasia.

Introduction

Autoantibodies are a hallmark in the diagnosis of many systemic autoimmune rheumatic diseases, including idiopathic inflammatory myopathies (IIM).[1-3] A number of autoantibodies, called myositis-specific autoantibodies (MSA), have been described in IIM.[4,5] The presence of MSA is a key feature for diagnosis of clinically distinguishable IIM subsets (polymyositis (PM), dermatomyositis (DM), inclusion body myositis, and myositis associated with malignancy and other connective tissue diseases).

Among MSA, autoantibodies against aminoacyl-tRNA synthetases (ARS) were detected in 25%–35% of IIM patients.[6] The anti-Jo-1 antibody directed against the antihistidyl–tRNA synthetase is the most common of anti-ARS autoantibodies, predominantly found in 15%–30% of patients with polymyositis and in 60%–70% of those with ILD.[7] Anti-ARS autoantibodies, especially the anti-Jo-1 antibody, characterize their own clinical IIM phenotype, which has become known as Anti-Synthetase Syndrome (ASyS) and which is characterized by multiple organ involvement, primarily ILD, and is often accompanied by myositis, non-erosive arthritis, Raynaud's phenomenon, "mechanic's hands," skin rashes, and constitutional symptoms, such as fever.[8,9] With proper diagnosis and early initiation of therapy, ASyS is potentially treatable. In this text, we report the rare appearance and disappearance of a liver mass in ASyS as the patient's condition improved.

Case report

A 58-year-old male with a history of diabetes mellitus and asthma presents to the emergency room complaining of swelling and pain of the lower extremities, generalized weakness, and weight loss for about 2 months' duration. Upon further questioning, he agrees that he has joint pain, stiffness, shortness of breath, and decreased exercise tolerance. He has also noticed a bluish discoloration of the fingers in cold weather. His weakness was throughout his body, but worse in the arms, and was associated with numbness and tingling, which had prompted a work-up for cervical radiculopathy the month prior at the neurosurgeon's office, whereby electromyography showed left ulnar neuropathy. A physical exam found loss of grip strength, "mechanic's hands," lungs

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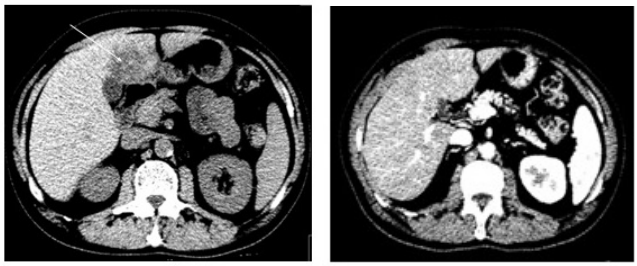
that are clear to auscultation, synovitis of the wrists bilaterally, tenderness and bony enlargement of the first, second, and third metacarpophalangeal joints, lower extremity edema, and proximal muscle weakness of three-fifths strength. Labs noted to be elevated are creatinine kinase, aldolase, and a positive result for anti-Jo-1 antibodies. Rheumatoid factor, anti-CCP, and ANA were negative, and liver function test unremarkable. A chest X-Ray revealed increased interstitial marking, warranting a chest CT, which showed patchy ground-glass areas in the lung bases suggestive of possible ILD. The patient was started on prednisone 60 mg and azathioprine 50 mg. A repeat chest CT 4 months after initiating medication showed resolution of ground-glass opacities, but noted a hypodense hepatic mass. A biopsy was offered but declined by the patient. Gradually, prednisone was tapered and azathioprine up titrated to 150 mg daily. Five months later, a follow-up abdominal CT showed resolution of the hepatic mass (Fig.1). The patient's disease is currently in remission, with resolution of symptoms at 2.5 mg prednisone daily and 150 mg of azathioprine.



a) b)

Fig. 1. *Abdominal CT scans before (a) and after (b) treatment*

Case discussion

The true population prevalence of ASyS is unknown. Several retrospective studies have found that the reported annual incidence of IIM has been 2 to 10 new cases per million adults per year and that ASS antibodies were detected in 20% to 40% of such cases. Due to the high association with malignancies, patients should be screened for age- and gender-appropriate malignancies.[10] Diagnosis is based on the constellation of symptoms, autoantibodies, and muscle histopathology. The use of electromyography and magnetic resonance imaging are controversial as they are costly and lack sensitivity and specificity.[1] Respiratory symptoms are by far the most commonly seen within the disease, where at least 60% of patients have such symptoms on presentation. Therefore, it is common that those patients presenting with unexplained ILD, should have ASyS in the differential diagnosis.[1,10] Those not presenting with respiratory symptoms should have pulmonary function tests, and a thoracic high-resolution CT. High-dose corticosteroids are the first-line treatment for ASyS.[11] Although corticosteroids are considered the mainstay of treatment, additional immunosuppressive agents, such as azathioprine and methotrexate, are often used, both as corticosteroid sparing agents and to achieve disease control. In those with severe or refractory disease, rituximab may be used. The long-term prognosis for people with ASyS varies based on the severity of the condition and symptoms present; although, with new therapy available, more cases are becoming chronic and requiring life-long therapy.[12]

