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ISSN 2158-0510

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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

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International Journal of Biomedicine 6(4) (2016) 255-258
doi: 10.21103/Article6(4)_OA1

ORIGINAL ARTICLE

Pulmonology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Influence of Natural Lung Surfactant Inhalations on Clinical Symptoms and Pulmonary Function Parameters in Patients with Bronchial Asthma. Communication 1

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Abstract

Background: Damage to lung surfactant (LS) enabling the lung local immunity may contribute to the development of bronchial inflammation in patients with bronchial asthma.

Methods and Results: A 40-day course of 16 LS (Surfactant-BL) inhalations at the dose of 25 mg was added to inhaled corticosteroids (ICS) and short/long-acting bronchodilators or combined inhalers in 14 patients with bronchial asthma. After 7 inhalations, patients demonstrated a significant decrease in shortness of breath and bronchospasm attacks, double reduction of ICS dose ($p=0.01$), and improvement of pulmonary function. Forced vital capacity (FVC) increases during treatment in a linear fashion ($y=62.9+5.60 \cdot x$; $p<0.05$), reaching the normal level (80%) after 9 inhalations (Day 15). Forced expiratory volume (FEV1) increases in a linear fashion ($y=50.7+4.15 \cdot x$; $p<0.05$) without reaching the normal level (80%) after 16 inhalations (Day 41). The FEV1/FVC ratio does not change significantly in the time period between Day 1 to Day 15. By Day 41 the value decreases significantly to 67.4±4.66% ($p<0.05$). The peak expiratory flow (PEF) parameter increases in a linear fashion ($y=53.9+5.00 \cdot x$; $p<0.01$) from 57.7±6.33% to 76.2±9.33% of the predicted value.

Conclusion: LS inhalations improve the condition of patients with bronchial asthma, allow ICS dose reduction by 2 times, and improve pulmonary function parameters. **(Int J Biomed. 2016; 6(4):255-258.)**

Key Words: lung surfactant • bronchial asthma • inhaled corticosteroids • dose • pulmonary function

Introduction

Conventional treatments for bronchial asthma (BA), such as inhaled corticosteroids (ICS) and short-term and long-term bronchodilators, are aimed at preventing bronchospasm episodes rather than restoring the structure and functions of the bronchial epithelium. Even though this kind of therapy significantly improves patients' quality of life, upon termination of daily medicine taking, bronchospasm episodes recur often with increased frequency and severity. In some patients long-term ICS use is accompanied by such complications as oral and oropharyngeal candidosis, dysphonia, and cough, which cause considerable discomfort. High doses of ICS equivalent to 1000 mcg of fluticasone propionate are accompanied by such complications as pneumonia, glaucoma, cataract, adrenal function suppression, osteoporosis, and diabetes.[1]

The evidence from our earlier work that long-term ICS in rats significantly decreases the LS content has led us to hypothesize that natural LS formulations may decrease patient dependency on ICS.[2] Deficiency or qualitative changes in LS are found in many lung conditions, including BA.[3,4] Apart from enabling the breathing mechanism, LS is known to provide molecular mechanisms of innate and adaptive lung tissue immunity and to have anti-inflammatory properties.[5,6] Although almost no research has been carried out so far to

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investigate the possibility of restoring pulmonary function in BA patients by inhaled LS formulations, the role of LS system impairment and the possible use of LS formulations for BA have been discussed in detail in the review by Hohlfeld.[7]

The objective of the study was to investigate the influence of inhaled natural LS formulation on the clinical manifestations and the dynamics of pulmonary function parameters in patients with partly controlled and uncontrolled persistent BA as well as to assess the feasibility of ICS step-down.

Methods

We examined 14 BA patients with partly controlled and uncontrolled persistent BA at the Pulmonary Centre of the Chita Road Clinical Hospital. The trial was carried out based on the approval of the Local Ethical Committee under Chita State Medical Academy of the Ministry of Health of the Russian Federation according to approved Protocol. The enrolled patients were diagnosed according to the 2016 GINA guidelines and had had a history of asthma for a period of time varying from 6 months to 24 years.[8] The treatment they had been receiving prior to their entry into the study included antibiotics during exacerbations, with 8 out of the 14 patients receiving short courses of systemic per-oral and/or intravenous glucocorticoids. After their condition became stable, they had received either inhaled ICS and short/long-acting bronchodilators or combined inhalers for 12 months to 12 years prior to enrollment. Upon enrollment the patients started a course of inhalations with Surfactant-BL (OOO Biosurf, Saint Petersburg, Russia), a natural LS formulation, given as add-on therapy. The surfactant was administered using the compressor nebulizer Boreal (Italy). The patients were instructed how to use it at the first visit (V1) and then continued to use it on their own for inhalations at home. The surfactant was taken daily at the dose of 25 mg for the first 7 days of the study and then at Days 10, 13, 16, 19, 22, 26, 30, 35 and 40 (a total of 16 inhalations), so that the intervals between inhalations were gradually increased. Patients were examined at 5 visits on Days 1(V1), 8(V2), 15(V3), 29(V4) and 41(V5). At each of the 5 visits the frequency of bronchospasm episodes, shortness of breath with exercise and speaking and frequency of attacks while sleeping were registered. For pulmonary function assessment, a spirometer MAC-1, VISMA-Planar (Belarus), and the computer software SpiroExpert were used. During the examination, 3-8 technically acceptable maneuvers were used to achieve result reproducibility based on the following criteria:

the difference between two highest FVC values ≤ 150 ml;

the difference between two highest FEV1 values ≤ 150 ml;

(for absolute FVC values < = 1 L with allowable differences between maneuvers not exceeding 100 ml).[9,10,11]

Lung function was assessed based on spirometric measurements: FVC - forced vital capacity (L), FEV1-forced expiratory volume in 1 second (L), FEV1/FVC ratio (%), and PEF - peak expiratory flow (L/s). The values of the above parameters were specified as a percent of the predicted value.[9]

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. Student's unpaired and paired t-tests were used to compare average values for data with normal distribution. Regression analysis was performed by the least squares method. The statistical significance of the b coefficients of the linear regression equations was evaluated using Student's t-tests. For evaluating the statistical significance of changes in patient condition registered on the basis of their complaints as well as ICS dose reduction or termination non-parametric Z-tests were used. A probability value of $p<0.05$ was considered statistically significant.

Results

Of 14 patients, 13 showed resolution of wheezing, decrease of frequency or resolution of attacks, and resolution of shortness of breath and bronchospasm episodes with moderate exercise (such as climbing upstairs to the first or second floor) since their V2. One patient of 14 suffered from asthma night attacks and his attacks stopped. Dynamics of patient complaints show statistically significant ($p=0.01$) differences of BA clinical manifestation frequency between baseline and after 7 inhalations of the formulation. In 11 (78.6%) out of 14 patients the ICS dose was halved, and in 1 (7.1%) patient ICS was later terminated. In 3 patients no significant ICS dose reduction could be achieved. Statistical analysis showed significant frequency of ICS dose reduction due to surfactant-BL inhalations ($p=0.01$).

Looking at the changes in pulmonary function parameters (Figure 1), it should be noted that FVC increases during treatment in a linear fashion ($y=62.9+5.60\cdot x$; $p<0.05$), reaching the normal level (80%) by V3 after 9 inhalations of Surfactant-BL. FEV1, the most important parameter of pulmonary function showing the severity of bronchial obstruction in BA, increases during treatment in a linear fashion ($y=50.7+4.15\cdot x$; $p<0.05$) without reaching the normal level (80%) by V5.

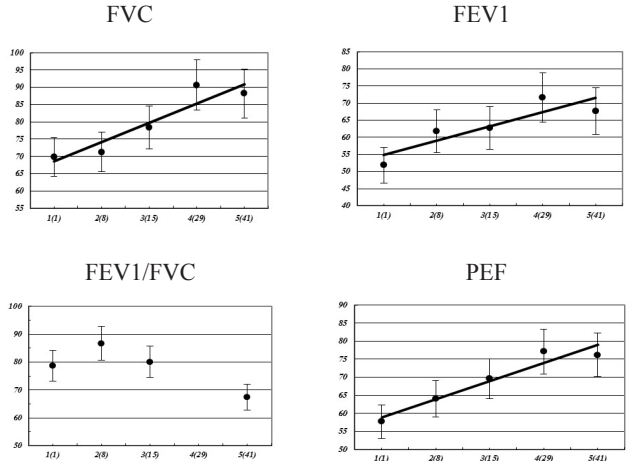

***Fig. 1**. Dynamics of pulmonary function parameters*
X-axis – the number of visit (day of visit); Y-axis – % from the predicted value; points with confidence interval - x ±2m;
——- lines of linear regression equations

The FEV1/FVC ratio does not change significantly in the time period between V1 and V3 staying at the mean value of 81.8±2.82%. However, by V5 the value decreases significantly to 67.4±4.66% ($p<0.05$). The PEF parameter showing resistance to airflow in the airways (Figure 1) increases during treatment in a linear fashion ($y=53.9+5.00 \cdot x$; $p<0.01$)) from 57.7±6.33% of the predicted value (V1) to 76.2±9.33% of the predicted value (V5), which suggests a decrease in resistance to airflow in the airways.

Thus, clinical evidence and pulmonary function parameters from patients with partly controlled and uncontrolled persistent BA who had received 16 inhalations of natural LS at the dose of 25 mg as add-on therapy to ICS and short/long-acting bronchodilators suggests a positive effect of surfactant therapy as part of combined therapy. As a result of this therapy, many patients reported lower frequency of asthma attacks, resolution or lower frequency of bronchospasm episodes with moderate exercise, and termination (1 patient) or double reduction of the ICS dose (10 patients out of 14). Another important result is that the objective evidence from changes in pulmonary function parameters showed significant functional improvement in the bronchi condition.

Discussion

Experiments on sensitized mice and guinea pigs showed prevention of bronchospasm in response to an antigen provocation following LS administration.[12] Improvement of pulmonary function due to LS administration has also been shown in human patients with BA in several studies. Babu et al. [13] demonstrated the feasibility of stopping early response to antigen provocation in atopic BA and increased FEV1 due to Pumactant inhalations. Kurashima et al.,[14] in a double-blind, placebo-controlled study of 12 patients with BA, registered a significant increase of FEV, FEV1 and MMF 20 minutes after administering 10 mg of surfactant formulation. Zagorulko et al.[15] showed that one time intratracheal administration of Suzakrin at the dose of 500 mg improves lung function parameters and normalizes LS phospholipid composition in children with BA. However no study has been carried out so far that would include BA treatment with LS formulations and register the dynamics of patient clinical status. Neither have there been any attempts to register the feasibility of ICS reduction due to surfactant therapy. It should be mentioned that multiple administration of 'empty' liposomes from phosphatidylcholine and cholesterol to rats [16] and multiple administarion of LS to newborns [17] enhances the synthesis of endogenous surfactant by its reutilization by the alveolocytes-II.[6] Furthermore, the effectiveness of surfactant therapy in adults has been shown in a number of studies on acute respiratory distress syndrome [18-20] and discussed in depth in reviews by Rosenberg et al. [6, 21] It should be noted that long-term courses of Sufactant-BL inhalations have been used before, for lung tuberculosis (TB).[22] The authors drew on the known evidence from surfactant system pathology in lung TB.[23] Adding surfactant inhalations to the standard anti-TB treatment scheme that includes chemotherapy and antibiotics resulted in significant resolution of lung infiltrations and foci in patients who had shown no positive X-ray dynamics to standard therapy during 3 months. In this study, 2 months after cessation of surfactant therapy 85% of patients showed bacillary elimination in the sputum (compared to 63% in control), over 90% of patients (compared to 61% in control) showed resolution or significant decrease of infiltrations, and 71% of patients (compared to 43% in control) showed closure of caverns up to 3 cm in diameter. Another study of surfactant therapy in lung TB yielded similar results, demonstrating a decrease of large caverns from 5 to 8 cm in diameter by 2-12 times.[24]

Therefore, we believe that the results of surfactant therapy given as 16 inhalations over 41 days as part of combined therapy of patients with BA, which included positive dynamics of clinical manifestations, high frequency of ICS dose reduction by half, and improved lung function parameters, are promising and can become a rationale for expanding this clinical trial.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

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

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International Journal of Biomedicine 6(4) (2016) 259-264
doi: 10.21103/Article6(4)_OA2

ORIGINAL ARTICLE

Pulmonology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Peculiarities of Airway Inflammation and Lipid Peroxidation in the Development of Hyperosmotic Airway Hyperresponsiveness in Patients with Asthma

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Abstract

The aim of our study was to evaluate the role of airway cellular inflammation and the lipid peroxidation level in the development of airway hyperresponsiveness (AHR) to inhalation of hypertonic saline (IHS).

Methods and Results: The study included the estimation of inflammatory-cellular composition, intracellular concentration of myeloperoxidase (MPO) in induced sputum (IS), serum levels of lipid hydroperoxides (LHP), ceruloplasmin, and vitamin E in 29 patients with asthma and 12 healthy persons. AHR to IHS was assessed by spirometry after 3-min IHS via ultrasonic nebulizer. Patients with asthma had higher indices of leukocytes destruction and cytolysis intensity with the increased leukocyte count in IS. Maximum values of neutrophils cytolysis intensity and leukocytic MPO were found in IS of the patients with AHR to IHS. After the bronchial provocation, serum concentration of LHP was higher in these patients in comparison with the patients without the AHR and control groups. In addition, patients with asthma had lower level of antioxidants than healthy subjects.

Conclusion: Marked inflammation involving MPO-activated leukocytes and intensive lipid peroxidation underlie the excessive airway response to IHS. **(Int J Biomed. 2016; 6(4):259-264.)**

Keywords: bronchial provocation • bronchoconstriction • hypertonic saline • myeloperoxidase • oxidative stress

Abbreviations

AHR, airway hyperresponsiveness; **CP**, ceruloplasmin; **IHS**, inhalation of hypertonic saline; **HOCI**, hypochlorous acid; **ICD**, index of cells destruction; **IC**, index of cytolysis; **IS**, induced sputum; **LHP**, lipid hydroperoxides; **LP**, lipid peroxidation; **MPO**, myeloperoxidase; **ROS**, reactive oxygen species; **RHS**, reactive halogen species; **VitE**, vitamin E.

Introduction

A high lability of the airways and bronchoconstriction caused by exogenous stimuli in patients with asthma are fully revealed under the inhalation of hyperosmotic solutions,[1,2] but in spite of the prevalence of hyperosmotic airway reaction, its mechanisms remain largely unexplored.

According to the view that oxidative stress plays a leading role in the pathogenesis of chronic obstructive pulmonary disease and asthma,[3,4] ROS, peroxynitrite and hypochlorite may serve as the inducers of bronchoconstriction.[3,5,6] Irreversible oxidative changes in proteins, lipids, and nucleic acids, as well as the damage to biomembranes by free-radical oxidation, are usually associated with the increased consumption and exhaustion of antioxidant protection.[7,8]

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If the genesis of AHR in asthma patients is associated with the stress damage to proteins and glycoproteins and to the inactivation of tissue receptors, the development of exudation and further stages of bronchial inflammation is caused by LP.[3] LP metabolites play an important role in enhancing airway response to osmotic stimuli and provoke the loss of asthma control.[9] It is established that LP processes are initiated not only by ROS, but also by halogen-containing reagents – the products of halogenides oxidation, which are catalyzed by MPO in the presence of H_2O_2.[10,11] Highly reactive hypohalous acids formed in the system of H_2O_2-MPO and their derivatives – secondary RHS along with ROS and reactive nitrogen species –determine the efficacy of the cellular response under induction and maintenance of inflammation,[10] thus providing the interrelation between oxidative and halogenating stress.[11]

The main source of ROS and RHS is primed neutrophilic leukocytes[12] depositing MPO in azurophilic granules and secreting the enzyme into extracellular media through degranulation.[10] In our previous studies we found the elevated level of MPO activity is associated with high intensiveness of degranulation and destruction of leukocytes in IS of asthma patients with AHR to hypoosmotic and cold triggers.[13,14]

In light of a well-established connection between oxidative stress and inflammation in asthma, the relationship between airway inflammation and LP in the formation of excessive airway response to osmotic stimulus deserves special attention. The aim of our study was to evaluate the role of airway cellular inflammation and the LP level in the development of AHR to IHS.

Materials and Methods

The study enrolled 27 patients aged from 31 to 55 years (mean age 41.0±1.8 years) of both sexes (17 females and 10 males) with mild, persistent, partly controlled and uncontrolled, physician-diagnosed asthma according to GINA criteria.[15] The control group included 12 healthy subjects without a history of smoking, upper airway pathology, allergic diseases, or acute respiratory infections during the previous 3 months, matched by age and gender with the asthma patient group. This study was performed in accordance with the Declaration of Helsinki and was approved by Local Committee of Biomedical Ethics of the Far Eastern Scientific Center of Physiology and Pathology of Respiration. Written informed consent was obtained from all participants. Preliminary examination included the analysis of clinical symptoms, the estimation of asthma control by an Asthma Control Test questionnaire (Quality Metric Inc., 2002), the assessment of lung function by standard spirometry (Easy on-PC, nddMedizintechnik AG, Switzerland) with further testing for reversibility of airway obstruction 15 minutes after inhalation of 200 mcg of salbutamol.

We conducted the bronchial challenge test with 3-minute IHS (4.5% NaCl at 37°C) via ultrasonic nebulizer to define the airway response to hyperosmotic stimuli. Inhaled corticosteroids and bronchodilators were withdrawn at least 12 hours before the challenge. Spirometry and blood sampling for biochemical study were done before and after the test. Osmotic AHR to IHS was diagnosed at the fall of FEV_1 by 10% or more from the initial value.

The collection of IS was performed using the standard methodology the day after the challenge test with IHS to improve the tolerance of the procedure. IS was collected after the estimation of lung function parameters and inhalation of salbutamol in the dose of 200 mcg. Sputum induction was done by successive 7-min inhalations of 3%, 4% and 5% solutions of NaCl via ultrasonic nebulizer (OMRON NE-U-17, Japan) at a constant flow of 3 ml/min. The inhalation was stopped when a satisfactory sputum sample was obtained or the FEV_1 value fell by more than 10% from the baseline.

The study of IS samples was done within 2 hours after collection. IS cell count was calculated with a haemocytometer. The smears were stained in a 4%-5% water solution of Romanowsky-Giemsa dye at pH 6.8. Only samples with the minimal level of squamous epithelium contamination (less than 20%) were included in the analysis.[16] The calculated number of neutrophilic and eosinophilic leukocytes, macrophages, lymphocytes and bronchial epithelium cells were expressed as a percent of total cell count.

MPO activity in leukocytes was evaluated by Graham-Knoll cytochemical method[17] in cytological smears of IS with additional Azure-2 staining. Images of microslides were captured by DCM 510 digital camera (Hangzhou Scopetek Opto-Electric Co.,Ltd.) and processed in ImageTool, Optika Vision Pro and MBF ImageJ software. Mean cytochemical coefficient (MCC) of MPO (in pixels) was calculated based on the optical density of the cells obtained by microdensitometry.

The degree and intensity of the destruction in neutrophilic and eosinophilic leukocytes were defined as one of 5 classes depending on the changes in structural integrity of cell elements. The degree of cell destruction was expressed as total ICD. In addition, IC was calculated as the ratio of the number of destroyed cells to the total cell count:

$$ICD=(n_1+n_2+n_3+n_4):100$$
$$IC= n_4: (n_0+n_2+n_3+n_4),$$

where 0, 1, 2, 3, and 4 – the class of destruction; n_0, n_1, n_2, n_3, and n_4– the number of cells of the corresponding destruction class.

The cytolysis intensity was evaluated as the ratio of ICD/IC.

The levels of oxidative stress and antioxidant protection were studied in the blood serum. To estimate the concentration of LP products (LHP), we used a method based on the capability of LHP to oxidize Fe^{2+} into Fe^{3+} with further reaction of Fe^{3+} with ammonium thiocyanate.

The content of VitE was assayed in blood lipid extracts by the color reaction with dipyridyl and $FeCl_3$. The content of CP in the blood serum was measured by simple calorimetric method developed by Ravin and based on the oxydation of p-phenylenediamine in the presence of ceruloplasmin.