In our case, we illustrate ASyS with liver involvement. Liver lesions are quite rare in ASyS. Upon review of the literature, we found that the most common liver abnormalities were abnormal liver function, chronic active hepatitis, and hepatomegaly. Few cases of liver conditions, such as nodular regenerative hyperplasia (NRH) or Budd-Chiari in ASyS, are reported. Our patient's lesion is likely NRH; however, it remains unconfirmed by biopsy. NRH of the liver is characterized by diffuse nodularity of the liver with little or no fibrosis.[13] It has been associated with autoimmune disease especially after drug treatment, notably azathioprine. NRH is one of the known causes of non-cirrhotic portal hypertension. Patients have presented with variceal bleeding and ascites. The natural history is unknown as there are limited data on the long-term prognosis and outcomes of patients with NRH. Experts believe that if NRH is identified, treatment should be directed at identifying an etiologic agent and removing it, if possible. Due to increased longevity of patients with ASyS, the physician should be aware of rare presentations, such as that of liver lesions and complications to monitor and follow.

In conclusion, ASyS is characterized by a variety of signs and symptoms leading to an array of presentations: myositis, ILD, polyarthralgia and mechanic's hands associated with anti-Jo1 antibodies. The physician should be vigilant when the patient is presenting with weakness, arthralgia, and shortness of breath. As more patients are achieving chronicity with the disease in remission, the physician should be aware of possible associated liver lesions and closely follow their evolution.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(2) (2017) 144-146
doi: 10.21103/Article7(2)_ShC1

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Results of Surgical Treatment of Lung Cancer in Patients of Different Age Groups

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Abstract

Background: Lung cancer is one of the most common cancers in the world. The main objective of our study was to analyze the results of the surgical treatment of non-small cell lung cancer (NSCLC) in patients of different age groups.

Methods and Results: We examined 280 patients (262/93.6% men and 18/6.4% women) aged from 39 to 75 years with NSCLC who underwent surgical treatment in the Ulyanovsk Regional Oncology Center in the period from 2010 to 2016. The mean age of patients was 64.9±10.1 years. Concomitant diseases were identified in 256(91.4%) patients: cardiovascular diseases in 170(60.7%), chronic obstructive pulmonary disease in 147(52.5%), lower extremity peripheral artery disease (stages II and III chronic ischemia) in 49(17.5%), a combination of concomitant pathology in 110(39.3%) patients. A total of 85(30.4%) pneumonectomies were performed, 56 of them in patients of young and middle age. Among early postoperative complications, the most frequent complications were purulent-inflammatory complications of the soft tissues of wounds (38.5%) and bronchopleural fistula (31.1%). The most severe complications, such as myocardial infarction, acute stroke, and acute limb ischemia, developed in patients with concomitant cardiovascular diseases, which caused the postoperative mortality of 4.6%. There were no statistically significant differences in the structure of postoperative complications depending on sex and age. **(International Journal of Biomedicine. 2017;7(2):144-146.)**

Key Words: non-small cell lung cancer • pneumonectomy • postoperative complications • concomitant diseases

Abbreviations

COPD, chronic obstructive pulmonary disease; **LC**, lung cancer; **MI**, myocardial infarction **NSCLC**, non-small cell lung cancer; **PAD,** peripheral artery disease.

Introduction

Lung cancer (LC) is the second most common cancer and the leading cause of cancer death in both men and women.[1-7] About 1 out of 4 cancer deaths are from LC. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined.[8] The number of LC patients increases with age, about 2 out of 3 people diagnosed with LC are 65 or older.[6-8,10,11] The risk of oncological pathology and the development of atherosclerotic occlusions of the main vessels sharply increase after age 50. In elderly patients, the presence of severe concomitant diseases limits radical surgical treatment. Postoperative mortality in LC patients older than 60 years is higher than in young and middle-aged patients.[1,2,12-14] According to the literature, cardiovascular diseases and malignant neoplasms are the most serious problem, as a main cause of premature death, for the developed countries.[4,7,12-14] The choice of the optimal treatment option for LC patients with concomitant cardiovascular diseases is still one of the

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most complex and controversial problems.[1,2,9,12-14] The main objective of our study was to analyze the results of the surgical treatment of NSCLC in patients of different age groups.

Materials and Methods

The study was retrospective, controlled, and non-randomized. The study was performed in the period from 2010 to 2016. The number of patients for primary selection was 310; the number of patients included in the subsequent analysis was 280.

Inclusion criteria were age 39 years and older, male and female, stage I-III NSCLC, surgery (pneumonectomy or lob-, bilobectomy), and systematic bilateral mediastinal lymphodissection. Exclusion criteria were myocardial infarction and acute cerebrovascular accident.

We examined 280 patients (262/93.6% men and 18/6.4% women) aged from 39 to 75 years with NSCLC who underwent surgical treatment in the Ulyanovsk Regional Oncology Center in the period from 2010 to 2016.

All patients underwent general clinical and laboratory, radiographic, endoscopic, ultrasound, histological methods of investigation and transthoracic echocardiography. In the preoperative period, primary LC and the presence of metastatic lesions were evaluated using chest radiography in two projections (direct and lateral), the double-contrast barium esophagram, CT of the chest, sputum cytology, bronchological examination, percutaneous transthoracic needle biopsy of peripheral lung lesions, and ultrasound examination of the organs of the abdominal cavity, retroperitoneal space and supraclavicular zones. All patients underwent surgical treatment: pneumonectomy or lob-, bilobectomy, systematic bilateral mediastinal lymphodissection.

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the by Ethics Committee at the Ulyanovsk Regional Oncology Center. Written informed consent was obtained from all participants.

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA).

Results and Discussion

The mean age of patients was 64.9±10.1 years and 28.5% of patients (n=80) were older than age 60. Patients older than age 70 accounted for 2.1% (Table 1).

Таблица 1.

Distribution of patients depending on sex and age

Age, y	Number of patients		
	Men	Women	Total
< 45	21	3	24 (8.6%)
46 – 60	166	10	176 (62.9%)
61 – 70	69	5	74 (26.4%)
≥71	6	0	6 (2.1%)
Total	262 (93.6%)	18 (6.4%)	280 (100%)

Stage I NSCLC was detected in 45(16.1%) patients, stage II NSCLC in 104(37.1%) patients, and stage IIIA in 131(46.8%) patients. Central and peripheral LC was determined in 63.9% and 36.1% of patients, respectively. Concomitant diseases were identified in 256(91.4%) patients: cardiovascular diseases in 170(60.7%), COPD in 147(52.5%), lower extremity PAD (stages II and III chronic ischemia according to the Fontaine classification modified by Pokrovsky) in 49(17.5%), a combination of concomitant pathology in 110(39.3%) patients. Concomitant pathology was identified in all patients older than age 60 and in 176(88%) patients under age 60.

A total of 85(30.4%) pneumonectomies were performed, 56 of them in patients of young and middle age; a greater number of pneumonectomies (65.9%) in this age group was associated with the detection of stage III NSCLC. In elderly and senile age patients, lob- and bilobectomy was performed in 51(63.8%) patients; at this age, the disease was more often detected at stages II and I.

The 5-year overall survival was 28.9% whereas the postoperative mortality was 4.6%. Mortality after pneumonectomy was 5.9% (5 of 85), after lob- and bilobectomy - 4.1% (98 of 195).

The postoperative period with complications was detected in 122(43.6%); the combined complications were observed in 36(12.9%) patients. There were no statistically significant differences in the structure of complications depending on sex and age. Among early postoperative complications, we identified bronchopleural fistula (31.1%), hemorrhagic complications (hemothorax, intrapleural hemorrhage) (11.5%), purulent-inflammatory complications of the soft tissues of wounds (38.5%), pleural empyema (5.7%), pneumonia (3.3%), myocardial infarction (2.5%), acute stroke (3.3%), acute limb ischemia (4.1%).

The obtained results indicated that the most frequent complications were purulent-inflammatory complications of the soft tissues of wounds (38.5%) and bronchopleural fistula (31.1%). The most severe complications, such as myocardial infarction, acute stroke, and acute limb ischemia, developed in patients with concomitant cardiovascular diseases, which caused the postoperative mortality of 4.6%.