Statistical analysis was performed using Statistica 8 software package (StatSoft Inc., Tulsa, OK, USA, 2008). All values are presented as mean ± standard error of the mean or as number (percentage). Student's paired t-tests was used to compare two groups for data with normal distribution. Multiple comparisons were performed with one-way ANOVA

and Tukey's HSD test. 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

According to the results of bronchoprovocation, patients were divided into 2 groups: Group 1 (n=10) with AHR to IHS and Group 2 (n=19) without the response to IHS. Patients with positive response to osmotic stimulus had the lowest values of FEF_{25-75}, what suggested a decreased conductance of distal bronchi (Table 1).

Table 1.

Comparative characteristic of the studied groups

Parameter	Group 1 (1)	Group 2 (2)	Control group (3)	Statistics
Age, y	43.0±2.9	39.8±1.8	37.1±3.0	ANOVA P=0.3178 Tukey's HSD P_{1-2}=0.6350;P_{1-3}=0.2852; P_{2-3}=0.6950
Height, cm	167.6±1.3	172.6±2.7	171.2±3.7	ANOVA P=0.5012 Tukey's HSD P_{1-2}=0.4704;P_{1-3}=0.7192; P_{2-3}=0.9345
Weight, kg	83.8±6.5	84.6±2.9	79.9±3.6	ANOVA P=0.7550 Tukey's HSD P_{1-2}=0.9923;P_{1-3}=0.8595; P_{2-3}=0.7444
Gender, n/% male female	5/50 5/50	8/42.1 11/57.9	6/50 6/50	X^2=0.256 P=0.8798
Asthma history, y	2.4±0.63	4.7±1.0	-	P_{1-2}=0.1271
ACT score	18.0±1.7	18.6±1.3	-	P_{1-2}=0.7849
FEV_1, %	89.8±4.9	95.8±5.7	103.6±1.6	ANOVA P=0.2397 Tukey's HSD P_{1-2}=0.6983;P_{1-3}=0.2172; P_{2-3}=0.5092
FEV_1/FVC	0.70±0.02	0.74±0.02	0.84±0.01	ANOVA P=0.0001 Tukey's HSD P_{1-2}=0.3196;P_{1-3}=0.0001; P_{2-3}=0.0012
FEF_{25-75}, %	53.0±2.0	70.9±5.1	98.8±3.77	ANOVA P=0.0000 Tukey's HSD P_{1-2}=0.0287;P_{1-3}=0.0000; P_{2-3}=0.0002
$\Delta FEV_{1 fenoterol'}$ %	16.7±6.5	11.1±3.9	-	P_{1-2}=0.4393
$\Delta FEV_{1\ IHS'}$ %	-15.8±2.1	-0.6±1.5	-0.98±1.1	ANOVA P=0.0000 Tukey's HSD P_{1-2}=0.0000;P_{1-3}=0.0000; P_{2-3}=0.9834
Amount of delivered aerosol during HIS, g	3.5±0.22	3.2±0.19	5.1±0.33	ANOVA P=0.0000 Tukey's HSD P_{1-2}=0.6752;P_{1-3}=0.0005; P_{2-3}=0.0000

In the inflammatory pattern of asthma patients there was a prevalence of eosinophils and neutrophils count against the background of an elevated number of lymphocytes and double reduction of macrophages in comparison with the control group (Table 2). The neutrophilic granulocytes tended to dominate in IS of patients of Group 1 than in those of Group 2 and the control group, and were accompanied by a lower macrophage count and an enhanced desquamation of bronchial epithelium.

Table 2.

Cellular composition of induced sputum

Parameter	Group 1 (1)	Group 2 (2)	Control group (3)	Statistics
Neutrophils, %	38.9±5.0	25.5±4.8	11.6±0.7	ANOVA P=0.0017 Tukey's HSD P_{1-2}=0.1045; P_{1-3}=0.0011; P_{2-3}=0.0677
Eosinophils, %	24.5±5.7	19.2±3.3	0.1±0.08	ANOVA P=0.0002 Tukey's HSD P_{1-2}=0.5656; P_{1-3}=0.0003; P_{2-3}=0.0010
Macrophages, %	30.2±8.2	49.3±5.3	84.8±0.9	ANOVA P=0.0000 Tukey's HSD P_{1-2}=0.0545; P_{1-3}=0.0000; P_{2-3}=0.0001
Lymphocytes, %	3.6±0.97	4.2±0.89	2.4±0.3	ANOVA P=0.3020 Tukey's HSD P_{1-2}=0.8747; P_{1-3}=0.6428; P_{2-3}=0.2709
Epithelium, %	2.1±0.86	0.5±0.15	0.9±0.2	ANOVA P=0.0254 Tukey's HSD P_{1-2}=0.0197; P_{1-3}=0.1422; P_{2-3}=0.7356

Analysis of cytomorphologic parameters of destruction and intensiveness of cytolysis in asthma patients showed an activation of these processes in comparison with the control group (Table 3). In Group 2, the ICD degree was increased and the destructive changes of eosinophils and neutrophils prevailed over their cytolysis. At the same time, in patients with a positive response to IHS, we observed a high degree of cytolic activity of neutrophils with high values of neutrophilic ICD/IC.

Table 3.

Cytomorphologic parameters of eosinophilic and neutrophilic destruction and intensiveness of cytolysis in induced sputum

Group	Eosinophils		Neutrophils	
	ICD	ICD/IC	ICD	ICD/IC
Group 1 (1)	0.40±0.03	0.29±0.04	0.38±0.02	0.44±0.02
Group 2 (2)	0.47±0.02	0.31±0.02	0.51±0.02	0.28±0.02
Control group (3)	0.22±0.06	0.08±0.006	0.19±0.09	0.11±0.007
ANOVA statistics	P=0.0001 Tukey's HSD P_{1-2}=0.3894 P_{1-3}=0.0096 P_{2-3}=0.0000	P=0.0000 Tukey's HSD P_{1-2}=0.8260 P_{1-3}=0.0000 P_{2-3}=0.0000	P=0.0001 Tukey's HSD P_{1-2}=0.1703 P_{1-3}=0.0483 P_{2-3}=0.0001	P=0.0000 Tukey's HSD P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.0000

MPO MCC in IS of Group 1 patients (142.5±10.98 px) was significantly higher than in Group 2 patients (97.89±11.43 px; *P=0.01*) and exceeded the value of the control group(84.8±4.06 px, *P<0.001*), which suggested the intensification of intracellular MPO activity.

Evaluation of the basic level of serum LHP in asthma patients with AHR to IHS revealed higher values of this parameter in comparison with the control group (Table 4). After the challenge with IHS, the concentration of LHP increased in both groups but to a greater extent in Group 1. In these patients, enhanced production of LHP after the test was well correlated with high responsiveness of small bronchi (ΔFEF_{50}) to IHS (r=-0.75; *P=0.02*).

Table 4.

LHP, VitE and CP before and after the challenge with IHS

Variable		Group 1 (1)	Group 2 (2)	Control group (3)	Statistics
LHP, nM/ml	Before TwIHS	16.5±0.6	14.6±0.6	12.4±1.2	ANOVA P=0.0109 Tukey's HSD P_{1-2}=0.2528;P_{1-3}=0.0082; P_{2-3}=0.1314
	After TwIHS	19.8±1.0	16.2±0.8		P_{1-2}=0.0112
	P	0.08	0.17		
ΔLHP, nM/ml		3.30±0.7	1.31±0.67		P_{1-2}=0.0706
VitE, mcg/ml	Before TwIHS	33.4±1.6	30.0±0.8	38.1±1.5	ANOVA P=0.0001 Tukey's HSD P_{1-2}=0.1351;P_{1-3}=0.0459; P_{2-3}=0.0000
	After TwIHS	34.8±1.8	29.9±1.2		P_{1-2}=0.0277
	P	0.49	0.91		
ΔVitE, mcg/ml		1.4±1.40	-0.1±1.08		P_{1-2}=0.4128
CP, mg/100ml	Before TwIHS	23.2±1.0	23.1±2.3	33.8±2.9	ANOVA P=0.0051 Tukey's HSD P_{1-2}=1.0180;P_{1-3}=0.0223; P_{2-3}=0.0065
	After TwIHS	23.3±0.9	21.8±1.1		P_{1-2}=0.3738
	P	0.85	0.51		
ΔCP, mg/100ml		0.10±0.6	-1.30±1.6		P_{1-2}=0.5418

TwIHS - test with IHS

There was initially a lower concentration of CP in both groups of patients in relation to healthy people. The challenge with IHS did not produce any dynamics of CP level in the studied subjects. In addition, a close correlation was found between the initial CP level and the response of small bronchi (ΔFEF_{50}) to IHS(r=0.73; *P=0.03*).

The level of VitE as another antioxidant in asthma patients initially was lower than in healthy subjects. Similarly to CP, there was no significant dynamics of VitE in both groups of patients after the test with IHS, so its level remained relatively constant.

Discussion

Most of the IS cells in patients with AHR to IHS were neutrophils, though eosinophils were also presented in this group and their inflammatory pattern may be characterized as combined neutrophilic-eosinophilic. The same phenotype has been observed before in asthma patients with the bronchial response triggered by cold.[18,19] However, it differed from the inflammatory pattern found in patients with AHR to hypoosmotic stimulus, in which eosinophilic granulocytes prevailed in IS.[20]

According to the literature, cytolysis along with cell degranulation serves as one of the forms of pro-inflammatory enzymes released from leukocytes.[10] Cell destruction of varying degrees does not always lead to cell death and may represent one of the morphological and cytological equivalents of degranulation. Cytolysis, rather than destruction, is associated with the influence of damaging factors that cause the loss of cell membrane integrity and isolation of the cell from its surroundings with further development or progression of inflammation.[4]

It is most likely that the increased cytolysis of neutrophils and the necrotic path of leukocyte death in patients with asthma who have AHR to IHS occurs due to a serious inflammatory stimulation of synthesis and secretion of MPO into extracellular space.

LP level is closely associated with the accumulation of MPO in leukocyte granules, which release under cellular destruction, and cytolysis and catalytic production of RHS. LHPs are considered to be traditional indicators of LP in biological systems. As is known, diene conjugates and LHP are formed by the free-radical mechanism of interaction between the primary RHS and unsaturated bonds of phospholipid acyl chains.[21] The role of the initiating link in RHS-induced LP is given to the reaction of HOCl with the hydroperoxide group, which is always present in the unsaturated lipid as a result of its natural oxygenation. This process is accompanied by the formation of peroxyl radicals with their further transformation into alkoxyl radicals.[11,21] It has been proven that MPO in the presence of its substrates (H_2O_2 and Cl^-) breaks down activated hydroperoxide with the formation of O-centered radicals identified as peroxyl and alkoxyl.[11] It is also supposed that with the hydroperoxides of fatty acids, it is possible that some quantity of singlet oxygen, besides O-centered radicals, could be formed. Inhibitory analysis conducted with HOCl "traps," interceptors of free radicals and MPO inhibitors, showed that the disruption of the hydroperoxy group in the presence of isolated MPO or activated neutrophils was caused by direct enzyme activity.[11]

An initially high content of LHP in the blood serum in patients with asthma, and its further increase after the challenge in Group 1, indicated the mobilization of a free-

radical oxidation cascade involving different organ systems. The obtained data on cytological and cytochemical analysis of IS corresponded to initially increased LHPs with their further growth after the bronchial challenge test in Group 1. The more intensive were the processes of neutrophils cytolysis and MPO accumulation, the higher were the level of LHPs in the blood. This serves as an evidence of the participation of RHS formed at MPO-dependent catalysis in the generation of LP products.

The scale of halogenating stress is determined not only by the amount and activity of MPO secreted into extracellular space but also by the efficacy of the natural interceptors of RHS. CP is the most effective interceptor of HOCl along with glutathione, taurine, ascorbate and urate, which also have very high reaction rate constants with HOCI.[22] The increase of neutrophilic and eosinophilic granulocytes in IS with high ICD and ICD/IC and elevated intracellular MPO in asthma patients with AHR to IHS indicates the activation of synthesis and release of MPO in these subjects. The deficit of CP observed in asthma patients most likely was caused by rising inflammation and the shift of redox status to prooxidants. In halogenating or oxidative stress, the low level of CP is associated with the disturbance of its ability to form complexes with MPO and inhibit both the chlorinating and peroxide activity of the enzyme.[23,24]

The low concentration of VitE found in asthma patients has an unfavorable prognostic effect on the treatment of the disease. VitE is able to regulate the biotransformation and pharmacological effect of drugs by changing the activity of xenobiotic-metabolizing enzymes, including the system of cytochrome P450. Cytochrome P450 3A4 is the most functionally important among the cytochrome P450 family as it metabolizes 637 substrates and participates in metabolism of 50% of currently used medicaments. ROS can interact with cytochrome P450, inducing inactivation of the enzyme. Vitamin C, vitamin A and VitE also influence the catalytic activity of cytochrome P450-3A4. As the reduction of cytochrome P 450 heme is the main stage in catalysis and is accompanied by generation of ROS, VitE influences catalytic functions of this hemoprotein. The influence of ROS "traps," including VitE, on electrocatalysis has been recently discussed in the literature.[25]

Enhanced inflammation with high activity of neutrophilic and eosinophilic leukocytes, deposition and exocytosis of MPO at cellular destruction, and cytolysis in combination with escalation of oxidative and halogenating stress are engaged in the development of AHR to IHS in asthma. In these patients, an elevated level of serum LHP is accompanied by incomplete neutralization of LP toxic molecules due to the decreased concentrations of antioxidants. Further study of MPO and LP as markers of osmotic AHR may be useful in connection with asthma therapy to achieve optimal disease control.

Sources of Funding

The study was supported by Russian Science Foundation (Grant #14-25-00019).

Competing interests

The authors declare that they have no conflict of interest.

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International Journal of Biomedicine 6(4) (2016) 265-270
doi: 10.21103/Article6(4)_OA3

ORIGINAL ARTICLE

Hepatology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Vitamin D Receptor *FokI* Gene Polymorphism Predicted Poor Response to Treatment in Chronic HCV Genotype 4

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Abstract

The aim of this study was to investigate the association between a genetic polymorphism of the vitamin D receptor (VDR) and antiviral responses in Egyptian patients with chronic hepatitis C virus genotype 4 (HCV-4).

Methods: Our study enrolled 100 HCV-4 patients who received pegylated interferon alpha-2a (pegIFNα-*2a) and* ribavirin for 48 weeks. Patients were divided into 2 groups according to their response to therapy: 50 were responders, and 50 were non-responders. All HCV-4 patients were further subjected to the following laboratory tests: HCV-RNA using quantitative PCR, vitamin D level using ELISA and VDR genotype using PCR-RFLP assays, and abdominal ultrasonography.

Results: There was a statistically significant difference in the frequency of the VDR polymorphism (*FokI* rs10735810) between responders (FF:60%, Ff:16%, ff:24%) and non-responders (FF:10%, Ff:26%, ff:64%) (*P<0.001*). There was a statistically significant association between VDR polymorphism with higher ALT levels (ff: 63.2±30.8 U/L, Ff: 48.5±19.5 U/L, FF: 54.4±10.8 U/L, *P=0.04*) and higher alkaline phosphatase levels (ff: 102.6±53.2 U/L, Ff: 100.3±66.4 U/L, FF:68.3±29.4 U/L, *P=0.007*). VDR polymorphism showed no association with baseline vitamin D levels (*P=0.21*).

Conclusion: VDR polymorphism plays a role in the treatment response of HCV and the modification of disease progression in Egyptians infected with chronic HCV-4. **(Int J Biomed. 2016; 6(4):265-270.)**

Key Words: HCV genotype 4 • response to treatment • vitamin D • FokI polymorphism (rs10735810)

Introduction

Chronic HCV remains a worldwide health problem with prevalence rates reaching alarming levels in some areas such as Egypt, where the prevalence is 15%-22%.[1,2] For genotype 4, which is the most prevalent form in Egypt, pegIFNα-*2a* and ribavirin remain the suboptimal "gold standard" for treatment with sustained virologic response rates (SVR) of about 40%-60%.[2] Despite the emergence of novel direct-acting antivirals, to date no interferon-free regimen has proved as effective as interferon-incorporating regimens and none has yet been approved for the treatment of HCV.[3,5]

In the quest to enhance treatment response there has been a relentless search for predictors of response, especially modifiable factors. Recently, the spotlight has been focussed on the role of vitamin D and its relation to progression and response to therapy of HCV.[6] A few studies have recently reported the association of serum vitamin D levels with fibrosis levels and response to treatment in genotypes 1,2,3 HCV.[7-9] Some other studies, however, have negated such evidence.[10] Two small trials have reported an improved SVR with vitamin D supplementation.[11,12] Vitamin D is known to have many immunemodulatory roles and possibly roles in modulating the process of fibrogenesis.[13,14] Immune-regulatory actions of vitamin D are thought to be exerted through the nuclear VDR, expressed in antigen-presenting cells and activated T cells.[13-15] VDR determines interference and/or direct interaction with vitamin D responsive elements in the promoter regions of cytokine genes. Analogous to IL28 gene polymorphisms, it was proposed that genetic polymorphisms affecting the vitamin D pathway may significantly affect response to therapy. Only a few studies have addressed a limited number of genetic polymorphisms where *the vitamin D 1α-hydroxylase* (CYP27B1-1260**)** promoter polymorphism and VDR polymorphisms (rs1544410, rs7975232 and rs731236) have shown significant association with response in

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genotypes 1,2 and 3.[8,16,17] The VDR *FokI* gene polymorphism has been implicated in many immunologic processes including breast and prostate cancer, autoimmune hepatitis, primary biliary cirrhosis, and TB;[6,18-20] its role in HCV is yet to be explored.

Our study aimed to assess the relation between VDR genetic polymorphism (*FokI* rs10735810) and response to therapy in HCV-4 patients. In addition, we also assessed the inter-relation between vitamin D levels and treatment response.

Materials and Methods

Patients

This is a retrospective study assessing the stored sera and whole blood samples of HCV-4 patients who received pegIFNα-*2a* and ribavirin. Fifty consecutive responders (RS) and 50 consecutive non-responders (NR) were selected for the study. Inclusion criteria included: treatment-naïve patients who received pegIFNα-*2a* (180 ug weekly) and weight-based ribavirin (1000-1200 mg daily) for 48 weeks according to current guidelines, compliance with treatment as defined by receiving ≥80% of drugs especially during the first 12 weeks of treatment, written consent of genetic testing and the presence of pre-stored serum and whole blood samples collected during the month before therapy and at the end of therapy. Patients without the previous criteria or with any of the following criteria were excluded: vitamin D or calcium supplementation during therapy or during the 6 months previous to therapy, bone and rheumatologic disorders and renal disorders of any severity. All patients had liver biopsies taken prior to initiation of treatment, and fibrosis was graded according to the METAVIR model. Clinical and demographic characteristics including age, sex, liver biopsy data, HCV viral load, hematologic indices and clinical biochemistry data were extracted from clinical databases.

Analysis of blood samples

A 5 ml whole blood sample was divided into 2 parts. The first part was subjected to DNA extraction and followed by assessment of VDR polymorphism rs10735810 (VDRP) by PCR-*RFLP* analysis. The second part was centrifuged at 3000xg to separate plasma for further assessment of vitamin D level (ng/mL) and quantitative HCV-RNA. Other laboratory measurements were assessed: CBC, INR, blood glucose, ALT, AST, albumin, bilirubin, alkaline phosphatase, α feto-protein, and creatinine.

PCR-RFLP analysis

Total DNA was isolated from mononuclear cells (MNC) using the extraction kit (Qiagene, USA) according to instructions of manufacturer. VDRP rs12979860 genotyping was assessed by RFLP-PCR method, EzWayTM Direct Taq PCR Master mix (Koma Biotech Inc., Seoul, Korea) in 25 µL reaction volume. The primers used for PCR-RFLP were Forward 5`-AGCTGGCCCTGGCACTGACTCTGCTCT-3`and Reverse 5`- ATGGAAACACCTTGCTTCTTCTCCCTC-3` (gene bank accession number: NG008731.1). The thermal cycling profile involved denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec for 35 cycles. Final extension was continued at 72°C for 10 min. The PCR products were separated by 2% agarose gel electrophoresis. 10uL of the PCR products were digested with 1 unit of the *FokI* restriction endonuclease (New England Biolabs, Hitchin, UK) in a total volume of 20uL at 37°C overnight. Both homozygous (FF and ff) and heterozygous genotypes (Ff) were estimated on 4% agarose gel.

Vitamin D serum level assessment

Vitamin D serum level was detected by ELISA 25-OH Vitamin D kit according to instructions of manufactures (DRG, international Inc., USA). The data are expressed as ng/mL. Grading of vitamin D levels was done as follows: normal (≥30 ng/mL), insufficiency (>10 - < 30 ng/mL), and deficiency (≤10 ng/mL).

qRT-PCR of HCV RNA

The AgPath-ID™ One –Step RT-PCR kit was obtained from Applied Biosystems (Foster City, CA, USA). HCV PCR was performed with lower limit of detection 15 IU/mL.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 22. The odds ratio (OR) and 95%CI were calculated to estimate the strength of associations between each genotype and alleles and patients and controls. *P* values were considered significant when $P < 0.05$.

Results

Patient characteristics are shown in Table 1.

Table 1. Baseline characteristics of patients

Variable	NR	RS	P
Sex Females (%)	28 (56)	12 (24)	0.001
Age, y	42.9±9.5	40.1±11.5	0.188
AST, IU/L	57.7±35.9	44.6±13.6	0.018
ALT, IU/L	61.5±31.2	52.6±10.1	0.060
Alk. Phosphatase, IU/L	113.0±60.2	67.2±26.6	<0.01
Bilirubin, g/dL	0.8±0.3	0.7±0.2	0.139
Albumin, g/dL	4.2±0.7	4.1±0.5	0.137
Creatinine, mg/dL	0.9±0.2	0.9±0.3	0.720
INR	1.1±0.1	1.1±0.1	0.702
WBC count/mL	5.9±1.5	6.6±2.0	0.044
Platelets /mL	223.6±67.4	273.0±94.7	0.003
	11.0±23.4	3.0±2.0	0.020
	F1: 14	F1: 14	0.819
	F2: 29	F2: 31	
	F3: 7	F3: 5	
HCV RNA, IU/mL x10^3	1288±2288	464±416	0.015
Vitamin D level, ng/mL	7.26±3.93	5.31±2.83	0.005

All HCV-4 patients suffered vitamin D deficiency or insufficiency (<30 ng/mL) prior to treatment. Although all patients were either deficient or insufficient in vitamin D at baseline, responders had a significantly lower baseline vitamin D level than non-responders (5.31±2.83 ng/mL vs. 7.26±3.93 ng/mL, *P=0.005*). There was a trend for higher baseline

vitamin D level in higher fibrosis stages (F1: 5.5±2.6 ng/mL, F2: 6.2±3.7 ng/ml, F3: 8.2±4.2 ng/mL; F1 vs. F3: *P=0.03*, F1 vs. F2: P=0.08, F1 vs. >F1: *P=0.19*).

At the end of treatment, serum vitamin D levels remained unchanged in non-responders (7.2±2.9 ng/mL vs 6.5±4.8 ng/mL at baseline and end of treatment respectively, *P=0.3*), while in responders serum vitamin D levels improved significantly (5.3±2.8 ng/mL vs 65.8±16.2 ng/mL at baseline and end of treatment respectively, *P<0.001*), Figure 1. According to standard cut-off values, all 50 responders reached normal vitamin D levels (> 30 ng/mL) at the end of treatment while among non-responders only 1(2%) patient improved from being deficient to insufficient and none reached normal levels (*P<0.01*, Table 2).

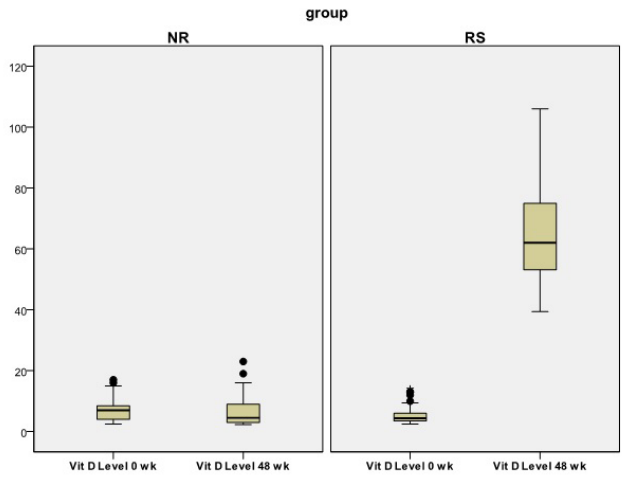

Fig. 1. *Vitamin D level in different studied groups*

Table 2. Vitamin D status before and after treatment

Vitamin D status	NR	RS	P value
Before treatment			0.25
Deficient	41	45	
Insufficient	9	5	
Normal	0	0	
After treatment			<0.001
Deficient	40	0	
Insufficient	10	0	
Normal	0	50	

VDRP

Of the 100 studied patients, 35 had the FF *FokI* variant, 44 had the polymorphic ff variant, and 21 were heterozygous Ff. Of the non-responders, 45(90%) patients had VDR FokI polymorphism, whether homo- or heterozygous (ff or Ff), in comparison to only 20(40%) patients in responders (*P<0.001*, Table 3). When analysing separate allelic combinations, 32(64%) patients with ff genotype were found in non-responders vs. only 12(24%) in responders (*P<0.001*), non-responders included 13(26%) patients with Ff genotype vs 8(16%) in responders (*P=0.22*). The FF variant was much more prevalent in responders than in non-responders: 30(60%) vs. 5(10%) (*P<0.001*), Figure 2.

Table 3. VDR genotype in responders and non-responders

Genotype	NR	RS	P value
ff	32 (64%)	12 (24%)	<0.001
Ff	13 (26%)	8 (16%)	0.22
FF	5 (10%)	30 (60%)	<0.001
(ff + Ff)	45 (90%)	20 (40%)	<0.001

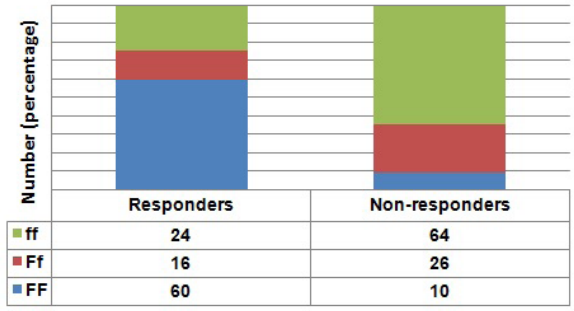

Fig. 2. *Proportion of different VDR FokI genotypes in RS and NR. Numbers indicate % of patients; P<0.001*

Correlation of VDRP with baseline patient characteristics and laboratory data revealed that polymorphism was associated with higher ALT (ff: 63.2±30.8 U/L, Ff: 48.5±19.5 U/L, FF: 54.4±10.8 U/L; *P=0.04*) and alkaline phosphatase (ff: 102.6±53.2 U/L, Ff: 100.3±66.4 U/L, FF: 68.3±29.4 U/L; *P=0.007*) levels. VDRP showed no significant association with fibrosis levels and baseline vitamin D levels; however, there was a trend toward higher vitamin D levels in patients with homozygous polymorphism (ff) versus patients with the wild FF genotype (7.0±3.7 ng/mL vs. 5.6±3.2 ng/ml, *P= 0.08*).

PCR products restricted with *FokI* were shown in Figure 3 and 4. The bands were shown after treatment with *FokI* as FF homozygous (266 bp), Ff heterozygous (266 bp and 193 bp) and ff homozygous (193 bp and 73 bp) according to restriction patterns.

Fig. 3. *Agarose gel electrophoresis showed FokI restriction patterns of the various genotypes of VDR.*

Lane M: DNA ladder (100,200,……bp)
Lane 1-3: 266 bp *PCR* products before treatment of enzyme *(FF genotype)*
Lane 4 and 5: 266 bp *PCR* products after treatment of enzyme *(FF genotype).*
Lane 6-9: 266, 193 and 73 bp *PCR* products after treatment of enzyme *(Ff genotype).*

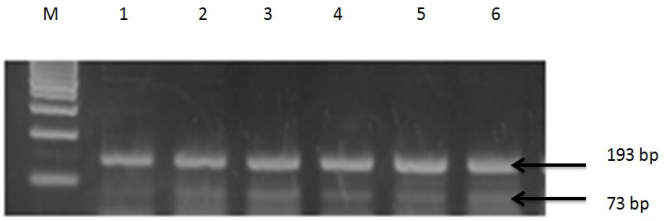

Fig. 4. *Agarose gel electrophoresis showed FokI restriction pattern of ff genotype of VDR.*

Lane M: DNA ladder (100,200,……bp)
Lane 1-6: 193 and 73 bp *PCR* products after treatment of enzyme *(ff genotype).*

Predictors of response

Multivariate analysis revealed that the following factors were associated with response (Table 4): lower age, alfa-fetoprotein, baseline vitamin D level, and absence of VDRP.

Table 4.
VDR polymorphism multivariate analysis with other predictors of antiviral response in HCV patients

Variable	OR	95% CI	P value
Age	1.1	1.0-1.3	0.008
Female gender	16.0	2.6-100	0.003
AFP	1.6	1.1-2.4	0.02
Baseline Vitamin D	1.4	1.0-2.0	0.02
VDR polymorphism	14.2	2.0-100	0.008

Discussion

The principle findings of our study are: a) the *FokI* VDRP is independently associated with poor response to therapy in HCV-4; b) chronic HCV-4 patients have a high prevalence of severe vitamin D deficiency; c) responders to treatment of HCV-4 have a remarkable improvement in serum vitamin D levels while non-responders do not; d) baseline vitamin D level does not correlate with a better response to treatment.

Immune-regulatory actions of vitamin D are thought to be exerted through the nuclear VDR, expressed in antigen-presenting cells and activated T cells. [13,14] VDR determines interference and/or direct interaction with vitamin D responsive elements in the promoter regions of cytokine genes. Immune-regulatory effects of vitamin D occur through many mechanisms, including the down-regulated expression of MHC class II, co-stimulatory molecules, and IL-12.[21,22] On the other hand, vitamin D enhances IL-10 production and promotes dendritic cell apoptosis.[21,22] A few studies have recently explored the impact of genetic polymorphisms affecting the vitamin D pathway on the course of chronic HCV and its response to treatment.[8,16,17,23] The CYP27B1-1260 promoter polymorphism (responsible for production of 1,25-dihydroxyvitamin D) has been shown to be a predictor of poor response to treatment in genotypes 1,2 and 3,[8] especially in difficult- to-treat patients.[16,23] These results have been negated, however, by 2 recent large studies that revealed no association between CYP27B1-1260 polymorphism and response to treatment in predominantly genotype 1 cohorts of patients.[17,24] Baur et al. reported that the bAt(CCA) haplotype was associated with impaired response to HCV treatment, a finding that was confirmed by Garcia-Martin et al.[17,24] A single nucleotide polymorphism of the VDR gene (rs2228570 T/C) also had a negative impact on treatment response.[17] The VDR *FokI* polymorphism restriction site is located on exon 2 in the 5′ coding region of the gene.[25] The *FokI* polymorphism has been correlated with many immunologic processes including cancers such as breast, prostate and colorectal cancer, autoimmune hepatitis and TB.[6,23-25] To our knowledge only one study has assessed the role of VDRP in HCV, in a mixed population including genotypes 1,2 and 3, VDRP showed no significant association with treatment response. [8] Our study is the first to assess the effect of VDRP in HCV-4. Our results show a significant and independent negative impact of VDRP on treatment response where 90% of non-responders were carriers of the restriction allele (f) in comparison to only 40% of responders ($P<0.001$). This significance was maintained in multivariate analysis ($P=0.008$). The impact of *FokI* polymorphism on response to treatment is plausible in view of the profound effects it has on the intracellular pathway of vitamin D signalling. VDR whose function is impaired by *FokI* polymorphism determines interference and/or direct interaction with vitamin D responsive elements in the promoter regions of cytokine genes.[26]

Our results also show an association between VDR *FokI* polymorphism and higher ALT and alkaline phosphatase levels. It might therefore be plausible that vitamin D deficiency or a VDR polymorphism impairing the vitamin D cellular pathway could lead to a proinflammatory milieu and promote hepatic inflammation, which is reflected in elevated ALT levels. This notion is supported by recent studies correlating low serum vitamin D levels with higher inflammatory activity indices in liver biopsies of HCV-infected patients.[7] Even patients with unexplained elevations of ALT have been shown to have lower serum vitamin D levels.[27] The correlation of VDR *FokI* polymorphism with higher alkaline phosphatase levels may also be partly explained by the concept of impairing the anti-inflammatory effects of vitamin D, as over 90% of HCV-infected patients have evidence of bile duct inflammation

Another possible explanation could be the deleterious effect of the VDR polymorphism on bone metabolism, thus increasing bone-specific alkaline phosphatase, although the results of studies testing the association of *FokI* polymorphism with osteoporosis or altered bone turnover have been discordant.[28,29]

All our HCV-4 patients had suboptimal vitamin D levels with the majority being severely deficient (86%). Possible explanations for the association of HCV with vitamin D deficiency have included decreased 25-alpha hydroxylation in the liver, HCV may have the ability to directly suppress 25-alpha hydroxylation through inducing cytokines and

oxidative stresses, and a recent study has shown that HCV alters lipid metabolism directly reducing production of 7-dehydrocholesterol, the precursor of endogenously-produced vitamin D.[30,31] The independent effect of HCV on vitamin D levels is strongly supported by our finding of the remarkable improvement in vitamin D levels after successful eradication of HCV and its persistence in non-responders. Further studies are definitely recommended to depict the exact mechanisms by which HCV alters vitamin D production and metabolism.

Perhaps an unexpected finding in our study was that vitamin D deficiency did not correlate with poor response to treatment. This comes in contrast to many studies that have shown that a lower vitamin D level is associated with poor response to interferon-based treatment in genotypes 1,2 and 3.[7-9] Our study is not, however, the only one to negate such evidence; a recent large study including 274 genotype 1 patients not only demonstrated a lack of association between vitamin D levels and response to treatment, but responders actually had lower baseline vitamin D levels than non-responders in univariate analysis (76.6nmol/L vs. 84.7 nmol/L, respectively; *P=0.03*).[10] Another large study including 310 genotype 1 patients also found no association. [24] In 2 studies that frankly showed no association of response with vitamin D level [our current study and Kitson et al.,2013] vitamin D levels were measured by LC–MS/MS methodology,[10,32] avoiding fallacies that may be induced by other commercially available kits. Another noticeable difference between the studies is the prevalence of advanced fibrosis. In the 3 studies that reported no association of vitamin D with response [our current study, Kitson et al.,2013, and Grammatikos et al.,2011] prevalence of advanced fibrosis was 12%, 14% and 19%, while Bitetto et al.[9] and Petta at al.[7], who both found a relation between vitamin D levels and response, had patients with a high prevalence of advanced fibrosis (29% and 28%).[7,9,10,24] This observation leads us to wonder whether the association of lower vitamin D with a poor response is not strongly confounded by the advanced fibrosis, even though strictly statistically speaking the multivariate analyses in these studies should have excluded this possibility.

Our study has some limitations. First is the retrospective nature of the study. Second, we had no standardization of the time (season) when samples were taken, which could influence vitamin D levels. Third, our cohort consisted of only Egyptian patients and thus results could not be extrapolated to other populations, especially because ethnic differences have been shown in the allelic frequencies in VDR polymorphisms.[33] Fourth, our study design also put some limitation on our interpretation of results; we started by selecting a fixed number of responders and non-responders (limited by samples availability) and compared them, rather than starting with a single cohort of patients and comparing all responders and non-responders. Strictly statistically speaking this approach did not allow us to express the SVR rate in each genetic variant, but rather the prevalence of each genetic variant in both responders and non-responders. Nevertheless, with the obvious high prevalence of the f allele in non-responders, it could be reasonably and confidently deduced that the f allele is strongly associated with a lower SVR. Fifth, our study did not assess IL28 gene polymorphisms, which have a strong impact on response.

In conclusion, the VDR *Fok*I gene polymorphism is associated with poor response to treatment in HCV-4 patients. Our study reveals significant vitamin D deficiency in HCV-4 patients that reverts with eradication of HCV, yet there is no association of vitamin D levels with response. A larger study assessing the FokI polymorphism, IL28 polymorphism, and vitamin D levels and possibly the effect of vitamin D supplementation is warranted.

Competing interests

The authors declare that they have no conflict of interest.

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International Journal of Biomedicine 6(4) (2016) 271-275
doi: 10.21103/Article6(4)_OA4

ORIGINAL ARTICLE

Pathophysiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Ion Exchange Properties of the Erythrocyte Surface Protein Band 3 and its Role in the Process of Oxygenation during CMV Infection in the Third Trimester of Pregnancy

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Abstract

The aim of our study was to determine the nature of the ion exchange properties of EPB3 and its participation in the process of oxygenation with CMV infection (CMVI) in the third trimester of pregnancy.

Material and methods: The study included 105 pregnant women (the third trimester of gestation from 26 to 32 weeks): 35 CMV-seropositive pregnant women (the main group - Group 1) with CMVI exacerbation and anti-CMV IgG antibody titer of 1:1600, 35 CMV-seropositive pregnant women (the comparison group - Group 2) with latent CMVI and an anti-CMV IgG antibody titer of 1:800, and 35 CMV-seronegative pregnant women (the control group).

The study of the protein spectrum of the erythrocyte membrane was carried out by analytical separation in the presence of 0.1% SDS on a one-dimensional 7.5%-10% gradient polyacrylamide gel. The oxyhemoglobin content was determined spectrophotometrically by the method of Malloy and Evelyn (1969); the total and bound-to-hemoglobin ATP and the total and inorganic phosphorus were determined by IS Luganov and MN Blinov (1975), the activity of ouabain-sensitive Na+/K+-ATPase by Kazennova's method (1986) and the levels of Na+ and K+ in plasma and RBCs by using "Vital Best" (Russia) sets.

Results: CMVI exacerbation in the third trimester of pregnancy is associated with a decrease in the content of EPB3 in RBC membranes due to increased proteolytic processes, which causes disturbances in its structural and functional properties. Thus, ion transport and association of the cytoplasmic domain of the protein with deoxyhemoglobin are changed and disrupt the processes of oxygenation. **(Int J Biomed. 2016; 6(4):271-275.)**

Key words: *cytomegalovirus (CMV) • erythrocyte surface protein band 3 (EPB3) • pregnancy • Na+/K+-ATPase • oxyhemoglobin*

Introduction

Erythrocyte, like every cell, has an outer shell, which separates its internal contents from the external environment. The inner layer directly adjacent to the cell cytosol is called the cytoplasmic membrane. The cell membrane provides elasticity, strength, and stability, and it has the ability to deform when red blood cells (RBCs) pass through the narrow holes of capillaries.[1] These properties are supported by the lipid-protein complex of the erythrocyte membranes.[2] Proteins belonging to the membrane, depending on their location and functional properties, are divided into peripheral and integral peptides. Of particular importance is the erythrocyte surface protein band 3 (*EPB3*), which has a wide range of properties.[3] EPB3 binds cytoskeletal proteins (spectrin and ankyrin) to the cytoplasmic membrane, which provides structural integrity and a biconcave shape to erythrocytes.[4] According to other research, terminal NH2-groups of EPB3 are associated with deoxyhemoglobin [5] and enzymes of glycolysis.[6] These data also show that deoxyhemoglobin competes with glycolytic enzymes for the binding site on the EPB3 that leads to the reversible displacement of the glycolytic enzymes from the membrane and changes their catalytic properties in response to changes in PO_2.[7] Also, it has been proved the role of the

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EPB3 – deoxyhemoglobin interactions in the regulation of erythrocyte volume modulated by activity of Na+/K+–channels and specific ATPases associated with them.[8] However, the stability of the proteinaceous cytoskeleton of RBC membranes can be exposed to toxic effects of various kinds, including the action of viruses. Previously we have shown the impact of the persistent herpesvirus and cytomegalovirus (CMV) infections on structural and functional properties of the peripheral blood erythrocyte, including during pregnancy.[9]

The aim of our study was to determine the nature of the ion exchange properties of EPB3 and its participation in the process of oxygenation with CMV infection (CMVI) in the third trimester of pregnancy.

Material and Methods

The study was conducted in the laboratory of pathogenesis and regenerative processes of the respiratory system in non-specific lung diseases and the department of pregnancy pathology of the Far Eastern Scientific Center of Physiology and Pathology of Respiration of the RAS (Blagoveshchensk, Russia). The study was approved by the local Ethics Committee. Written informed consent was obtained from all participants.