To reduce the number of these complications, it is necessary to improve the preoperative diagnosis and preparation, surgical intervention techniques, and postoperative care. Thus, radical surgical treatment according to indications can be applied to young, middle-age, and older patients with operable NSCLC and allows achievement of satisfactory long-term results and of satisfactory short- and long-term functional results. At the same time, the high number of postoperative complications, in our opinion, is due to the imperfection of preoperative preparation in NSCLC patients with concomitant diseases. In this regard, improving the preoperative diagnosis and preparation in NSCLC patients has important value.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

This study was funded by the Ministry of Education and Science of the Russian Federation (the science project No. 18.7236.2017/BCh).

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International Journal of Biomedicine 7(2) (2017) 147-149
http://dx.doi.org/10.21103/Article7(2)_ShC2

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Results of Surgical Treatment of Patients with Critical Limb Ischemia and Stenotic Lesions of the Brachiocephalic Arteries

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Abstract

The aim of our study was to evaluate the results of the surgical treatment for patients with critical limb ischemia (CLI) and stenotic lesions of the brachiocephalic arteries.

Methods and Results: We examined 72 patients (68/87.2% men and 4/7.3% women) aged from 46 to 78 years (mean age, 62.2±4.3 years) with CLI and stenotic lesions of the brachiocephalic arteries. Conservative treatment was performed in 17(23.6%) patients and surgical treatment in 55(76.4%). It has been carried out 73 surgical operations: femoral popliteal bypass (5/6.8%), lumbar sympathectomy (4/5.5%), thrombectomy of occluded aortofemoral graft (2/2.7%), limb amputation (4/5.5%), iliofemoral bypass (4/5.5%), aortofemoral bifurcation bypass (10/13.1%), endovascular surgery (1/1.6%), limb amputation at thigh level - 4(5.5%), thrombectomy of occluded distal arteries (4/5.5%), femoro-femoral cross-over bypass (1/1.6%), resection of popliteal artery aneurysm and prosthesis of the popliteal artery (1/1.6%), semi-closed loop endarterectomy of occluded arteries of the lower limbs (8/10.9%), carotid endarterectomy (23/31.5%), and carotid-subclavian bypass (2/2.7%). After the surgical intervention, we observed the disappearance or reduction of pain, restoration of sensitivity and motor activity, and healing of trophic ulcers in 75% of patients. In the late postoperative period, we detected the progression of limb ischemia in 4(5.5%) patients; in connection with that, we performed limb amputation at thigh level. Ischemic stroke with a lethal outcome developed in one patient (1.4%).

Conclusion: In patients with multifocal atherosclerosis, multilevel reconstructive surgical interventions must be performed in stages, due to the high operational risk, and risk of complications, secondary amputations and lethality in the postoperative period. **(International Journal of Biomedicine. 2017;7(2):147-149.)**

Key Words: critical limb ischemia • multifocal atherosclerosis • postoperative complications • concomitant diseases

Abbreviations

BAs, brachiocephalic arteries; **CLI**, critical limb ischemia; **MFA**, multifocal atherosclerosis; **PAD,** peripheral artery disease.

Introduction

Critical limb ischemia (CLI), which is at the end of the peripheral artery disease (PAD) spectrum, is associated with excessively high risk for cardiovascular events, including myocardial infarction, and death.[1,2] CLI with multifocal atherosclerotic lesions and concomitant diseases is an urgent problem of vascular surgery.[3-6] Recently, the number of patients with MFA and CLI has increased, largely the result of an increase in the number of elderly patients.[3,6-9] According to the literature, CLI complicates the course of eliminating atherosclerosis of the lower limbs in 33% of patients.[6,10-12] As a result, the lethality and number of amputations now reaches 14.0% and 20.4%, respectively, in CLI patients after reconstructive interventions on the vascular bed.[5-7,13]

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Reconstructive surgical interventions provide preservation of limbs in the postoperative period in terms up to 5 years in 80% of patients, up to 10 years - only in 50%.[4-7]

To this day, the choice of the optimal treatment option for patients with CLI and MFA remains one of the most difficult and controversial problems for representatives of various surgical schools and directions due to the high risk of complications with reconstructive interventions, which is due to the age of patients and the severity of the concomitant pathology.

The aim of our study was to evaluate the results of the surgical treatment for patients with CLI and stenotic lesions of the brachiocephalic arteries (BAs).