The study included 105 pregnant women (the third trimester of gestation from 26 to 32 weeks): 35 CMV-seropositive pregnant women (the main group - Group 1) with CMVI exacerbation and anti-CMV IgG antibody titer of 1:1600, 35 CMV-seropositive pregnant women (the comparison group - Group 2) with latent CMVI and an anti-CMV IgG antibody titer of 1:800, and 35 CMV-seronegative pregnant women (the control group).

Inclusion criteria for the main group were a relapse of CMVI identified by molecular biological and serological methods and anti-CMV IgG antibody titer of 1:1600, as well as herpes virus infection (HHV-1,2) remission during the entire gestation period.

Exclusion criteria were primary CMVI, an aggravation of other inflammatory diseases of extragenital localization and sexually transmitted infections.

The relapse of CMVI was diagnosed in a comprehensive study of the peripheral blood to check for the presence of IgM or a fourfold or more increase in anti-CMV IgG antibody titer in paired serum in the dynamics after 10 days, an avidity index > 65%, and the presence of CMV DNA in samples of blood, urine, buccal epithelium, and cervical mucosa.

Blood samples (5 ml) were collected from the ulnar vein in standard vacuum tubes with EDTA to obtain the samples of mononuclear cells. For serological tests, we used blood that does not contain anticoagulants. Mononuclear cell isolation for PCR was carried out with Ficoll-Urografin (d-1,077g/ml) ("DNA-Technology", Russia). Serological studies were performed in paired serum samples at intervals of 10-14 days. The morning urine specimens for PCR analysis were collected in a sterile container (60 ml). Buccal epithelium and contents of the cervical canal were collected in standard sterile plastic tubes (0.5 ml) with a physiological solution.

For CMV verification, the type-specific antibodies (IgG and IgM) and avidity index were determined by ELISA on the microplate reader "StatFax 2100" (USA) using the sets of CC "Vector-Best" (Russia). CMV DNA was detected by PCR on a DT-96 machine using sets of "DNA-Technology" (Russia).

The study of the protein spectrum of the erythrocyte membrane was carried out by analytical separation in the presence of 0.1% SDS on a one-dimensional 7.5%-10% gradient polyacrylamide gel.[10] Polypeptide bands were stained in a 0.1% solution of Coomassie R-250, a 50% alcohol solution, and a 7% acetic acid solution. The electrophoregrams identification was carried out at a wavelength of 590-600 nm using BioDocAnalize machine (Germany). The oxyhemoglobin content was determined spectrophotometrically by the method of Malloy and Evelyn [11] using StatFax-1900 (US); the total and bound-to-hemoglobin (Hb) ATP and the total and inorganic phosphorus (Pt and Pi) were determined by IS Luganov and MN Blinov,[12] the activity of ouabain-sensitive Na+/K+-ATPase by Kazennova's method, [13] and the levels of Na+ and K+ in plasma and RBCs by using "Vital Best" (Russia) sets.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard error of the mean (SEM) were calculated. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results

The EPB3 level in Group 1 was 1.22 times less than in the control group ($P=0.0000$) and 1.19 times less than in Group 2 ($P=0.0000$) (Table 1), which indicated an increase in the proteolytic degradation of protein components of the erythrocyte membrane and the destabilization of its properties caused by the increased expression of CMV antigens during CMVI exacerbation. In Group 2, we did not find statistically significant differences compared to the control group.

In assessing the state of the energy balance of RBCs of peripheral blood, which reflects the state of the processes of protein phosphorylation, we identified a reduction in the levels of total and bound-to-hemoglobin ATP that causes disorders in the structural and functional properties of the membrane and its components, including EBP3. The total ATP level was 1.4 times less than in the control group ($P=0.0002$) and 1.2 times less than in Group 2 ($P=0.0202$) (Table 1). The level of bound-to-hemoglobin ATP was 1.29 times less than in the control group ($P=0.0000$) and 1.2 times less than in Group 2 ($P=0.0000$) (Table 1). In Group 2, we found statistically significant differences compared to the control group only for the level of bound-to-hemoglobin ATP ($P=0.0004$).

The obtained results showed an increase in the content of total and inorganic phosphorus in RBCs of peripheral blood in pregnant women of Group 1 compared to the control group, which indicated an increase in ATP hydrolysis, as well as in the catalytic activity of glycolytic enzymes responsible for the synthesis of 2,3-DPG. In Group 2, we found statistically significant differences compared to Group 1 only for the level of total phosphorus.

Table 1.

EPB3, ATP, the ionic composition of the blood plasma and RBCs, and the Na^+/K^+-ATPase activity in pregnant women with CMVI in the third trimester of pregnancy

Variable	Group 1 (1)	Group 2 (2)	Control group (3)	Statistics
EPB3, %	15.00±0.12	17.83±0.17	18.34±0.19	ANOVA *P=0.0000* Tukey HSD Post-hoc Test P_{1-2}=*0.0000* P_{1-3}=*0.0000* P_{2-3}=*0.0731*
Total ATP, μmol/ml	0.51±0.03	0.64±0.03	0.71±0.04	ANOVA *P=0.0002* Tukey HSD Post-hoc Test P_{1-2}=*0.0202* P_{1-3}=*0.0002* P_{2-3}=*0.3094*
АТФ per 1 g Hb. μmol/ml	4.4±0.05	5.3±0.07	5.7±0.09	ANOVA *P=0.0000* Tukey HSD Post-hoc Test P_{1-2}=*0.0000* P_{1-3}=*0.0000* P_{2-3}=*0.0004*
Pt, μmol/ml	0.158±0.005	0.145±0.003	0.139±0.003	ANOVA *P=0.0020* Tukey HSD Post-hoc Test P_{1-2}=*0.0443* P_{1-3}=*0.0017* P_{2-3}=*0.5036*
Pi, μmol/ml	0.102±0.005	0.093±0.002	0.087±0.002	ANOVA *P=0.0072* Tukey HSD Post-hoc Test P_{1-2}=*0.1386* P_{1-3}=*0.0052* P_{2-3}=*0.4101*
Na^+/K^+-ATPase, μmol Pi/mlEr/hr	2.02±0.10	2.85±0.11	3.15±0.10	ANOVA *P=0.0000* Tukey HSD Post-hoc Test P_{1-2}=*0.0000* P_{1-3}=*0.0000* P_{2-3}=*0.1055*
plasma Na^+, mmol/ml	145.93±0.72	144.40±0.68	143.80±0.49	ANOVA *P=0.0561* Tukey HSD Post-hoc Test P_{1-2}=*0.2117* P_{1-3}=*0.0522* P_{2-3}=*0.7842*
Na^+ in RBC, mmol/ml	125.53±1.13	122.27±0.45	121.23±0.79	ANOVA *P=0.0012* Tukey HSD Post-hoc Test P_{1-2}=*0.0190* P_{1-3}=*0.0013* P_{2-3}=*0.6552*
plasma K^+, mmol/ml	3.89±0.07	3.71±0.03	3.35±0.03	ANOVA *P=0.0000* Tukey HSD Post-hoc Test P_{1-2}=*0.0223* P_{1-3}=*0.0000* P_{2-3}=*0.0000*
K^+ in RBC, mmol/ml	2.75±0.18	3.52±0.06	4.14±0.09	ANOVA *P=0.000* Tukey HSD Post-hoc Test P_{1-2}=*0.0001* P_{1-3}=*0.0000* P_{2-3}=*0.0013*

In Group 1, the identified patterns in decreasing levels of total and bound-to-hemoglobin ATP were associated with a decrease in the catalytic activity of ouabain-sensitive Na+/K+-ATPase that, apparently, caused a disorder in phosphorylation of the transmembrane domain of EBP3 in the process of its catalytic cycle, and in the transport and translocalization of ions Na+ and K+ across the plasma membrane. In Group 1, we found significantly decrease in the Na+/K+-ATPase activity (in 1.56 times) compared to the control group (*P=0.0000)* and Group 2 (in 1.4 times) (*P=0.0000*). In Group 2, the Na+/K+-ATPase activity did not change significantly compared to the control group.

In assessing the ionic composition of the blood plasma and RBCs in pregnant women of Group 1 and Group 2, we revealed the opposite changes in the content of Na+ and K+ (Table 1). Thus, we observed a moderate increase in the concentration of Na+ ions in the plasma and RBCs in Group 1 compared to the control group (*P=0.0522* and *P=0.0013)* and Group 2 (*P=0.2117* and *P=0.019*). In Group 2, we did not find statistically significant differences compared to the control group. When calculating the ratio between the content of Na+ ions in the plasma and erythrocytes, we found a moderate reduction in this parameter only in Group 1(1.16±0.01 vs 1.19±0.01 in the control group, *P <0.05*), which indicated an increase in the intracellular Na+ level, resulting in an increase in the dehydration and modulation of RBC volume.

In analyzing the content of K+ ions in the blood plasma (Table 1), we found a significant increase in this parameter in Groups 1 and 2 compared to the control group (*P=0.0000* in both cases). In Group 1, this increase was more pronounced. In RBCs, on the contrary, the levels of K+ ions decreased in Groups 1 and 2 compared to the control group (*P=0.0000* and *P=0.0013)*. In Group 1, this increase was more pronounced. The ratio between the contents of K+ ions in the plasma and

erythrocytes was significantly increased in Groups 1 and 2 compared to the control group, indicating a decrease in intracellular ion concentration.

The established changes in the ratio of Na+ and K+ ions and their transport through the transmembrane domain of EBP3 will contribute to disrupting the formation of electronegative potential of the erythrocyte membrane, causing a violation of the hemoglobin oxygenation process and the formation of its oxygenated form. This statement is confirmed by our results. Thus, in Group 1, we observed a decrease in the level of oxygenated Hb on the background of a low total Hb level compared to Group 2 and the control group (Table 2). Indicators of oxyhemoglobin in Group 1 were significantly decreased compared to Group 2 and the control group. In Group 2, we found statistically significant differences compared to the control group only for the level of total Hb.

Table 2.

The levels of total Hb and oxyhemoglobin in RBCs of the peripheral blood of pregnant women with CMVI in the third trimester of pregnancy

Variable	Group 1 (1)	Group 2 (2)	Control group (3)	Statistics
Total Hb, g/l	11.00 ±0.22	12.00 ±0.15	13.00 ±0.20	ANOVA $P=0.0000$ Tukey HSD Post-hoc Test $P_{1-2}=0.0011$ $P_{1-3}=0.0000$ $P_{2-3}=0.0011$
Oxy-Hb, %	89.00 ±0.55	97.00 ±0.23	98.00 ±0.45	ANOVA $P=0.0000$ Tukey HSD Post-hoc Test $P_{1-2}=0.0000$ $P_{1-3}=0.0000$ $P_{2-3}=0.2338$

Discussion

According to the study, CMVI exacerbation in the third trimester of pregnancy increases the expression of the antigen on the surface of RBCs, which facilitates the penetration of toxic proteins tegument deep into the cells. This is followed by the launch of a cascade of hydrolytic and proteolytic processes, disturbing the protein-protein and lipid-protein interactions and leading to a redistribution in the ratio of proteins. The proof of this fact was the decreasing EBP3 content in RBC membranes of peripheral blood of CMV-seropositive pregnant women with CMVI exacerbation compared with the latent course of CMVI and CMV-seronegative pregnant women. These changes may be the result of possible conformational changes due to oxidative modification, which breaks the association of EBP3 with ankyrin and the plasma membrane as a whole.[4] A disturbance in the structural integrity of EBP3 increases its affinity to deoxyhemoglobin in the cytoplasmic part, inducing thereby conformational and functional changes in the transmembrane domains of the protein.[3,7] These changes lead to a change in electronegativity of the erythrocyte membrane through inactivation of interfacial interactions with the ion transporter (Na+/K+-ATPase), weakening phosphorylation of the transmembrane domains of EBP3, resulting in compensatory increases in the catalytic activity of the glycolytic enzymes associated with the cytoplasmic domain of the protein, which regulate the synthesis of 2,3-DPG.[14,15] As a result, the number of bonds between deoxyhemoglobin and 2,3-DPG is increased, which reduces the affinity of hemoglobin for oxygen and the formation of its oxygenated forms. This fact is confirmed by a decrease in the EBP3 level and in the activity of the Na+/K+-ATPase in erythrocytes of the peripheral blood in CMV-seropositive pregnant women; we also found an increase in the content of inorganic phosphate and a reduction in the amount of ATP and total and oxygenated Hb in Group 1 compared with the latent course of CMVI.

Conclusion

Thus, CMVI exacerbation in the third trimester of pregnancy is associated with a decrease in the content of EPB3 in RBC membranes due to increased proteolytic processes, which causes disturbances in its structural and functional properties. Thus, ion transport and association of the cytoplasmic domain of the protein with deoxyhemoglobin are changed and disrupt the processes of oxygenation. The latter circumstance forms a threat for the development hypoxia-related disorders in CMV-seropositive women in the third trimester of pregnancy.

Competing interests

The authors declare that they have no conflict of interest.

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International Journal of Biomedicine 6(4) (2016) 276-278
doi: 10.21103/Article6(4)_OA5

ORIGINAL ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Role of Nerve Growth Factor in Assessing the Severity of Clinical Manifestations and Outcomes of Perinatal CNS Lesions in Infants

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Abstract

The purpose of our research was to analyze the association between the serum beta-NGF level and the severity of neurological deficit (ND) in children in the first year of life. Our results suggest a possible functional link between the low level of NGF and the development of severe ND. The obtained results allow us to consider the serum beta-NGF level as a useful marker of the ND severity in young children.**(Int J Biomed. 2016; 6(4):276-278.)**

Key words: nerve growth factor • neurological deficit • central nervous system • perinatal lesions.

Introduction

Despite the current improvement in perinatal care, a high incidence of perinatal lesions *of* the central nervous system (CNS) still persists.[1] Perinatal hypoxia is a common complication of pregnancy and childbirth, an important cause of central nervous system damage in newborns, leading to serious long-term neurological complications. Neurological deficit (ND), as a consequence of perinatal damage, ranges from mild behavioral disorders to cerebral palsy, epilepsy, mental retardation, etc.[2] In addition to the evaluation of the functional activity of nerve cells, an assessment of their trophic supply with the participation of neurotrophins (NTs) is very important.[3] Several neurotrophins have a multifunctional role both in the central and peripheral nervous system.[4] These neurotrophic factors are important regulators of neuronal development, proliferation, differentiation and maturation of the peripheral and central nervous system.[5] Experimental animal models showed that these neurotrophins could be effective in restoring neuronal cells after brain ischemia,[6] suggesting that they might be used as therapeutic agents for treating this kind of brain damage.[4]

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NGF (nerve growth factor) is most interesting among NTs as a marker of neurologic deficit (ND). NGF is widely expressed in various tissues; it may have a neurotrophic effect on damaged neurons and promote neurogenesis.[7] NGF supports the survival and differentiation of neurons during brain development.[8] It has been shown that NGF reduces neural degeneration[9] and promotes peripheral nerve regeneration in rats.[10] It appears globally neuroprotective to the developing brain in a neonatal model of cerebral hypoxia-ischemia.[11] A certain NGF level in the blood is an indication of the normal function of glial cells.[12] Exogenous administration of NGF and other NTs has been shown to prevent or significantly reduce severe ND, apoptosis, and brain-cell death. [13-16] The mechanisms underlying this neuroprotective role of NGF are not fully characterized, but several studies have shown its positive influence on cerebral blood flow, cellular Ca2+ homeostasis, and antioxidant activity. [13,14,17-19]

The purpose of our research was to analyze the association between the serum beta-NGF level and the severity of ND in children in the first year of life.

Material and Methods

The study included 419 patients (52% boys and 48% girls) aged from 0 to 6 months. The main group (Group 1) included 336 patients in the first year of life who were

hospitalized with perinatal nervous system lesions of varying severity; the control group included 83 apparently healthy children. Children in the control group (Group 2) passed standard clinical examinations in specified periods of observation at the stage of outpatient services.

Groups 1 and 2 were divided into two subgroups according to age: Group 1a (n=163) and Group 2a (n=43) between the ages of 1 to 3 months; Group 1b (n=173) and Group 2b (n=40) between the ages of 4 to 6 months. In accordance with the ND severity, the main group was also divided into subgroups: mild degree (n=122), moderate degree (n=118), and severe degree (n=96).

All of children underwent the somatic and neurological examination. To assess the damage to the nervous system, YA Yakunin's classification (1979) was used. ND was evaluated by a quantitative method based on the 3 points according to the severity (0 - norm, 1 - mild ND, 2 - moderate ND, and 3 - severe ND).

The serum level of the beta subunit of human NGF (beta-NGF) was measured by EIA (Beta-NGF, «RayBio» Russia.). The test was conducted according to the standard guidelines suggested by the manufacturer. Sensitivity was 1 pg/mL and cross-reactivity with other neurotrophic factors was less than 0.3%. All assays were performed in triplicate and results were expressed as pg/mL.

Statistical analysis was performed using StatSoft Statistica v6.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Analyzing the frequency of clinical neurological manifestations in the main group, we found that the dominant neurological syndromes were intracranial hypertension syndrome (91%), two-sided pyramidal insufficiency (89%), vegetative-visceral dysregulation (54%), movement disorders (43 %), various paroxysmal states (17%) and liquorodynamic disorders, (28%), and organic lesions of CNS (21%).

The structure of the ND severity was as the following:

• Group 1a: mild degree of clinical manifestations was detected in 60(36.8%) children, moderate degree in 58(35.6%) children, and severe degree in 45(27.6%) children.

• Group 1b: mild degree of clinical manifestations was detected in 62(35.8%) children, a moderate degree in 60(34.7%) children, and a severe degree in 51(29.5%) children.

• Control group: In each age subgroup, reference values for the studied parameters were determined using the accumulated average; the obtained values for two subgroups coincided with each other, and the baseline values for comparison in this regard were identified as common.

The correlation analysis showed the presence of significant high direct links between the low level of NGF and the clinical condition of the child in all groups: Graefe's symptom (r=0.67, $P<0.05$), decrease in muscle tone (r=0,84, $P<0.05$), reduction of tendon reflexes (r=0.77, $P<0.05$), asymmetry of folds (r=0.83, P<0.05), the presence of pyramidal signs (r=0. 89, $P<0.05$).

In the control group, the serum level of beta-NGF was the same for Group 2a and Group 2b (243±168.62 pg/ml). Thus, we found no age-related effect on the beta-NGF levels.

In the Group 1a subgroup with the mild clinical manifestations of ND, the average beta-NGF level significantly increased compared to the control group. In Group 1a subgroup with the moderate and severe clinical manifestations of ND, the serum level of beta-NGF significantly decreased by 17% and 31%, respectively (Table 1).

In the Group 1b subgroup with the mild clinical manifestations of ND, the average beta-NGF level was also significantly increased compared to the control group. In the Group 1b subgroup with the moderate and severe clinical manifestations of ND, the serum level of beta-NGF significantly decreased by 21.5% and 40%, respectively (Table 1).

ANOVA showed a main effect of the ND severity ($P<0.000$) in both age groups. In fact, post-hoc comparisons between groups revealed a decrease in the beta-NGF levels with the severity of ND.

Table 1.

The serum level of beta-NGF (pg/ml) in the studied groups

Group	Control group (1)	Mild degree of ND (2)	Moderate degree of ND (3)	Severe degree of ND (4)	Statistics
Group 1a	243±168,62 (n=43)	342,1±92,37 (n=60)	201,9±72,79 (n=58)	166,8±75,57 (n=45)	*ANOVA P=0.000* *Tukey HSD Post-hoc Test* $P_{1-2}=0.0000$ $P_{1-3}=0.2168$ $P_{1-4}=0.0047$ $P_{2-3}=0.0000$ $P_{2-4}=0.0000$, $P_{3-4}=0.3402$
Group 1b	243±168,62 (n=40)	339,4±97,61 (n=62)	190,7±61,29 (n=60)	145,7±69,49 (n=51)	*ANOVA P=0.000* *Tukey HSD Post-hoc Test* $P_{1-2}=0.0ooo$ $P_{1-3}=0.0590$ $P_{1-4}=0.0001$ $P_{2-3}=0.0000$ $P_{2-4}=0.0000$, $P_{3-4}=0.0950$

These results reflect the association between beta-NGF level and the ND severity.

Previous experimental and clinical studies have shown that hypoxic-ischemic brain injury (HIBI) determines an increased expression of NGF and other neurotrophic factors in CNS. The increased expression of NGF in HIBI patients plays a key role in response after injury, and may have a beneficial impact on the regenerative capacity of the injured tissues[20] that we observed in mild degree of ND. Our results suggest a possible functional link between the low level of NGF and the development of severe ND. Indeed, both in animal models and in humans, a low level of circulating NGF is associated with sensory and/or sympathetic neuronal deficits and even cell death.[21]

Thus, the obtained results allow us to consider the serum beta-NGF level as a useful marker of the ND severity in young children and open up new possibilities for the pathogenetic choice of drug therapy and evaluation of its effectiveness.

Competing interests

The authors declare that they have no conflict of interest.

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International Journal of Biomedicine 6(4) (2016) 270-282
doi: 10.21103/Article6(4)_OA6

ORIGINAL ARTICLE

Child and Adolescent Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Comparative Characteristics of Physical Development of Schoolchildren in Moscow and Kiev

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Abstract

The aim of this study was to find peculiarities in processes of growth and development of the child and adolescent in different megalopolises, *Moscow and Kiev,* on the background of various social and economic changes in society and standards of living of the child population. Comparison analysis of physical development of Moscow and Kiev children did not show statistically significant differences in body length except in boys aged between 10 and 16 years and in girls aged between 12 and 17 years. The changes in physical development of Kiev children demonstrate a domination of gracilization, accompanied by significant low values of body mass with high values of body length, especially in girls. The study showed that modern adolescents of both cities exceed their peers from previous generation in body length. Chest circumference in all ages of both sexes and body mass starting from 10 years of age were higher in adolescents from Moscow. We found a negative trend to the increase in the number of overweight Moscow children in the studied dynamics. **(Int J Biomed. 2016; 6(4):279-282.)**

Key Words: physical development • children • adolescents • body mass • body length

Introduction

Due to the incompleteness of the processes of growth and development of young people of school age, children and adolescents have an increased sensitivity to the adverse effects of exogenous factors, and, simultaneously, to the positive impact of preventive measures aimed at preserving and improving health in organized children's groups.[1-3]

The importance of studies of growth and development of the younger generation for childhood hygiene has been repeatedly noted by Russian scientists.[4-6,11] One of the most essential directions of such studies is to establish changes in the physical development of children in "homogeneous groups," the characteristic of morphological and/or functional changes in the development of the child population.

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The aim of this study was to find peculiarities in processes of growth and development of the child and adolescent in different megalopolises, Moscow and Kiev, on the background of various social and economic changes in society and standards of living of the child population. We studied the interdependence of growth and development on children (between 8 and 17 years old) – the residents of the two capitals – by comparing the results of the two parallel studies.

Material and Methods

Our study was carried out by a natural hygienic experiment with the use of epidemiological studies in schools of Moscow and Kiev. Written informed consent was obtained from the *child's parents.* The work did not infringe on the rights and did not endanger the welfare of study subjects and met the requirements of biomedical ethics.

The participants were children and adolescents of the Moscow and Kiev regions who were born and lived in these

cities (Russians, Belarusians, Ukrainians and also children from mixed marriages); 762 students from Moscow region and 612 from Kiev region were examined in the dynamics of learning from 1st to 11th grade.

We studied the physical development of children by unified methods with the use of standard tools,[4] as well as the somatometric indicators of physical development (body mass and body length, chest circumference) . The evaluation of the physical development of Moscow schoolchildren was carried out by regional modified scales of regression of body mass (BM) on body length (BL), and in Kiev by aged-sex regression scales of BM on BL. In fact, the principle of building these standards is identical, but in each state they are approved by the Ministry of Health alone.

The 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. For data with normal distribution, inter-group comparisons were performed using Student's t-test. 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

It has been shown that physical development in the two cities is subject to the general regularities: sex dimorphism and heterochrony of development. However, children and adolescents of Moscow and Kiev were found to have some differences in the main physiometric indicators of physical development: the respiratory capabilities of children in Kiev in all age-sex groups were significantly lower than in Moscow groups.[7,10]

The studies showed that modern adolescents of both cities exceed their peers from previous generation in body length. Moscow children also have higher BM and chest circumference (ChC) than their peers of the 1960s and 1980s, while modern children of Kiev have less BM and ChC than their peers of 30 to 50 years ago.[8,9]

The findings of the change in total body sizes in Moscow children show positive shifts in physical development, which may be associated with positive changes in the socioeconomic situation. The changes in physical development of Kiev children demonstrate a domination of gracilization, accompanied by significant low values of BM with high values of BL, especially in girls.

Comparison analysis of physical development of Moscow and Kiev children didn't show statistically significant differences in BL except in boys aged between 10 and 16 years (Moscow children had significant higher values) and in girls aged between 12 and 17 years (Kiev girls had significant higher values) (Tables 1, 2).

The analysis of annual increase showed that in both cities girls had maximum BL increase from 11 to 12 years of age, and boys from 13 to 14. In addition, it should be noted that the growth processes of the majority of Moscow girls are completed by age 16 (average annual increase from 16 to 17 was less than 1 cm), while the growth processes of Kiev girls continue longer (average yearly increase from 16 to 17 years old is 1.82 cm). Thus, we can suppose that definitive body sizes in Kiev girls will be greater than in Moscow girls.

Table 1.

Comparison analysis of physical development of Moscow and Kiev boys aged between 8 and 17

Age (y)	City	BL(cm)	BM (kg)	ChC (cm)
Boys				
8	Moscow	129.9±5.0	26.5±3.6	64.1±3.2
	Kiev	128.9±4.3	27.2±3.2	62.5±3.47
	P	*>0.05*	*>0.05*	*<0.05*
9	Moscow	135.6±5.7	31.1±4.0	66.1±3.11
	Kiev	135.9±5.06	31.6±4.2	64.6±3.83
	P	*>0.05*	*>0.05*	*<0.05*
10	Moscow	140.9±5.6	35.4±4.8	67.7±4.32
	Kiev	138.9±6.34	32.7±4.8	66.6±4.65
	P	*<0.05*	*<0.05*	*>0.05*
11	Moscow	146.0±6.6	40.0±5.9	70.8±4.11
	Kiev	147.7±5.48	39.7±5.8	70.7±4.08
	P	*>0.05*	*>0.05*	*>0.05*
12	Moscow	151.20±0.81	44.50±1.07	74.10±0.80
	Kiev	152.87±0.59	40.95±0.62	72.83±0.41
	P	*>0.05*	*<0.01*	*>0.05*
13	Moscow	158.60±0.96	50.90±1.36	77.90±0.88
	Kiev	158.58±0.66	47.77±0.58	75.70±0.46
	P	*>0.05*	*<0.05*	*<0.05*
14	Moscow	165.70±0.96	53.60±1.31	79.90±0.86
	Kiev	165.61±0.63	52.62±0.67	78.21±0.53
	P	*>0.05*	*>0.05*	*>0.05*
15	Moscow	172.21±0.84	63.31±1.46	85.48±0.86
	Kiev	171.24±0.53	59.53±0.78	84.20±0.58
	P	*>0.05*	*<0.05*	*>0.05*
16	Moscow	175.9±0.8	67.5±1.6	88.4±0.9
	Kiev	173.32±0.63	58.99±0.87	84.66±0.50
	P	*<0.05*	*<0.001*	*<0.001*
17	Moscow	177.5±0.8	69.7±1.6	90.8±0.9
	Kiev	175.68±0.59	63.48±0.83	88.74±0.69
	P	*>0.05*	*<0.001*	*>0.05*

The BM of modern children and adolescents from 10 years old was higher in Moscow children. Boys aged 10, 12-13 and 15-17 and girls aged 10, 14-15 and 17 had statistically significant differences. ChC was higher in all age groups of both sexes in Moscow children, but boys aged 8-9, 13, and 16 and girls aged 8-9, and 16 had statistically significant differences.

The analysis of harmonicity of physical development of modern Moscow children aged between 8 and 17 years showed that 66.3% of participants had normal physical development. About 22.3% were underweight and 11.3 % were overweight.

In comparison with previous studies,[6] a clear trend to being overweight has appeared among Moscow children since 1980. In the 1980s, 6.6% of children were overweight; in the

1990s, 7.0%; and in the 2000s, 11.2%. Consequently, over the last 30 years the number of children and adolescents who are overweight and obese has increased almost twice.

Table 2.

Comparison analysis of physical development of Moscow and Kiev girls aged between 8 and 17

Age (y)	City	BL (cm)	BM (kg)	ChC (cm)
Girls				
8	Moscow	129.2±5.6	25.7±3.7	62.5±3.35
	Kiev	127.9±4.51	26.6±3.6	61.3±3.37
	P	*>0.05*	*>0.05*	*<0.05*
9	Moscow	134.5±5.6	29.6±4.5	65.1±3.32
	Kiev	134.7±4.64	30.7±4.0	63.9±3.39
	P	*>0.05*	*>0.05*	*<0.05*
10	Moscow	140.2±6.0	34.6±5.3	67.5±4.10
	Kiev	137.4±5.16	32.2±4.9	65.7±4.50
	P	*>0.05*	*<0.05*	*>0.05*
11	Moscow	146.2±7.2	39.6±5.9	70.1±4.59
	Kiev	146.4±5.58	37.3±5.0	69.0±4.63
	P	*>0.05*	*>0.05*	*>0.05*
12	Moscow	153.20±0.64	44.40±0.97	74.60±0.79
	Kiev	155.13±0.59	44.09±0.64	73.14±0.47
	P	*<0.05*	*>0.05*	*>0.05*
13	Moscow	159.40±0.60	50.70±0.97	78.40±0.66
	Kiev	160.06±0.64	48.79±0.59	78.26±0.45
	P	*>0.05*	*>0.05*	*>0.05*
14	Moscow	162.60±0.64	53.20±1.09	80.40±0.67
	Kiev	161.99±0.43	50.45±0.41	79.83±0.41
	P	*>0.05*	*<0.05*	*>0.05*
15	Moscow	164.25±0.5	56.2±1.02	82.10±0.6
	Kiev	163.38±0.49	52.25±0.57	81.04±0.40
	P	*>0.05*	*<0.001*	*>0.05*
16	Moscow	164.63±0.53	57.41±0.99	82.71±0.61
	Kiev	165.16±0.53	55.29±0.64	84.38±0.41
	P	*>0.05*	*>0.05*	*<0.05*
17	Moscow	164.52±0.63	58.05±1.35	83.47±0.80
	Kiev	166.98±0.45	53.95±0.61	83.04±0.45
	P	*<0.01*	*<0.05*	*>0.05*

An increase in the prevalence of excess weight has been observed in modern children. In boys age 8, it was 8.0%; in girls, 5.6%. In boys age 12, it was 16.8%; in girls, 11.0%. the maximum prevalence of excess weight was noticed in boys age 15(17.0%) and in girls age 14(13.1%). This trend was more expressed among boys from 8 to 15 years old: the incidence of overweight boys has increased 3 times. By the age of 17, 13.8% of boys and 10.1% of girls were overweight.

The statistical analysis showed that the level of harmonicity of physical development is mainly kept in the dynamics of study. Pearson's contingency coefficient for boys and girls from 8 to 15 years old was 0.60 (*P<0.001*), 15 to 17 years old was 0.73 (*P<0.001*) and 0.58 (*P<0.002*), respectively. About 18.6% of underweight boys changed to the group of normal physical development by 15 years old, 8.5% of boys with normal physical development moved into the underweight group, and 6.8% moved into the overweight group (Figure 1). Unfortunately, overweight boys from 8 to 17 years old remained in the overweight group. Thus, the boys' overweight group was replenished in the dynamics of maturity. The most dynamic changes were observed in underweight girls. By 15 years old, about 31% of girls change their group to normal physical development (Figure 2), 5.2% of girls moved into the underweight group and 3.5% - into the overweight group. As with the boys, the girls' overweight group was replenished in the dynamics of maturity.

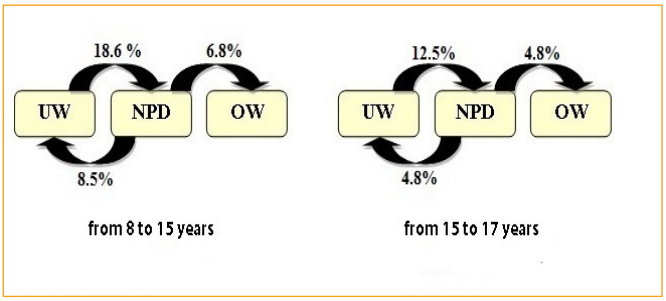

Fig. 1.

UW - underweight boys; NPD - normal physical development; OW- overweight boys

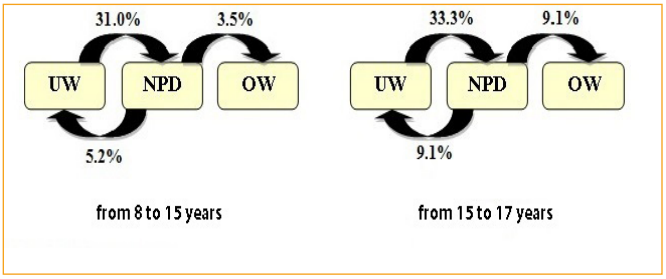

Fig. 2.

UW - underweight girls; NPD - normal physical development; OW- overweight girls

Conclusion

In sum, the studies showed that modern adolescents from Moscow and Kiev did not have statistical differences in BL except for boys aged between 10 and 16 and for girls aged between 12 and 17. ChC in all ages of both sexes and BM starting from 10 years of age were higher in adolescents from Moscow. In most age groups of both sexes, the differences were statistically significant.

The analysis of harmonicity of physical development of Moscow children showed the prevalence of an overweight condition in the adolescent population for the last 30 years. In addition, we found a negative trend to the increase in the number of overweight Moscow children in the studied dynamics.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 6(4) (2016) 283-286
doi: 10.21103/Article6(4)_OA7

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Comparative Analysis of *in vitro* Performance of Total-Etch and Self-Etch Adhesives

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Abstract

The aim of the study was *in vitro* assessment of shear bond strength and micro-leakage after application of total-etch and self-etch adhesive systems.

Materials and Methods: Four adhesive systems were chosen for assessment of adhesion performance: Contax (DMG, GmbH), Bond Force (Tokuyama Dental Corp. Japan Mfr), Te-Econom Bond (Ivoclar Vivadent, Liechtenstein) and Swisstec SL Bond (Coltene, Switzerland). The assessment of bond strength was performed on 20 tooth samples, which were prepared in accordance with the UltraTest technique for shear bond strength (SBS) estimation. The test was conducted at a crosshead speed of 1.0 mm/min and results were fixed in kilograms. The assessment of SBS was performed on enamel and dentin separately. Microleakage assessment of self-etch and total-etch adhesive systems was performed on 20 extracted non-carious upper human premolars with immersion in 1% methylene blue solution after thermocycling.

Results: Good SBS results and microleakage values on the dentin substrate were obtained after application of the Contax self-etch bonding agent. But the values of bond strength to enamel and the extent of dye penetration within the composite-enamel interface were still better with the total-etch approach. **(Int J Biomed. 2016; 6(4):283-286.)**

Key words: adhesive technique • enamel • dentin • shear bond strength • micro-leakage

Introduction

In dentistry, the etch-and-rinse technique is still considered to be the gold standard of the bond strength of adhesives to enamel.[1] Many studies have demonstrated that if there is a large area of available enamel to be bonded and only a small area of dentin, the total-etch technique is the preferred alternative since it has been shown to result in a stronger bonding to enamel than the self-etch technique.[2,3] Conversely, if a preparation side has a substantial area of dentin to be bonded and a lesser area of enamel, the group of self-etch adhesives is more often preferred.[4,5]

The self-etch adhesive technique, in comparison to total-etch, creates a thinner hybrid layer and mainly relies on the formation of multiple chemical bonds between active groups of monomer and calcium ions of hydroxyl apatite, and less on collagen fiber hybridization.[6-9] The thinner hybrid layer may be the reason for poor resistance to debonding stresses. In addition, several studies have indicated a decrease in the adhesion strength of self-etch adhesive systems, which might be largely associated with the chemical instability of the material composition.[10,11]

The chemical instability of self-etch adhesive materials also indicates the need for strict storage conditions. Many of them should be refrigerated. Also, the storage during shipment and transportation is not always as prescribed. Thus, many factors may account for bad bond strength after a bonding agent application.

The adhesion performance of total-etch and self-etch bonding agents is of clinical importance. Many studies have indicated that in the total-etch approach the successful hybridization of an etched dentin substrate is not always predictable, whereas in the self-etch technique the strength of composite adhesion to enamel is often questionable. [12-16]

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The aim of the study was in vitro assessment of shear bond strength and micro-leakage after application of total-etch and self-etch adhesive systems.

Materials and Methods

All adhesive procedures were performed by the same operator in accordance with the manufacture's protocol. In each case, the light activation was done using a halogen light-curing unit (Bluephase 20i (G2), Ivoclar Vivadent) with a light intensity around 700 mW/cm2.

Four adhesive systems were chosen for assessment of adhesion performance: Contax (DMG, GmbH), Bond Force (Tokuyama Dental Corp. Japan Mfr), Te-Econom Bond (Ivoclar Vivadent, Liechtenstein) and Swisstec SL Bond (Coltene, Switzerland). Chemical composition of used adhesive systems is presented on Table №1.

Table 1.

Chemical composition of adhesive systems

Bonding agent	Type of system	Composition
Contax (DMG, GmbH)	Self-Etch	Contax-Primer: water, carboxylic acid, sodium fluoride. Contax-Bond: Hydrophilic and acidic Bis-GMA-based resin matrix, catalyst.
Bond Force (Tokuyama Dental Corp. Japan Mfr.)	Self-Etch	Phosphoric acid monomer (3D-SR monomer), Bis-GMA, 3G (TEGDMA), HEMA, Alcohol, Water, Camphorquinone
Te-Econom Bond (Ivoclar Vivadent, Liechtenstein)	Total-Etch	HEMA, di- and monometha-crylates, inorganic fillers, initiators, stabilizers, alcohol solution
Swisstec SL Bond (Coltene, Switzerland)	Total Etch	Methacrylates, polyalkenoate methacrylized

The composite filling material of choice for the application of Contax and Bond Force was palfique ESTELITE paste; for Te-Econom Bond - Te-Econom Plus and Swisstec SL Bond, we used the Swisstec light curing composite.

The assessment of bond strength was performed on 20 tooth samples, which were prepared in accordance with the UltraTest technique for shear bond strength (SBS) estimation. The test was conducted at a crosshead speed of 1.0 mm/min and results were fixed in kilograms. Tooth samples (Fig. 1a and Fig. 1b) were divided into two groups: Group 1 (n=10) for the assessment of SBS on enamel, and Group 2 (n=10) for the assessment of SBS on dentin.

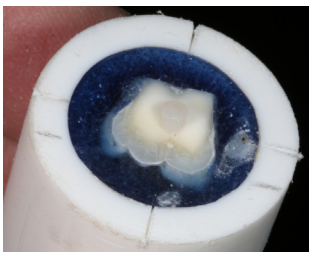

Fig. 1a. *Tooth sample for SBS test on dentin*

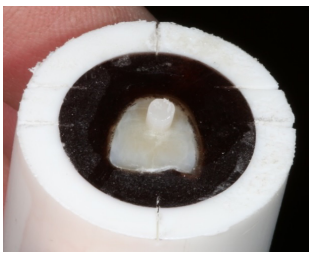

Fig. 1b. *Tooth sample for SBS test on enamel*

Each sample of both groups was subjected to the 4 following SBS tests (in accordance with the number of adhesives under the study) and every consecutive test was performed after grinding off the remnants of the existing bonded area.

Microleakage assessment of self-etch and total-etch adhesive systems was performed on 20 extracted non-carious upper human premolars. Round artificial cavities (3 mm in diameter, 1 mm deep) were prepared on two approximal surfaces of each tooth with half in enamel and another half in root dentin. All samples were randomly divided into two groups: Group A for assessment of microleakage at the enamel margin, and Group B for assessment of microleakage at the dentin margin. Artificial cavities were filled with composite, polished, and thermocycled (500 cycles in separate water baths of 5°C and 65°C±2°C with a dwell time of 20 seconds in each bath and a transfer time of 1 second). After thermocycling, the apices of tooth samples were sealed with sticky wax and coated with nail varnish, with the exception of the restoration site and a 1 mm distance around of it. The teeth were stained in 1% methylene blue solution for 24 hours and sectioned through the centers of restorations (Fig.2).