Materials and Methods

We examined 72 patients (68/87.2% men and 4/7.3% women) aged from 46 to 78 years (mean age, 62.2±4.3 years) with CLI and stenotic lesions of BAs who underwent surgical treatment in the department of vascular surgery of the Regional Oncology Center. Inclusion criteria were patients with lower limb ischemia (Fontaine III or IV) of atherosclerotic genesis in combination with stenotic lesions of BAs, who have had the disease for at least three years and are undergoing in-patient treatment courses at least twice a year. Diagnostic tests included ultrasound duplex scanning of the vessels, assesment of tissue blood flow in the distal parts of limbs using laser Doppler flowmetry, and aortic angiography. The indications for surgery were the decompensated stage of peripheral circulation, ineffective conservative therapy, and angiographic signs of the presence of blood flow in the deep femoral artery, its branches, the distal portion of the popliteal artery, and at least one of the tibial arteries. The decision to conduct operative intervention was strictly individual, taking into account the patient's condition, the nature and degree of arterial damage, the state of the distal bed, the stage of the disease, and the presence and severity of the concomitant pathology. Contraindications were the total occlusion of the deep and superficial femoral arteries, occlusions of the popliteal, anterior and posterior tibial arteries, severe limb ischemia with the presence of tension and contraction of the calf muscle, and the presence of wet gangrene. The choice of the method of reconstructive surgery was determined by the level of damage, the prevalence of the obliterating process, and the severity of the concomitant pathology.

Conservative treatment was performed in 17(23.6%) patients and surgical treatment in 55(76.4%). It has been carried out 73 surgical operations: femoral popliteal bypass (5/6.8%), lumbar sympathectomy (4/5.5%), thrombectomy of occluded aortofemoral graft (2/2.7%), limb amputation (4/5.5%), iliofemoral bypass (4/5.5%), aortofemoral bifurcation bypass (10/13.1%), endovascular surgery (1/1.6%), limb amputation at thigh level - 4(5.5%), thrombectomy of occluded distal arteries (4/5.5%), femoro-femoral cross-over bypass (1/1.6%), resection of popliteal artery aneurysm and prosthesis of the popliteal artery (1/1.6%), semi-closed loop endarterectomy of occluded arteries of the lower limbs (8/10.9%), carotid endarterectomy (23/31.5%), and carotid-subclavian bypass (2/2.7%).

The study was conducted in accordance with ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

In the postoperative period, all patients received anticoagulant and disaggregating therapy. Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA).

Results and Discussion

Given the etiological factors, the following nosological forms of PAD were identified: atherosclerosis (70/97.2%), Buerger's disease (1/1.4%), and diabetes mellitus (1/1.4%). Critical ischemia of the lower limbs was detected in 70(97.3%), upper limbs in 2(2.7%) patients. Hemodynamically insignificant and significant stenoses of BAs were detected in 47(65.2%) and 25(34.8%) patients, respectively. Trophic changes in distal sections of the lower limbs were observed in 53(73.6%) patients. Ischemic rest pain was in 19(26.4%) patients. Angiographic and ultrasound investigations of arterial pools revealed that all CLI patients had multifocal vessel lesions, namely, lesions of the arteries of the lower limbs and BAs. Trophic lesions and clinical manifestations were determined by the level and amount of obliterated arteries.

In patients with critical stenosis of BAs and arteries of the lower limbs, surgical interventions were performed in stages (with a minimum interval between stages): first on the major arteries of the head, then on the arteries of the limbs. The primary restoration of blood flow in BAs was performed to reduce the risk of fatal complications in CLI patients. The main clinical criteria for the effectiveness of treatment were the preservation of the functioning limb, epithelization of ulcers, and the absence of rest pain and neurological symptoms.

After the surgical intervention, we observed the disappearance or reduction of pain, restoration of sensitivity and motor activity, and healing of trophic ulcers in 75% of patients. In CLI patients, the risk of amputation of the affected limb and a lethal outcome during the first year after surgery is extremely high. In the late postoperative period, we detected the progression of limb ischemia in 4(5.5%) patients; in connection with that, we performed limb amputation at thigh level. Ischemic stroke with a lethal outcome developed in one patient (1.4%). Prognosis after surgery in elderly patients with CLI is determined by the type, level and volume of the vascular reconstruction, the initial degree of limb ischemia and the presence and severity of concomitant pathologies, as well as the severity and duration of the recovery period. All of the above have a direct impact on the quality of life of geriatric patients and the results of reconstructive surgery.[6,13]

Very important is not only the careful selection of patients with a comprehensive assessment of the reserve and compensatory capabilities of the body, but also the targeted preoperative preparation and prevention of cardiac, pulmonary and other complications, and wide involvement of specialists in related fields that can reduce the risk of postoperative complications and lethality. [6,7,13]

Thus, surgical treatment of CLI patients with MFA is a complex task due to the multilevel lesion of the vascular bed

and severe concomitant diseases. Using a combination of open and endovascular methods to restore blood flow, an individual approach to the choice of surgical method, and adequate medication support leads to success in preserving the limbs and improving the quality of life of these patients.