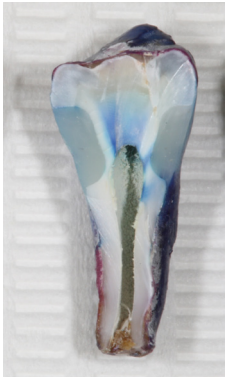

Fig. 2. Microleakage assessment

Enamel and dentin dye penetration (DP) was assessed using the following scale of 0-3 scoring system:

0 - no DP

1 - DP up to one-half of the cavity wall length

2 - DP up to the full length of the cavity wall, not including the axial wall

3 - DP to the full extent of the cavity wall, including the axial wall

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard deviation (SD) were calculated. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results

According to obtained SBS test results (Table 2), the enamel bonding capacity of Contax was not as strong as that of Te-Econom Bond and Swisstec SL Bond, but differences in values were not statistically significant. But with the dentin substrate, the bonding capacity of Contax was better (2.8 times) than that of Swisstec SL Bond ($P=0.000$). At the same time, the average microleakage value of Contax to a dentin tissue was 5 times better than that of Swisstec SL Bond ($P=0.0119$).

The bonding capacity of Bond Force to enamel or dentin hard tissues was 1.42 and 1.66 times lower, respectively, than the capacity of Te-Econom ($P=0.0202$ and $P=0.0001$). Microleakage parameters of the compared bonding agents

in relation to enamel surface were 2.44 times better for Te-Econom than for Bond Force (*P=0.0111*). Thus, good SBS results and microleakage values on the dentin substrate were obtained after application of the Contax self-etch bonding agent. But the values of bond strength to enamel and the extent of dye penetration within the composite-enamel interface were still better with the total-etch approach.

Discussion

Table 2.

Shear bond strength and micro-leakage values of total-etch and self-etch adhesive systems under study

Bonding agent, LOT, exp date, time of test running	Group 1	Group 2	Group A	Group B
Contax (DMG, GmbH), LOT 743584, 2018-02, 27.11.2016 (1)	10.56±3.26	12.49±1.72	1.1±1.0	0.3±0.48
Bond Force (Tokuyama Dental Corp. Japan Mfr.), LOT 313 MM, 2019-06, 27. 11.2016 (2)	8.71±2.34	7.92±2.95	2.2±0.79	0.5±0.53
Te-Econom Bond (Ivoclar Vivadent, Liechtenstein), LOT V11012, 2018-09, 26. 11.2016 (3)	12.4±1.63	13.14±2.17	0.9±0.99	1.1±0.99
Swisstec SL Bond (Coltene, Switzerland), LOT G43043, 2018-08, 28.11.2016 (4)	12.4±3.18	4.42±2.1	0.5±0.7	1.5±1.09
ANOVA Tukey HSD Post-hoc Test	P=0.0106 P_{1-2}=0.4251 P^{1-3}=0.4299 P^{1-4}=0.4299 P^{2-3}=0.0202 P^{2-4}_{3-4}=0.0202 =NaN	P=0.0000 P_{1-2}=0.0004 P^{1-3}=0.9191 P^{1-4}=0.0000 P^{2-3}=0.0001 P^{2-4}_{3-4}=0.0079 =0.0000	P=0.0009 P_{1-2}=0.0393 P^{1-3}=0.9565 P^{1-4}=0.4333 P^{2-3}=0.0111 P^{2-4}_{3-4}=0.0006 =0.7407	P=0.0086 P_{1-2}=0.9469 P^{1-3}=0.1466 P^{1-4}=0.0119 P^{2-3}=0.3702 P^{2-4}_{3-4}=0.0457 =0.6961

Strong adhesion of composite to tooth substrates may be of primary importance for the long-term stability of a tooth colored restoration. However, high values of SBS are not always associated with long-term stability of a tooth-composite interface.[17,18] Deep adhesive tags, which are usually produced by total-etch adhesives, help to provide the initial stability. However, the formation of zones of incomplete infiltration is more likely for the same total-etch bonding agent because of dimensional weakness of a denuded dentin collagen network. [19,20]

The presence of micro-cracks on a surface of dentin or enamel may also lead to the formation of longer adhesive tags in those zones, which can be of great benefit in the beginning. However, micro-cracks are prone to microleakage, which could be the main reason for bond degradation in the end.

In this study, an accurate assessment of a bond failure pattern was not undertaken. But preliminary visual analysis of macro images of torn-off surfaces, estimation of the extent of micro-leakage in tooth samples, and SBS test results demonstrated that the Contax 2-bottle self-etch adhesive system (DMG, GmbH) is a reliable bonding agent in relation to a long-term prognosis. In addition, it was shown that total-etch adhesives adhered better to enamel.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 6(4) (2016) 287-289
doi: 10.21103/Article6(4)_OA8

ORIGINAL ARTICLE

Biomedical Technology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Applying Transpalpebral Rheoophthalmography to Monitor Effectiveness of the Treatment of Patients with Glaucoma

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Abstract

Assessment of ocular hemodynamics is an important element in diagnosis of all forms of glaucoma. Existing methods, unlike the transpalpebral rheoophthalmography (TP-ROG), evaluate only the posterior eye segment. This study evaluates the opportunities of new TP-ROG technology to assess the effectiveness of the glaucoma treatment. The proposed TP-ROG method is characterized by ease of use and the lack of direct contact between the surface of the eyeball and the electrodes; this method is highly informative and quite accurate, which allows us to objectively assess the state of ocular hemodynamics in patients with primary open-angle glaucoma **(Int J Biomed. 2016; 6(4):287-289.)**

Key Words: transpalpebral rheoophthalmography • ocular hemodynamics • microcirculation • primary open-angle glaucoma

Introduction

It is known that alterations in ocular blood flow play a prominent role in primary open-angle glaucoma (POAG) processes. The main collector of blood flow, performing the function of nourishing the inner shells of the organ of vision is the vascular tract, which contains more than 80% of the incoming blood in the eye. In this connection, the search for the most informative and accurate methods of determining the blood flow in the uvea is important and has continued over the years.[1]

The method of rheoophthalmography (ROG) allows us to objectively assess the state of blood flow in the main hemodynamic system of the eye—the vascular tract. The principle of this method is based on recording the changes in the total resistance (impedance) while passing a high-frequency electric current through the ocular tissue.[2-4] ROG was developed by Katznelson to assess the uveal blood flow currently and is used as a diagnostic test and criterion of effectiveness in treatment of glaucoma, myopia, degenerative diseases of the retina, and traumatic injuries.[1]

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In 2012, scientists at the Moscow Helmholtz Research Institute of Eye Diseases and the Bauman MSTU together developed a new method of rheoophthalmography examination, which differs from the classical ROG method in that it lacks direct contact between the eyeball surface and electrodes, which certainly is an advantage of the new method. During TP-ROG, electrodes are superimposed on the closed eyelid and, to improve the accuracy of existing studies, the bipolar technique is replaced by a tetrapolar technique that allows considering features of the anatomical structure of the vascular bed of the eyeball.[4,5] As a result of the recording, processing, and analyzing of recorded signals using specially developed software, the following indexes can be calculated: rheographic index (RI), which shows the value of systolic blood flow and depends on the value of stroke volume and vascular tone; the period of maximum filling (PMF), which increases with increases in the vessel tone and with decreases in the elasticity of blood vessels; and the index of elastic modulus (IEM), which characterizes the structural properties of the vascular wall, its elasticity and tone, as well as other hemodynamic characteristics.[6]

TP-ROG registration is carried out using a tetrapolar lead system. The main elements of this system are four reusable metal electrodes (internal diameter, 4 mm) and a substrate with elements of attachment and positioning. A structure in

the form of an elastic hat, which is specially designed for TP-ROG, provides an attachment point for the lead system with the possibility of adjustment and optimization for each patient, the correct positioning, and the desired force to fix electrodes on the upper eyelid.[7,8] During the examination, with the patient in a supine position, the electrode lead system is set on the closed eye and fixed by a knitted hat; the other eye remains open during the investigation in order to reduce the number of involuntary eye movements and the number of artefacts in the recorded signal. TP-ROG signal registration lasts no more than 2 minutes.[4,5] Research of the myopic eye hemodynamics by TP-ROG shows that the new method is characterized by being highly informative and sufficiently accurate; TP-ROG allows us to objectively assess the state of blood supply to the myopic eye.[2]

Despite achieving the target level of intraocular pressure (IOP) with the help of medical or surgical treatment, involution and metabolic disorders, changes in cerebral blood flow, and reduction in the activity of antioxidant systems are responsible for the gradual decline of visual function in POAG patients. To correct the noted disorders during the integrated treatment of POAG, several methods are used: neuroprotective, anti-sclerotic, and vasoactive drugs; vitamins and antiplatelet agents; and various methods of physiotherapy.[1,7] Therapeutic effects of physical factors that are used in the treatment of POAG, aimed at restoring the conduction of optic nerve fibers, improve microcirculation and hemodynamics and affect the regulatory structure of the brain. Physical therapy techniques also provide general sedative, antispasmodic, hypotensive, distracting and analgesic effects, and normalize the functional state of the central and peripheral nervous systems.[1]

In recent years, laser therapy has been widely used in medicine. The physiotherapy impact of low-power laser radiation leads to activation of cellular metabolism, improvement of ocular hemodynamics indicators, and increased trophic support for eye tissues. That is why this kind of feedback can be used as a component of complex treatment of patients with POAG. Currently, we are working on implementing the ROG technique in clinical practice for monitoring the glaucoma treatment.

The purpose of this study was to determine the capabilities of TP-ROG in monitoring the effectiveness of POAG treatment.

Materials and Methods

There are 4 stages of glaucoma. In formulating a diagnosis, the stages are designated by Roman numerals: from I - early-stage up to IV - end-stage. In this case, the state of the visual field and optic disc are taken into account. Stage I: peripheral visual field (PVF) is normal, but there are defects in the central field of vision; excavation of the optic disk is expanded, but does not reach the edges. Stage II: PVF is narrowed with the nasal side of more than 10°; the observed changes in excavation do not reach the edge of the optic disc. Stage III: PVF is narrowed concentrically (the nasal side up to 15° or less from the point of fixation); subtotal excavation reaches the edge of the optic disc. Stage IV: complete loss of vision or saved light perception with irregular light projection. The light perception may be a small island of the residual field of vision in the temporal sector.

Low-level laser therapy (LLLT) was conducted in 11 patients (17 eyes) aged between 58 and 76 years with POAG stages II and III; the IOP normalization was reached without antihypertensive regime.

Before LLLT (from 6 to 18 months), patients underwent sinus-trabeculectomy. Prior to surgery, all patients were on maximal hypotensive mode. Postoperatively, all patients had a course of conservative therapy, including anti-oxidants, vitamins B, antiplatelet agents, and neuroprotective agents. LLLT was performed using MACDEL-08, the operating principle of which consists in the activation of neuronal cells of the retina and optic nerve by observing the patient moving a speckle formed by helium-neon laser radiation (λ=0.63 um). During TP-ROG signal processing, three main hemodynamic parameters are calculated: RI, PMF, and IEM.[6,9,10] Before TP-ROG, all patients underwent a comprehensive eye exam: visometry, computer perimetry, gonioscopy, ophthalmoscopy, pneumotonometry, tonography, morphometric analysis of optic disc parameters using the Heidelberg Retina Tomograph (HRT), and the determination of the critical flicker fusion frequency (CFFF).

The exclusion criteria were as follows: a) pathology of the organ of vision associated with impaired intra-ocular blood flow: vascular occlusion, diabetic retinopathy, etc.; b) the terminal stage of POAG; c) secondary glaucoma; d) inflammatory diseases and injuries of the eyeball; e) ophthalmic treatment at least 6 months prior to the survey; f) severe pathology of systemic hemodynamics, including atrial fibrillation, heart failure (>2A degree), clinically significant carotid artery stenosis., etc. MACDEL-08 is used for laser treatment of sensory disorders in ophthalmic practice. The operating principle of this device is projecting the laser speckle pattern on the retina. The laser speckle pattern is contrast, and the size of speckle is perceived even at reduced function (up to 0.02-0.03).

This study was performed in accordance with the Declaration of Helsinki and was approved by Local Committee of Biomedical Ethics of the Moscow Helmholtz Research Institute of Eye Diseases. Written informed consent was obtained from all participants. The statistical analysis was performed using the statistical software Statistica 6.0. 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

In our study, we formed 2 groups: Group A included 9 eyes (POAG stage II) and Group B included 8 eyes (POAG stage III). Visual acuity before the LLLT course was as follows: Group A - from 0.4 to 0.9 (0.70±0.17); Group B – from 0.1 to 0.8 (0.53±0.25). The LLLT course using MACDEL-08 was well tolerated by all patients. Seven patients reported subjective improvement in visual acuity on the 3rd and 4th days after initiation of therapy. All patients showed

improvement of visual acuity after the LLLT course: from 0.6 to 1.0 (0.87±0.16) and 0.3 to 1.0 (0.69±0.25) in Groups A and B, respectively; $P<0.05$ on both cases.

Both groups showed a statistically significant increase in the RI level (from 12.81±1.12 mOhm to 14.58±1.05 mOhm in Group A and from 9.57±0.89 mOhm to 11.31±1.29 mOhm in Group B; $P<0.05$ in both cases) and a statistically insignificant decrease in levels of PMF (from 0.27±0.02 sec to 0.23±0.02 sec in Group A and from 0.31±0.03 sec to 0.28±0.02 sec in Group B; $P>0.05$ in both cases) and IEM (from 0.31±0.03 sec to 0.28±0.02 sec in Group A and from 0.33±0.03 sec to 0.29±0.03 sec in Group B; $P>0.05$ in both cases). The achieved levels of rheogram indicators remained unchanged throughout the period of observation. Increased RI indicates an improvement in microcirculation. The dynamics of PMF and IEM indicate no significant effect on the elastic properties of intraocular vascular walls.

In conclusion, the proposed TP-ROG method is characterized by ease of use and the lack of direct contact between the surface of the eyeball and the electrodes; this method is highly informative and quite accurate, which allows us to objectively assess the state of ocular hemodynamics in POAG patients. The TP-ROG method has shown its capabilities to monitor the effectiveness of POAG treatment.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 6(4) (2016) 290-293
doi: 10.21103/Article6(4)_CR1

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Monitoring Extremely Premature Newborns with IVH using NIRS: One Patient Case Study

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Abstract

The present study investigates the possibility of detecting changes in cerebral oxygenation and consequently of intraventricular hemorrhage (IVH) in preterm children using paired data from the NIRS-monitor and the patient vital signs monitor. During the study, one patient's data were analyzed. The patient was born at the gestational age of 25+6 weeks. He was monitored for a period of 72 hours. IVH was first detected by the ultrasound diagnostics in 1000 minutes after birth. Analysis of the paired data showed that HR is not a useful IVH indicator. Comparison of the regional cerebral (cSrO2) and peripheral blood oxygen saturation (SpO2) changes and cerebral fractional oxygen extraction (OEF) with its second derivative represents a more beneficial method. It shows the tissue oxygen absorption changing (ie, periods of great change in hemodynamic, at the beginning or end of IVH). By comparing the calculated results and the medical records of the IVH course, it can be argued that this method can be efficiently used to determine the start and end of the bleeding into the brain ventricles. **(Int J Biomed. 2016; 6(4):290-293.)**

Key Words: near-infrared spectroscopy • extremely premature newborns • intraventricular hemorrhage • blood oxygen saturation

Introduction

Each year about 15 million babies are born prematurely worldwide (before 37 completed weeks of gestation), and this number is steadily growing. One of the most frequent and serious complications observed in prematurely born children (<28 weeks) is intraventricular hemorrhage (IVH).[1]

IVH is a bleeding into the central nervous system that occurs most frequently in premature infants in the cerebral ventricles, because the blood vessels in these areas are very fragile, and adaptive changes in the bloodstream of newborns and fluctuations in blood pressure may cause a rupture of the blood vessels and subsequent bleeding. Other risk factors are mechanical ventilation, hypercapnia, patent ductus arteriosus, volume expansion in the first days of life, and fetal growth retardation. About 90% of cases of bleeding in the brain ventricles occur during the first 72 hours after childbirth, hence this period must be closely monitored.[2-4]

Clinical manifestations most often include convulsions, worsening of breathing difficulties, fontanelle bulging, and coma. Asymptomatic IVH is no exception.

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There are 4 grade of IVH:

Grade 1 - subependymal hematoma;

Grade 2 - bleeding into the brain ventricles, ventricles have normal volume;

Grade 3 - bleeding into the brain ventricles, ventricles are expanded;

Grade 4 - bleeding into the brain parenchyma accompanied or not accompanied with the presence of blood in the ventricles (periventricular hemorrhagic infarction). Infarction is typically one-sided, large, probably venous origin. [3, 4]

Bleeding of Grades 1 and 2 is limited to the ventricles and in most cases is spontaneously absorbed without any consequences and with favorable prognosis for the child's further development. Hemorrhage of Grade 3 is bleeding into the ventricles on a larger scale, which is usually healed without any residual effects, but in this case there is a greater risk, for example obstruction of the blood brain pathways, blood clot formation, and hydrocephalus. IVH Grade 4 is the cause of brain tissue damage, usually with serious consequences for the further development of the child. Severe psychomotor retardation affects are observed in up to 90% of the children with Grade 4 bleeding. Bleeding is not always isolated. Bleeding of this grade is usually associated with the cerebral lesions such as periventricular leukomalacia, pontosubikulární necrosis etc., which contribute to an unfavorable prognosis. [3,4]

In most cases, bleeding is diagnosed by ultrasound diagnostics, which is usually done with a long interval (4-6 hours) between particular diagnostics. Ultrasound diagnostics cannot detect the start time of bleeding. The grade of IVH is determined by the extent of the bleeding.

Treatment of bleeding is possible. In most cases the blood is spontaneously absorbed and the rupture heals itself. An important point is a regular monitoring of bleeding and timely diagnosis of complications (eg, the development of hydrocephalus). Complications such as hydrocephalus are resolved surgically. Therefore, ultrasound is useful for diagnosis and also in the subsequent period during the monitoring of the healing process, but does not allow continuous monitoring because of the large intervals between the times of diagnosis. [2-4]

Near-infrared spectroscopy (NIRS) is a noninvasive spectroscopic method widely used in research. It is an instrument for continuous measurement of tissue oxygen saturation that uses the infrared region of the electromagnetic spectrum. The method is not very sensitive, but NIRS is able to penetrate deeper into the sample. The method is very popular in neonatology, particularly in premature babies. It enables monitoring of the regional tissue oxygenation, for example oxygenation of the brain tissue.[5,6]

At the beginning of the 21st century in Drexel University (PA), Alper Bozkurt and his research team, having researched the principle of NIRS, assumed a possible correlation between cerebral blood flow, cerebral oxygenation, and subsequent occurrence of intraventricular / brain hemorrhage in premature infants.[7]

Ying Zhang and his team analyzed the 3-hour records of 5 preterm infants with IVH and 12 preterm infants without IVH. The main conclusion of this study was that infants with IVH had lower coherence between arterial blood pressure (BP) and deoxygenated hemoglobin (HHb) illative from NIRS. Parameters derived from the analysis of transfer function, however, did not show significant deviation from the norm. Because of this, the hypothesis that IVH could be detected by analyzing changes in BP was rejected.[8]

The aim of this study was to verify the possibility of detecting the period of time with large changes in hemodynamics and consequently the beginning or end of IVH using the values of regional cerebral (cSrO2) and peripheral blood oxygen saturation (SpO2), cerebral fractional oxygen extraction (OEF) and its second derivative.

Methods

During the study, one patient's data were analyzed. The study was approved by the Czech Technical University Ethics Committee. Written informed consent was obtained from the child's parents. The patient was born at the gestational age of 25+6 weeks. He was monitored for a period of 72 hours. IVH was first detected by the ultrasound diagnostics in 1000 minutes after birth (Table 1).

In this research, we used data of regional oxygenation obtained with the device INVOS 5100C. The INVOS 5100C is a system that enables real-time monitoring of changes in regional blood oxygen saturation in the brain (cSrO2) or other tissues under the sensor for adults, children, infants and newborns. The NIRS device had the sampling rate set to approximately 6 times per minute.[9] We used also a patient vital signs monitor, Delta, with the sampling rate set to 1 sampling per minute. Subsequent data processing was done in MATLAB.

Tab. 1.