In conclusion,

- with MFA, multilevel reconstructive surgical interventions must be performed in stages, due to the high operational risk, and risk of complications, secondary amputations and lethality in the postoperative period;
- complex surgical treatment of patients with CLI and stenotic lesions of BA with the minimal time interval between stages allows reducing the number of amputation of the lower limbs to 5.5% and lethality to 1.4%.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

This study was funded by the Ministry of Education and Science of the Russian Federation (the science project No. 18.7236.2017/BCh).

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International Journal of Biomedicine 7(2) (2017) 150-153
http://dx.doi.org/10.21103/Article7(2)_PV1

POINT OF VIEW

INTERNATIONAL JOURNAL OF BIOMEDICINE

Creativity as a Determinant of Intraspecific Aggressive Properties of the Psyche of Homo sapiens

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Abstract

A sharp and non-linear qualitative change event, namely, the emergence of creativity in the structure of the psyche of Homo sapiens (HS) about 50,000 years ago, created developmental bifurcations, as a result of which HS attained extraspecific and intraspecific domination. Creativity, a new quality of HS psyche, for the first time in the history of hominids enabled HS to separate the image of the goal (IG) from the reactive behavior, transforming IG into an abstract, symbolic object. The emergence of this creative construct, the ambivalent structure existing in the interpretive environment, created a new need for hominids: a relationship with the virtual product of the psyche, a symbol, which has the signs of objective reality in perception. Thus, a fundamentally unsolvable frustration was created: a frustration caused by the inability to achieve equilibrium relations with the controlling symbolic image. Behavior aimed at satisfying needs and eliminating frustration, accompanied by the ordering and structuring of society, acquires under certain conditions the elements of aggression, transforming into sheer aggression. We believe that in the periods preceding the aggression, there is a deliberate deformation of the psychological image of the enemy, the future victim, resulting in the aggressor perceiving the enemy as lacking in human qualities, those qualities that are the attributes of species identity. In this process, the aggressor's psycho-filters and ethical restrictions are eliminated. We understand intraspecific aggression as the antagonistic contact between the frustration constructs, aimed at reducing the tension caused by frustration. **(International Journal of Biomedicine. 2017;7(2):150-153.)**

Key Words: creativity • symbol • aggression • Homo sapiens

The purpose of this research was to describe the fundamental mechanism by which the intraspecific aggression of Homo sapiens (HS) emerged, resulting from the emergence of new psychic qualities of hominids: creativity and symbolic thinking.

To achieve this goal, we used our previously published materials,[1,2] along with the works of cited authors.[3-6] We view the psyche as a distributed system consisting of functional subsystems, where the emergence of new subsystem, creativity,[1] created conditions for a sharp jump-over to a new quality level of the system as a whole, and changed the functionality of the psyche and its transition into a new class with the possibility of implementing goals at a new level.

The emergent phenomenon, a sharp non-linear enhancement of the psychic qualities of HS, which to various degrees is also noted in other hominids, created a developmental bifurcation, the result of which was that HS gained extraspecific and intraspecific domination. Creativity has given to the HS psyche the possibility of separating IG from reactive behavior, i.e. creating the forms of behavior going beyond utilitarian, vital forms.

The visualized IG, the creative construct (CC) having the initial applied (learning) value, transformed into an abstract image becomes an independent object of perception of HS.[1,2] The system of primary abstract representations, transforming over several generations because the means of fixation were lacking, inevitable becomes more complicated, which increases the levels of uncertainty of the setting, the evaluation criteria, and the information volume. Only an individual tolerant of uncertainty (a conditional priest-divergent) is capable of ordering and systematizing primary mythologies, becoming an intermediary between the population and a chaotic set of representations, structuring them and reducing the level of anxious expectations. Integration of CC by the

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conditional priest for several generations creates a stable virtual construction,[7] which reflects reality in the form of sensual-specific associations, which we are aware of as real. A conditional leader (a convergent) having another set of characterological qualities, is effective in making managerial decisions and initiating the coordinated vector actions of the population (mainly within the coordinates, which are generally accepted in the population).