Basic patient's data and parameters of monitoring

Gender	male
Gestational age at birth	25 + 6 weeks
Diagnosis	IVH Grade 1
First detecting IVH by UZ	The 2nd day of life (≈1000 minutes after birth)
Character of bleeding	unilateral, small size
Symptoms	no
The period of monitoring	4320 minutes (72 hours)
Monitored parameters	$cSrO_2$, SpO_2, HR

Data processing

Using MATLAB, a summary table for a patient was created, which contained minute paired data from two devices. The table contains the values of HR (heart rate), SpO2 and cSrO2 and calculated OEF for each minute of recording. Dropouts of measured signals were checked and complemented by linear approximation. OEF was calculated according to the equation:

$$OEF = \frac{SpO2 - cSrO2}{SpO2} \quad (1)$$

Fig. 1 shows changes in time of cSrO2 (blue) and SpO2 (yellow) oxygen saturation. The sudden decrease in cerebral oxygen saturation (from the 419th minute) presumably marks the start of the IVH. The patient had IVH Grade 1, which was absorbed during the return to the normal hemodynamic, which happened around the 1320th minute of life. There was a sharp increase in regional cerebral oxygen saturation. The red line shows the time-point of US diagnostics, when IVH was first detected.

OEF values are directly proportional to the difference between SpO2 and cSrO2, and indirectly proportional to peripheral blood oxygen saturation (Fig.2). The OEF coefficient is a normalized value that increases considerably after the expected IVH start (ie, from the 419th minute). The red line shows the time-point of US diagnostics when IVH was first detected.

To detect great changes in hemodynamics, which could indicate the beginning or the end of bleeding, we used the method of second difference. This method determines the time of the changes in the tissue oxygen absorption according to the equation:

$$2.diff_i = \left| |x_i - x_{i-20}| - |x_{i-20} - x_{i-40}| \right| \quad (2),$$

where i is a minute of life, and x is a value of OEF.

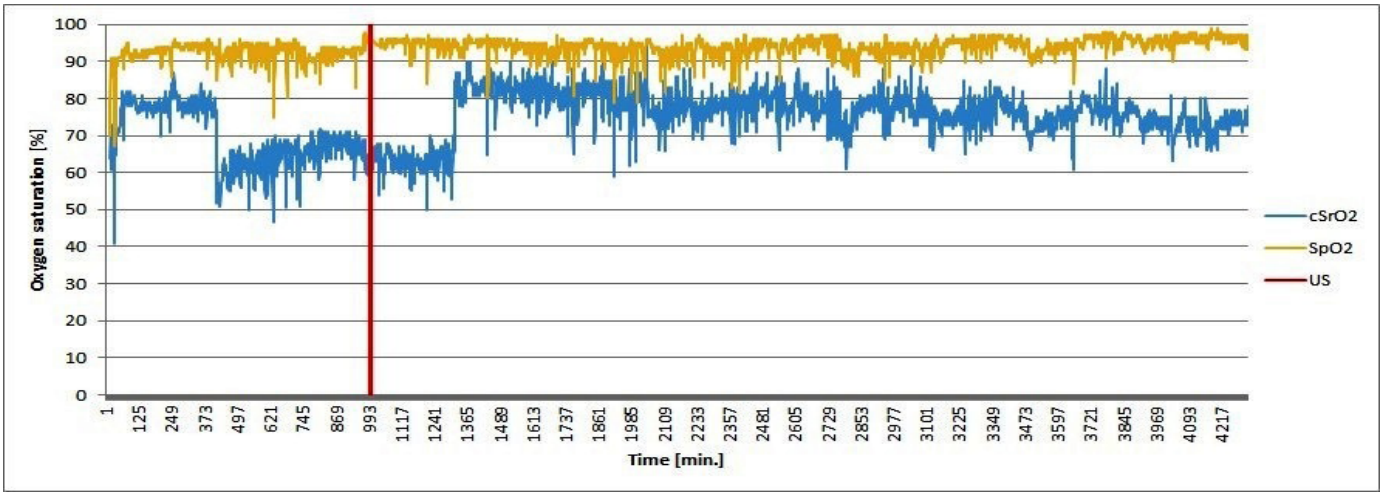

Fig. 1. *SpO2 and cSrO2 changes during the 4320 minutes (72 hours) monitoring.*

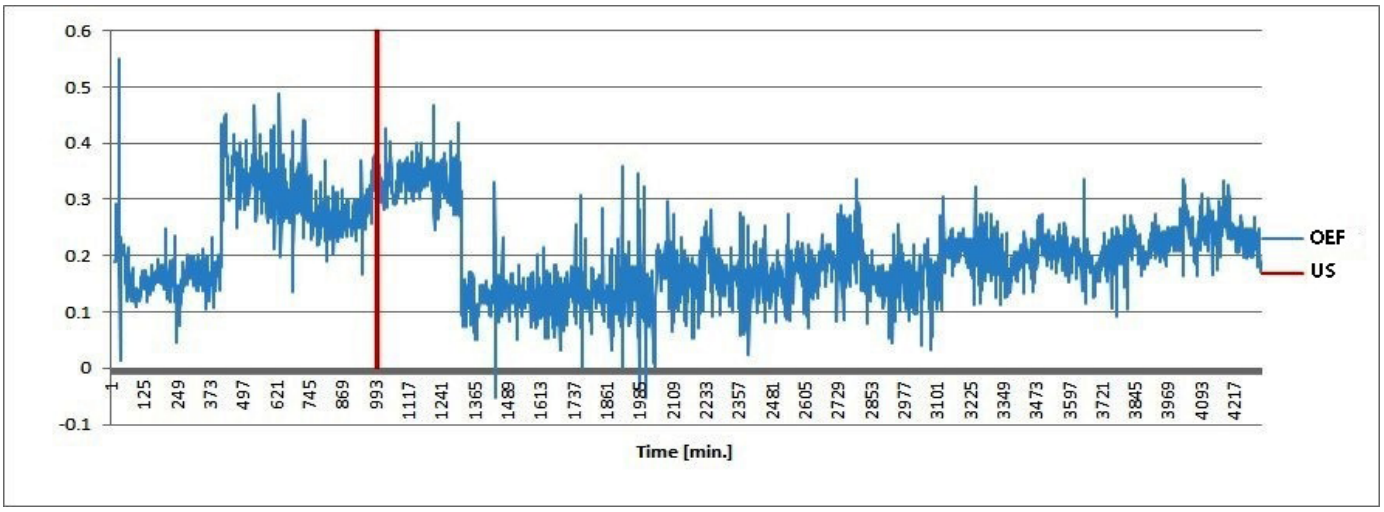

Fig. 2. *OEF changes during the 4320 minutes (72 hours) of monitoring.*

It is assumed that at the point where the second derivative of OEF is greater than 0.25, there is a significant change in hemodynamics that may indicate the beginning or the end of hemorrhage.

Fig. 3 is a graphical representation value of the second derivative of OEF. The first time period when the second differential values are greater than 0.25 (the 74th minute of life) can be omitted because of its origin; interference is the result of mechanical operations at the beginning of monitoring. Considering the medical records of the IVH course, presented above, other places where the second derivative of OEF is greater than 0.25 (432th and 1320th minutes of life) are probably the beginning and the end times of bleeding. The red line shows the time-point of US diagnostics when IVH was first detected.

The HR dynamic is shown in Fig. 4. These changes cannot be combined with the process of IVH. The HR values obtained before the 739th minute of life were subtracted from SpO2, and values obtained starting from the 740th minute were subtracted from ECG. HR values did not show significant deviation from the norm.

To determine the possibility of IVH detection by HR, we performed a correlation analysis of HR and OEF. The correlation coefficient was calculated as Pearson's correlation coefficient according to the equation 3:

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2 \sum(y-\bar{y})^2}} \qquad (3),$$

where x and y are each minute values of HR and OEF, and $\bar{x}$ and $\bar{y}$ are mean values of HR and OEF. The correlation coefficient is $r_{4320\,min}$ = - 0.03476, which means that there is no correlation between the HR and OEF values recorded during 4320 minutes.

Correlation analysis was also performed for the HR and OEF values recorded during the period of 120 minutes. The interval chosen was from the 359th minute to the 479th minute of after birth, so that the expected start of IVH (the 419th minute of life) was in the middle section of the correlated signals. The correlation coefficient is $r_{120\,min}$=-0.01497, which means that there is no correlation between the records of HR and OEF taken during the 120-minute period, wherein the putative start of IVH (the 419th minute of life) was in the middle of the correlated signals. The hypothesis that IVH could be detected by changes in HR analysis was rejected.

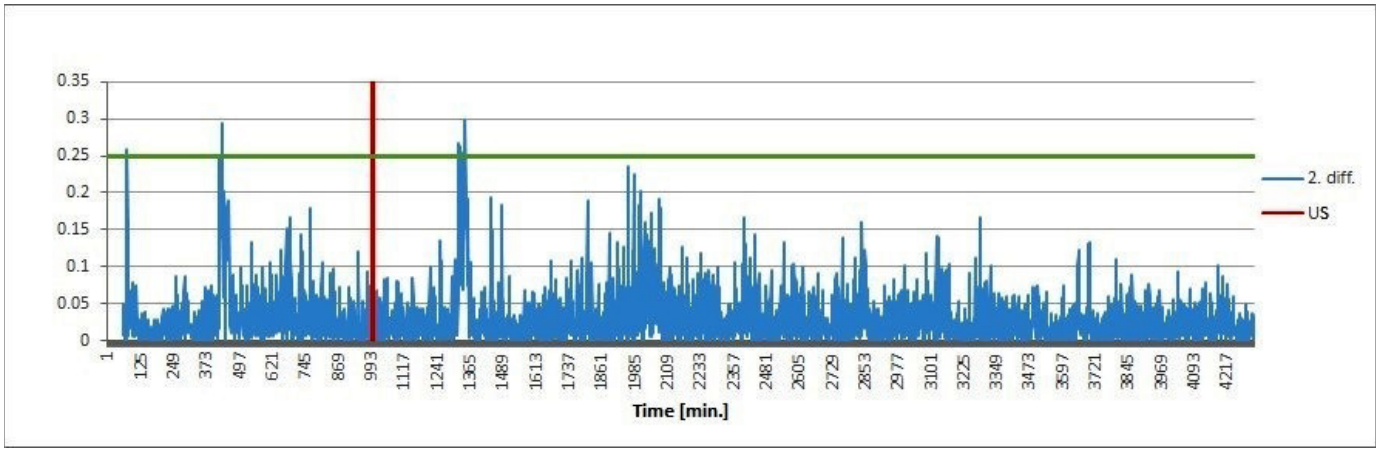

Fig. 3. *Changes of the OEF second derivative during the 4320 minutes (72 hours) of monitoring.*

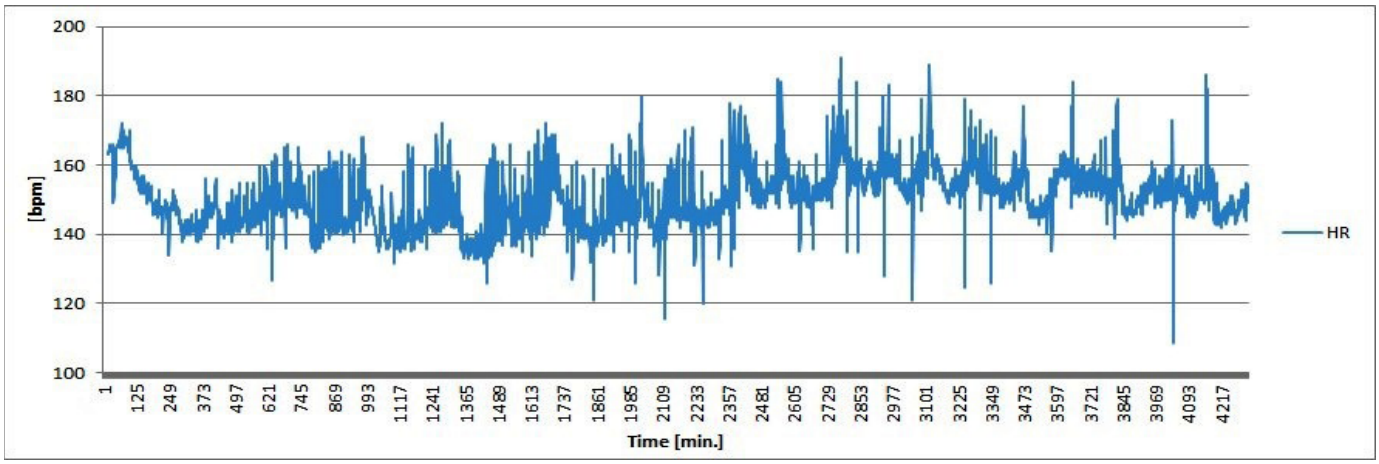

Fig. 4. *HR changes during the 4320 minutes (72 hours) of monitoring.*

In conclusion, changes in regional cerebral oxygenation changes and subsequent IVH can be detected in preterm children using paired data from NIRS and the patient vital signs monitor. Analysis of the paired data showed that HR is not a useful IVH indicator because it does not correlate with the changes of cSrO2. Comparison of cSrO2 and SpO2 changes and OEF with its second derivative represents a more beneficial method. It shows the tissue oxygen absorption changing (ie, periods of great change in hemodynamic, at the beginning or end of IVH). By comparing the calculated results and the medical records of the IVH course, it can be argued that this method can be efficiently used to determine the start and end of the bleeding into the brain ventricles.

Acknowledgments

We would like to thank Ing. Petr Kudrna, Ph.D from the Department of Obstetrics and Gynecology of the First Faculty of Medicine and General Teaching Hospital for the considerable help in collecting and providing access to the patient's data.

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International Journal of Biomedicine 6(4) (2016) 294-297
doi: 10.21103/Article6(4)_CR2

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Superior Sagittal Sinus Thrombosis Presenting with Hallucinations in the Puerperium: A Case Report

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Abstract

Cerebral venous sinus thrombosis is an uncommon cause of stroke presenting with varied presentation patterns. We report a case of a 21-year-old woman with superior sagittal sinus (SSS) thrombosis (SSST) developing after childbirth, presenting with visual hallucinations, severe headache, and tonic-clonic seizures. Time-of-flight magnetic resonance angiography (TOF-MRA) demonstrated the presence of thrombus in SSS. She was treated with low molecular weight heparin (LMWH) followed by warfarin. She had excellent recovery a few weeks after admission and was regularly followed up. Although this condition can be presented with different neurological symptoms, it does not typically present with hallucinations. We suggest that CSVT should be suspected even when a patient presents with an atypical picture in a category of patients at higher risk. **(Int J Biomed. 2016; 6(4):294-297.)**

Key words: cerebral venous sinus thrombosis • hallucinations • tonic-clonic seizure • puerperium

Introduction

Cerebral venous sinus thrombosis (CVST) is less common than other types of stroke but can be more challenging to diagnose due to its varied presentation patterns; for this reason, it seems to be overlooked not only by general practitioners but also in some specific cases by neurologists as well.[1] CSVT is a multifactorial condition with sex-related specific causes. Among its important etiological factors, pregnancy, puerperium, oral contraceptive use, coagulopathies, intracranial infections, cranial tumors, lumbar puncture, malignancy, dehydration, inflammatory bowel disease, connective tissue disorders, Behcet`s disease, parenteral infections, and various drugs can be implicated.[2] Among other causes, the inherited pro-thrombotic tendencies, such as factor V Leiden mutation, protein S or C and anti-thrombin III deficiencies are also important. However, in 30% of patients the etiology cannot be determined.[3] The clinical signs and symptoms of CVST are relatively nonspecific. The presentation of CVST can include headache, vomiting, papilledema, mental status changes, seizures, and focal neurologic deficit (motor and/or sensory).[4] SSST may present with unilateral paralysis that extends to the other side secondary to extension of the clot into the cerebral veins. Because of the location, this may present as a unilateral lower extremity weakness or paraplegia.[5] Although CVST may present with neuropsychiatric symptoms, it does not typically present with hallucinations.[4]

Because of the very nonspecific and variable clinical picture of CVST, brain imaging plays a crucial role in the diagnosis. The accuracy of magnetic resonance venography (MRV) and TOF-MRA for depiction of cerebral venous thrombosis is better compared to computed tomography angiography (CTA) and MRI.[6]

Treatment options for CVST include anticoagulants, thrombolytic therapy and, in some cases, surgical thrombectomy. The use of heparin and oral anticoagulants is based on a rationale of reversing the causal thrombotic process and of preventing complications.[7] CVST can result in death or permanent disability, but usually has a favorable prognosis.[8]

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Case report

A 21-year-old woman presented in the neurologic department complaining of severe headache, speech disturbances, hallucinations and tonic-clonic seizures. She was 4-day postpartum following Caesarean section. She had hypertension and problems with vision (scotomes and blurred vision) during the pregnancy. After antihypertensive therapy with α-methyldopa 250 mg, 4 times daily, the arterial blood pressure was normalized and the vision went to normal. There was no history of epilepsy.

During the hospitalization in the neurologic clinic, a bilateral headache was persistent, and she had hallucinations and two tonic-clonic seizures. In the physical examination, there was no nuchal rigidity but she was dehydrated. There was no neurological deficit. The seizures were controlled with intravenous lorazepam, which was commenced with carbamazepine tablets.

Laboratory blood analysis was performed and the following results were obtained: ESR, 110 mm/hr; RBC count, 4.59 x 10^{12}/L; hemoglobin, 12.3 g/dL, WBC count, 14.7 x 10^{9}/L; hematocrit, 36.4 %; platelets, 206 000; serum glucose level, 6.76 mmol/L; serum creatinine, 0.66 mg/dL. The international normalized ratio (INR) and partial thromboplasitin time (PTT) were within normal ranges: 0.9 and 25, respectively. D-Dimer was increased to 752 ng/mL; thrombin time (TT) was 16 seconds. During follow-up, cranial CT and TOF-MRA were obtained, which demonstrated the thrombosed SSS with partial recanalization (Figures 1 and 2).

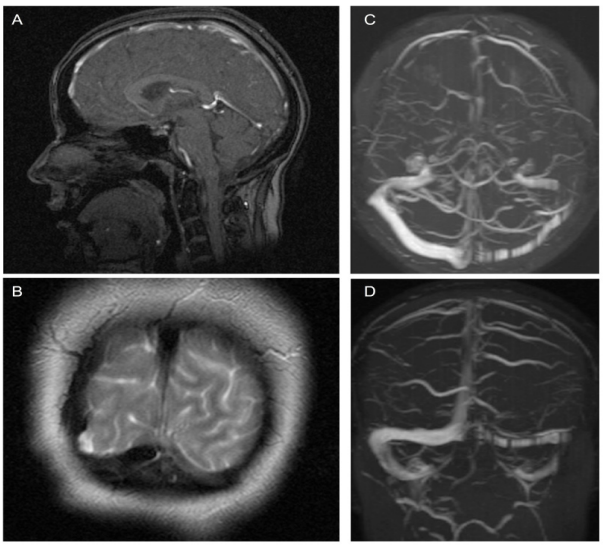

Fig 1. *SSST with partial recanalization. A- sagital view, B- axial view. TOF-MRA 2D images, C- axial and D- coronal view.*

Treatment started as soon as the diagnosis was confirmed. Initial anticoagulation therapy was used with LMWH for 2 weeks followed by warfarin, broad spectrum antibiotics (parenteral ceftriaxone 2g/day for 10 days), and hydration with physiologic solutions. She had an excellent recovery and she is on regular follow-up.