The cumulation and combination of behavioral coordinates, transformed into ethical coordinates (a derivative of the conditional priest) with the control signals that initiate the concerted actions (a derivative of the conditional leader), transforms the population into a society. The conditional priest and the leader are symbionts performing this transformation, which does not exclude the competition between them. Without a perceptual source, an applied image (IG-CC) in the past acquires the properties of an independent object, which is projected into visual forms[1] with the qualities of an artistic image. The conditional priest maintains a visualized and verbalized virtual product (CC), filling it with semantic content (so that the population appeals to him in order to obtain uncontested arguments), translating control signals, usurping the feedback channel with CC.

For each generation, the virtual images, passed from their predecessors and accompanied by mythologems, are an objective fragment of the habitat, relations with which have the character of bilateral (direct-inverse) connections. Thus, objective reality is endowed with unaccustomed properties (mental product) through the integration of fragments of objective and virtual reality into a single whole, which determines the place and role of the individual in this coordinate system. The result of the above is the formation of a unified information space with reference points of social and ethical coordinates and fixation of the individual's position in this system, leading to the possibility of predictive assessments, a decrease in the level of anxiety, and an increase in social negentropy. As experience of the divergence between forecasts and results accumulates, the idea of one's own insolvency and defenselessness grows. A derivative of this experience is that the control functions are projected onto an external object with a potential superior to that of HS and that the regulation and control functions are delegated to the channeled priest and leader. Thus, the outwardly projected CC is transformed through certain phases (1st - an applied phase [training], 2nd - a symbolic image separated from reactive behavior, 3rd - a visualized projection that does not have a perceptual source [artistic image] and receives semantic content) and is endowed with regulatory and control functions in relation to the individual and the society. In other words, the psyche of HS produces CC, the projection of which acquires, for human perception, the properties of objective reality with regulatory and control functions that can initiate both individual and concerted social effects, which are not always deterministic and equivalent to the conditions of objective reality.

Lack of the possibility of feedback relations with the controlling CC initiated a fundamentally new way for the hominids to communicate: a verbal method, supplemented by address code actions (rituals), behavioral algorithms, the substantive part of which was established by intermediaries (priests), i.e., accumulators, carriers of a new kind of anthropological information, channeled relationships with an external controlling object (CC).

A population that transforms into a society establishes a system of interrelated symbols (ethical coordinates) as a standard coordinate system of representations about the boundaries of what is possible and admissible, acquiring stability and reducing the level of anxiety. The creativity of HS, contributing to the emergence and development of symbolic thinking and the expansion of the boundaries of goal-setting beyond the achievable,[1,2] provided the prerequisites for the emergence of frustrations, removing HS beyond the utilitarian forms of vital behavior, which created the possibility of the ambiguous (interpretative) assessment of target images with the extrapolation of virtual structures into objective reality. That is to say, the new intraspecific need that arose for the first time in the history of hominids, namely, the relationship with the symbolic image (CC) which is perceived to have the signs of objective reality created a situation of unsolvable frustration: the impossibility of achieving equilibrium relations with the controlling virtual product (CC). The need, the most important disposition of the motive, is defined as the state of dissatisfaction of the organism (personality), the deficit of what is required to maintain homeostatic equilibrium. CC, for the considered aspect of HS activity, is the leading link in the conscious regulatory process, purposeful behavior, where IG is the virtual construction: the symbol. Due to the ambivalence of the symbol (image+sense) that exists only within interpretations,[8] the one-to-one isomorphic coordinates of the correspondence of any operational image to the final, reference IG (CC) are excluded, and there are convincing signs of homomorphism, where the complete correspondence to the standard (CC) is not possible, but some degree of similarity is achieved. In other words, if the controlling CC, as a symbol, locates the expected result beyond the achievable one, then it creates a primary, unsolvable frustration, which has an inexhaustible negentropic potential for improving the homomorphic sociopsychological constructions of approaching the ideal, the symbolic IG (CC).

The population, transforming into society (regardless of the scale), forms the mechanisms of regulation and management (hierarchy, construction of intrasystem coordinate principles, etc.) and, by so ordering, increases the measure of social negentropy. One of the significant setup principles is the formation, training, and introduction of a set of characteristics (formal and informal) of conformity and belonging to a particular society (from tattoos to confessional attributes), which are identification identifiers, the information and control signals of the recognition filter (ours - alien). We believe that the addressee of the aggressive behavior, the individual (socium), when passed through the aggressor's recognition filter as "alien," is not subjectively considered as belonging to the same species (in the biological sense) as the aggressor. A deficit model of the opponent's psychotype (the future victim) is formed, with the identification of negative features and differences. The concomitant reduction of the restrictive social structure (responsibility, punishment) as

it regards the aggressor's behavior toward the addressee is a significant addition, which increased the potential for aggression. Demonstrative examples are interracial relations (until recently), as well as interethnic, religious, and other conflicts. The topic of a special study will be changes in the individual and social psyche and psychology in the periods preceding aggression, forming the installation of systems of recognition filters according to the principle "ours – alien." We believe that the result of this process is the deficit states of the psyche, which include the persistent ideas about intraspecific differences (down to antagonistic) and initiate the aggressive behavior and aggression.

In biology, intraspecific aggressive forms of behavior, which have the species-protect functions, are the basis for optimal sexual selection and uniform dispersal (swarming of bees), that optimize the interspecific relationships and trophic connections within the biocenosis and increase the sustainability and diversity of species. The only exception to this series is HS, whose aggressiveness is a phylogenetically altered program at the genetic level, which reflects the instinct of the struggle for existence.[5] Typical of HS is the development of a negative mental state as the response to the impossibility (difficulty) of meeting certain needs that would eliminate frustration.[9] This state includes the whole spectrum of negative emotions (anger, anxiety, irritability), which increase the level of aggression and the likelihood of aggressive actions. The supposed relative rarity of aggressive clashes between primitive societies of antiquity can be explained by the small size of societies that had territorial resources for extensive growth (or displacement) in directions that did not have the specific population resistance. Another reason could be the similarity (affinity) of the totemic values of geographically close communities, which provided the reasons and methods for non-aggressive contacts and associations. Population growth, reduction of compensatory territories, and competition for resource potential led to direct contacts between societies with differences (up to antagonistic ones) in systems of ethical coordinates.

Zones and periods of uncertainty at the borders of these contacts became the arena of a meeting of societies with a stable social structure and symbols. We consider these situations as a dynamic contact between frustration designs that differ in informative filling of the frustration constructs with the potential for antagonistic relations and the likelihood of aggressive actions. Uncertainty in the contact zone is defined as the variability in the choice of forms of behavior from among a set of alternatives and the absence of clear criteria for optimality and efficiency. That is to say, the frustration construct (we stand in solidarity with L. Berkowitz)[10] does not produce aggression immediately, but demands the creation of appropriate conditions with the possibility of exhausting arguments, maturing the antagonistic relations and choosing in favor of aggression, including (in the final stages) the identification by the "ours-alien" filter, the result of which can initiate aggression (the effect of "attack"[11]). Aggression, under the conditions described, is the result of the antagonistic contact between the frustration designs, the ultimate goal of which is the realization of the expectations that cause frustration, thus reducing frustration tension. In the stage that precedes aggression, the opposite side is endowed with properties deforming the attributes of species identity, with a decrease in the filters of the species protection functions, which in turn leads to the elimination of restrictive psychological and other mechanisms in relation to the opponent (victim). Productive, successful aggression leads to domination by the aggressor and regression (destruction) of the frustration construct of the victim, fixing aggression as a way of reducing the frustration tension.

Having considerably simplified the psychological mechanisms that form intraspecific aggressive behavior and aggression, not limited to the scheme we outlined, we aimed to emphasize the existing consistency and dependence of HS intraspecific aggression and the new function - creativity. In our opinion, these traits of HS exist regardless of historical epochs and social formations. A principled scheme of intraspecific aggression of HS, in our opinion, is not contradictory; it supplements the main statements of a number of theories: theory of evolutionism; theory of motivation; theory of cognition; theory of social learning.[12] In the next paper, we intend to consider the differences between and the interdependence of individual and social forms of intraspecific aggression.

Conclusion

Intraspecific HS aggression is the final stage of negative development of the specific form of adaptive purposeful behavior and realization of a new function unique to HS: the satisfaction of the need for homeostatic relations with the controlling mental construct, a symbol, which is a frustration construct without the opportunity of solution. The negative result of the adaptive period of contact between frustration constructs, which are transformed into antagonistic constructions (aggressor-adversary), initiates the realization of an aggressive potential, which results in a temporary decrease in the stress caused by frustration. The addressee of aggression is endowed with certain properties that reduce and deform the signs of species identity, which leads to the elimination of the species protection filters, the removal of restrictive psychological and other mechanisms, leading HS aggression beyond the limits of intra- and extraspecific regulators of species sustainability and diversity.

Conflicts of interest

There are no commercial interests or conflicts of interest to declare.

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