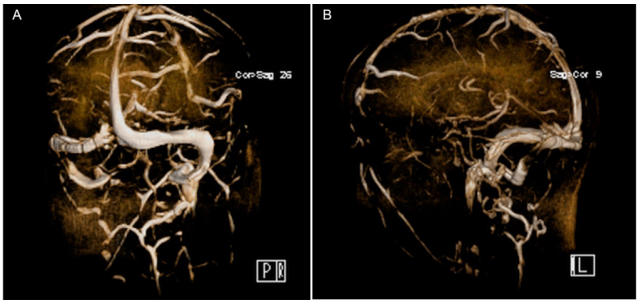

Fig 2. *TOF-MRA imaging with 3D reconstructions, SSST with partial recanalization. A-posterior and B-lateral view.*

Discussion

CVST can affect all age groups, particularly women of childbearing age.[9] Pregnancy and puerperium are prothrombotic risk factors. Acute blood loss during delivery, prolonged lying in bed, postpartum infections, sweating, and hyperlipidemia dramatically increase the chance of venous thromboembolism.[10] Cesarean delivery and pregnancy-related hypertension also increase CVST risk.[10,11]

CVST is a rare pathology that presents with a wide spectrum of manifestations. Our patient with cesarean delivery presented with SSST. SSS is most commonly involved with thrombosis. Most patients present with rapid neurological deterioration.[12] The clinical picture is determined by the age of patient, site of CVST, and the presence or absence of parenchymal lesions.[11,13] When a focal brain injury occurs, most common clinical presentations are hemiparesis and aphasia, but other cortical signs and sensory symptoms may be also observed, together with psychosis in rare cases.[14]

Our patient presented with a severe progressive bilateral headache, tonic-clonic seizures, and hallucinations. The headache was bilateral and worsening with sleep. Headaches are the most frequent presenting symptoms (70.6%) of CVST, followed by seizures (47%) and paresis (43%),[11] and have been described as its only clinical presentation in 15% of patients. There is no typical pattern of headaches in CVST. A headache can be of any grade of severity, usually is global and persistent, and has an acute onset.[15]

Intracranial hypertension with local inflammatory reaction determining dilation of vessels in the walls of the affected sinuses is possible, and extravasations of inflammatory proteins could explain the bilateral pain. The pain may also be caused by the stretching of the nerve fibers in the walls of the occluded sinus.[13,15]

There are controversial data on the frequency of seizures after CVST; a prevalence of 10% to 50% has been reported.[16] Most of the seizure types are focal seizures. Rarely, life-threatening generalized tonic clonic-seizures can be seen.[17,18]

No clinical trials have studied either the optimal timing or medication choice for anticonvulsant in CVST. Whether to initiate anticonvulsants in all cases of CVST or wait for initial seizures before treatment is controversial. Because seizures

increase the risk of anoxic damage, anticonvulsant treatment after even a single seizure is reasonable.[14]

In our patient, we made definite diagnosis by MRV, which is the most sensitive diagnostic investigation, but also TOF-MRA has a high accuracy. It has to be underlined that very frequently delays in diagnosis of CVST are common and significant. MRV allows direct visualization of the dural venous sinuses and the large cerebral veins.[19]

Neuroimaging techniques have allowed a more rapid and accurate diagnosis of these conditions, enabling earlier therapeutic interventions.[4] Neuroimaging techniques have also demonstrated that the prevalence of CVST is higher than previously reported.

Treatment options for CVST include anticoagulants, thrombolytic therapy and, in some cases, surgical thrombectomy.[20] Our patient was treated with anticoagulation therapy and had very good recovery. It has been conclusively shown that intravenous heparin is the first-line treatment for CVST because of its efficacy, safety and feasibility. The initial anticoagulation therapy has 3 aims in CVST: a) to prevent thrombus growth, b) to facilitate recanalization, and c) to prevent deep venous thrombosis or pulmonary embolism.[14] Post-acute treatment with oral anticoagulants is recommended for up to 6 to 12 months.[21]

Prognosis of CVST is quite variable, the outcome ranging from total recovery to death. The disease had fatal outcomes during the pre-imaging era, when early diagnosis and effective therapies were not possible, and only supportive care was available. Currently, more clinical studies are reporting a better outcome. Neuropsychiatric manifestation, as in our patient, and pseudotumor cerebri-like presentations carry favorable prognosis while an acute fulminant course, bilateral hemorrhagic infarctions and diffuse cerebral edema are associated with relatively poor outcomes.[22] Favorable outcomes in obstetric CTSV have been attributed to the assumption that the occlusion is limited and transient with rapid recanalization, or by development of collaterals.[23] Mortality rates range from 6% to 10%, and independent survival is reported in 90% of patients.[24]

In conclusion, CVST is a rare pathology that needs a complex diagnostic evaluation. CVST must be considered in young women presenting with any neurologic manifestation related to CNS during puerperium and pregnancy but also when presenting with atypical symptoms, such as hallucinations. TOF-MRA and MRV are the best means of investigation because they make early diagnosis and treatment possible. Generally, correcting the cause can prevent complications.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 6(4) (2016) 298-302
doi: 10.21103/Article6(4)_PV1

INTERNATIONAL JOURNAL OF BIOMEDICINE

POINT OF VIEW

Creativity as a Determinant of the Development of Homo Sapiens

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Abstract

This work was undertaken to determine the genesis and role of creativity (Cr) in the formation of mental qualities that gave Homo sapiens (HS) the evolutionary advantages in intra- and interspecific competition during the period of the intraspecific bifurcation of hominids on the border of the Middle and Upper Paleolithic. Creativity allowed HS to design the adaptive forms of purposeful behavior corresponding to the conditions and the degree of uncertainty, and create stable mental constructs, in the absence of perceptual sources, that do not require reactive behavior. Visualization of a target image, which originally had an applied and instructional value in the process of semantic filling, was transformed into a symbol, which, getting the qualities of the perceptual source, loses its connection to the primary value and initiates the creation of qualitatively new needs for hominids: bilateral (direct and inverse) relations in the system "subject-symbol." The ability to produce the prognostic hypothesis with expansion of the operating range of the HS mind allowed the ability to search and change the tactics of an adaptive behavior, which gave the results: improvements in quality of life and an increase in life expectancy (genetically fixed); domination in intra- and interspecific competition; the emergence of new operating systems of the psyche, including the emergence and development of symbolic thought. As a criterion of creativity, with the potential ability for quantitative measurement, we propose the value of deviation of creative oscillations ("proposal" of a creative individual) from the boundaries between stereotypes: for the cognition sphere, a deviation from the border between recognizable and unrecognizable; for the social sphere, between acceptable and unacceptable. (**Int J Biomed. 2016; 6(4):298-302.)**

Key Words: creativity • genetic mutation • shizotimiya • interspecific competition

The aim of our study was to determine the genesis and the role of creativity of HS in the formation of adaptive forms of behavior in conditions of intra- and interspecific competition.

To build a model of creativity as a mental function, along with the works of cited authors, we used our previously published materials.[1] Creativity (a species-specific feature of the psyche of HS compensating the lack of biological adaptability), as the ability to produce the predictive hypotheses that cannot be derived directly from the initial conditions, formed about 50,000 years ago, presumably as a result of genetic mutation [2] that does not exclude the possibly interfering evolutionary and other exogenous factors. It is necessary to emphasize the existence of various exogenous (including biogenic) factors which change the external behavioral manifestations of mental activity not only in humans, but also in other species. For example, Toxoplasma gondii alters the behavior of rodents up to eliminating the instinct of self-preservation while maintaining newly acquired psychic qualities after full recovery.[3] In the available literature, we have not found works about the genetic fixation of the new mental properties in rodents that underwent toxoplasmosis. It was noted that there is a similarity in some syndromic forms of schizophrenia and toxoplasmosis in humans,[4] as well as a significant correlation between toxoplasmosis and expressed anxiety.[5] Not having sufficient grounds to assert the identity of states defined as schizophrenia with the effects of toxoplasmosis, we would like to emphasize their considerable syndromic similarity. It is possible that Toxoplasma gondii, in the considered historical period, could not be the single biogenic factor with potential effects on the brain (mind) of man.

We are interested in an historical period having significant time assumptions (for thousands, sometimes tens of thousands of years) as a result of new archaeological finds—the completion of the Middle Paleolithic (300,000-40,000 years ago), the time when the tribal system replaced

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the primitive herd. This is a period of significant rapid climate changes during the ice age,[6] increased intra- and interspecific competition, disappearance of species that did not have a sufficient range of adaptability, and the emergence of the modern physical type—Cro-Magnon man (HS).

HS existed in small groups with a dominant leader, the hierarchical distribution of functions and benefits, and the suppression of individual traits. The emergence of communities, the development of intergeneric relationships, and exogamous marriage in the face of increasing competition for food resources with competing subspecies, Homo Neanderthalensis (HN), led to a blurring of the boundaries between the possible and permissible, an increase in the degree of uncertainty on the boundaries of hierarchic subsystems (genuses) within and outside of the community. We understand uncertainty as the variability of choice for a variety of alternatives and the absence of clear criteria of optimality and efficiency. There are conceptions of uncertainty as a fundamental property of nature.[7]

Benefits that include the ability to search and change tactics while ensuring competitiveness within and between species, and an increase in the probability of the occurrence of posterity (inheritance and accumulation of parent qualities) reconcile the tolerant individuals to uncertainty.

Emergence of new mental characteristics (Cr) allows us to construct the target images, adaptive forms of purposeful behavior appropriate for the condition and the degree of uncertainty. The abstracted images of hunting objects with detailed scenes achieving the desired results (the cave of Altamira)[8] demonstrate the HS ability to create consistent themes of purposeful behavior, achieving future results, and the image of the goal (IG), which has no perceptual sensory source in the process of its creation. Through the operating systems of the psyche, presumably integrable by Cr, the structure of purposeful behavior is created, which includes IGs that are outside the boundaries of the immediate satisfaction of vital needs. In other words, a new feature of the species (Cr) allows us to create stable mental constructs in the absence of a perceptual source and does not require a reactive adaptive behavior. In particular, an abstracted and coded IG is created, which actualizes the need to achieve this goal by following purposeful behavior that ensures satisfaction of this need, in the absence of which the "anxious waiting" state is formed. Adaptive behavior gets a vector of satisfaction for a qualitatively new need: the relationships with the coded and visualized IG, which is not associated with reactive behavior. We believe that the visualization of IG, having initially utilitarian and instructional value, inevitably becomes the subject of supplements, discussion, verbalization, and interpretations (ie, semantic content), transforming into a symbol (image+sense).[9]

Cr, implying the capacity for symbolic thinking, includes:

- a selective attention to contradictions;
- deviations from the standard;
- a tendency to allocate the minor, non-obvious features of the information field as reference by following construction of an integrated mental product (image); and
- the ability to design a set of solutions on the basis of monosemantic data (divergent thinking).[10]

Creative individuals are characterized by the identification of the subjective "I" with an imaginary (external) objective "I", by abstraction, and the "transfer" (imagination, empathy) of personal properties to a third party (an external object), a kind of schism of integral personal presentation.[11]

We believe that the appearance of Cr created an evolutionary bifurcation that gave a branch of HS an evolutionary advantage that enabled it to produce the divergent forecasting models and generate the adaptive behaviors adequate to different degrees of uncertainty. A possible effect of cumulative accumulation of qualitative changes of the psyche is the appearance at the end of the Middle Paleolithic (about 40,000 years ago) of the samples of fine art of Cro-Magnons around the habitat, signs of the cultural environment, and domestication and involvement of dogs in economic activity (about 33,000 years ago).[12]

In HS, in incomplete or "open" vitally important situations to the integration of new elements (deficiency), there is an inevitable development of the state of dissatisfaction, a deficit of that condition which is required to maintain a homeostatic balance; and the need to fill the missing fragments in the imaginative mapping of reality is formed. We believe that Cr provides a constructive way to complete the deficient image of reality by using the brain's operating systems (thinking, imagination, memory, etc.). Thus, anticipatory and perspective designing of an integral image of a future result (IG), completion of which is experienced as "insight," is realized, as well as the method of meeting the arising need, which reduces the level of risk in the process of achieving the result with elimination of "anxious waiting," which leads to a relationship of equilibrium with the object (IG).

Individuals (tolerant to uncertainty) design (through Cr) the abstracted IGs which are visualized through graphic forms with acquisition of more sophisticated color, contour, perspective, and plot, and transformed into art forms—the symbols. The result is unique to the species: the ability to create stable mental constructs with the following projection into external environments as symbolic forms, visualization of which as artistic images appeals to the arsenal of ideas. These forms are perceived in the inverse projection (reflection of reflection) as objectively existing (beliefs, myths, etc.). An image having the applied instructional value becomes an independent mental construct (symbol), acquiring the quality of the perceptual source, which creates an afferent information flow and motivates the formation of the mental activity vector to achieve the goal: the parametric balance with the symbol. The symbol dissociation, eliminating the "image" (category of representativity), retains only "sense" as the addressee of subject appeal (ie, symbol, imagery) becomes exclusively virtual, without losing the qualities of objectively existing for the subject. Obviously, this is the mechanism that forms the coded images of the primary myths initiated by the abstractive (symbolic) IG, followed by transformation (complications, interference, interpretation by the second signaling system) in totems, cults, ideologies, etc. In certain conditions (resonance, including induction), this construction, being entirely a

product of the second signaling system, can acquire the properties of the dominant focus with appropriate mechanisms (neural, metabolic, and behavioral) of implementation. The combination of these constructs, creating a system of representations (virtual system of ethical coordinates) about relationships within and outside society, reduces the level of uncertainty, creates and regulates the coordinate system of the social environment and its relations with the outside, and reduces the level of suspense. Intra-group relations of the ethical coordinate construct reduce the degree of uncertainty (the creator of social negentropy), namely, relations between a creative person resistant to uncertainty (conditional priest, ideologist - "divergent") and another person, the advantage of which is the ability to adopt and implement adequate solutions in the current coordinate system (conditional leader-"convergent"), as well as interference of functions in one individual. These will be issues for the next study.

According to modern ideas, the meeting of 2 subspecies of Homo: HN (natives) and HS (aliens from the south) occurred in Eurasia about 50,000 years ago. HN and HS are the most adapted modern gregarious predators. HN and HS lived together on the "feeding" landscapes of Eurasia for 6,000 years in conditions of increasing competition (with an increase in population) for food resources. HS, having a new feature of the species (Cr), compared with HN, acquired a much greater range of adaptive behaviors, as well as a strategic advantage in conditions of interspecific competition, which resulted in dominating and then crowding out the competitive subspecies (HN). It should be noted that this particular historical period (date of occurrence from 50,000 to 138,000 years ago)[13] is a time when the Y-chromosomal Adam occurred, whose actively functioning genes (without alleles in the X-chromosome) are presented only in the genotype of heterogeneous gender, appearing phenotypically only in representatives of heterogametic sex.

The vector of subsequent analysis is based on near-zero probability of female authorship as the creator of the cave art images that had practical importance and required the knowledge, skills and experience of direct contact with the image object that was not familiar to the average woman, regardless of historical era. Regardless of the prevalence (in different historical periods) of matriarchal or patriarchal society, while hunting the male groups were structured organizations with the undisputed leader (possibly only during the hunting time) and the distribution of functions on the pack model. Anthropometrically, psychologically and physiologically, the appropriate psychosomatic conditions are more typical for men.

We believe that a point mutation in a Y-linked gene led to development of a new quality of the psyche—creativity. Sexual differences for mammals, including humans, are provided by a pair of sex chromosomes (XX and XY). Reduction or translocation of genes, useless or harmful to the females, led to the separation and concentration of genes, providing the development of physiology and psychology of the male type. The SRY gene located on the short arm of the Y-chromosome plays the most important role in initiating male sex determination. The peculiarity of the human Y-chromosome is a short length of regions, which are exchanged with loci of other chromosomes, in particular, with the X-chromosome, except for the pseudoautosomal regions occupying about 5% of the Y-chromosome's length.

Sequencing the genome of the Neanderthal in comparison with genome of the Cro-Magnon man, in the framework of modern features, revealed a difference between them of 0.16%. Not found in Neanderthals were genes associated with mental activity and energy exchange, having adaptive character,[14] and genes associated with the development of schizophrenia and bipolar disorder,[15] as well as a reduced number of genes on the site responsible for the development of speech.[16] Genes that have been found in Cro-Magnons and are absent in Neanderthals and chimpanzees emerged during evolutionary bifurcation (or became the cause of it). The number of nucleotide substitutions that have semantic meaning, is 1% of the total differences between the human and chimpanzee genome (ie, 1 million base pairs, 78 single-nucleotide differences) with the concentration of these differences in the DNA regions encoding 5 genes.[17] We believe that the focused search in this area can reveal a substrate (genetic) basis of Cr.

Genetic variations associated with increased risk of schizophrenia and bipolar disorder were found in a large sample of HS.[18] In a creative environment, variations in genes associated with an increased likelihood of these diseases are higher by 17%-25%.

A key feature of schizoid thinking,[19] along with a tendency toward complex mental operations (generalization, abstraction) is choosing as reference points the insignificant, latent signs, and then using them for generalization and abstraction compared to the most frequent stereotypes entrenched in the experience. In particular, there is a change in the logical course of judgment, when the conventional story and the logic of the sequence are understood, but not accepted, and the formation of the judgment is formed in several directions. All of this leads to the creation of stand-alone logic with non-obvious conclusions that differ from the findings of individuals having the same original data. In this paper we do not discuss changes in thinking that reached the level of the marked clinical manifestations. We are interested in basic mechanisms of changes in thinking that give the opportunity to find qualitatively new forms of adaptive behavior, avoiding stereotypes. We would like to emphasize the common features for the schizoid and creative personalities: an inability to filter out irrelevant information; a propensity to build mental (associative, shaped) structures that differ from the social standards; cognitive disinhibition; sthenic emotions; splitting of the personal views, as well as other features confirming the similarity of cognitive processes involved in creativity and mental illness. Through MRI,[20] it was found that creative individuals and groups with the confirmed signs of schizothymia, when solving cognitive tasks, displayed the same objective equivalents: deactivation in the right parietal region and precuneus—in the areas responsible for concentration.

A study of single nucleotide polymorphisms of genes of modern man found 8 genetically determined variants of the

clinical forms of schizophrenia, which depend on the structure of clusters. In addition, if each gene had a tenuous connection with the development of the disease, a cluster association increased the risk by 70%-100%.[21] Regarding the combination of genes that have a limited number of variations, it was stated that there is the simultaneous presence of a predisposition to the schizoid state with characteristic changes of thinking and of creativity. It should be noted that the average life expectancy (26-32.3 years)[6] in this historical period at low-progredient forms of endogenous diseases (debuting in 20-25 years) reduced the probability, because of high-risk habitats, of reaching the final or even psychotic deployed stages. Thus, new features of the psyche for hominids, acquired as a result of a genetic mutation or other reasons, with schizoid initiation in the mature age (for the historical period) can form deviations (in the modern sense, disorders) in thinking, such as ideas of influence, relationships, harassment, and mania states in varying degrees of severity. In other words, schizoid accentuation or endogenous disease in the prodromal stage may lead to the construction of sustainable ideas about an exogenous impact (in relation to the individual), the presence of an external object, perceived by the individual perceptually with the corresponding effects on the subject. This stimulates the creation, development, and interference of logic circuits forming and unifying the perceived metamorphosis. The result is the construction of predictive models of development and outcome of the situation, with variations of personal and social behavior. We believe it possible, among other things, to consider the development of a second signaling system as a need to express and describe something that has no obvious perceptual source for the audience. Thus, a stable endogenous psychic structure, becoming a representation, is transformed by a subject into visualized and verbal forms (as the semantic content - symbols), transformed into the belief (an inverse projection) and initiates the adaptive forms of behavior in the relationship system: an individual-a symbol (in certain circumstances: society-symbol). Artistic images, originally of an applied nature, with the accumulation and complexity of semantic content, are dissociated from an initial sense and acquire an independent significance, becoming abstract symbols forming a qualitatively new demand (and forms of adaptive behavior): parametric and homeostatic equilibrium of relationship with the symbol.

The logic of the discussion leads to the conclusion that just the carriers of Cr, having the above properties of the psyche (sthenic emotions, the tendency to oscillation) with deviation from the allowable and acceptable borders, allow us to find new optima of the individual and system functioning (ie, to determine the vectors of adaptation for society to the changing parameters of an external [or internal] environment). As a criterion of creativity, with the potential ability for quantitative measurement, we propose the value of deviation of creative oscillations ("proposal" of a creative individual) from the boundaries between stereotypes: for the cognition sphere, a deviation from the border between recognizable and unrecognizable; for the social sphere, between acceptable and unacceptable.

The described time interval, ending the Middle Paleolithic and beginning the Upper Paleolithic, for HS is a time of formation (as a result of improved quality of life) of a more improved structure of biological age: late ripening - late aging (for HN: early ripening - premature aging) with an increase in life expectancy and fixing the potential ability for adaptive variation at the genetic level, which increased the average life expectancy in the Upper Paleolithic (the next stage after a long break is the Middle Bronze Age).[6] We believe that an increase in life expectancy to more than 30 years for the Cr carriers in the prodrome of endogenous disease (unlike accentuation) could reveal a side effect of one of the variants of the genetic mechanism for an increase in population adaptability: an emergence of the expanded forms of endogenous diseases, which dramatically increase risks for their carriers.

Conclusion

Creativity that expanded the adaptive capacity of HS—as the ability to assimilate and process the volume of information in excess of the standards and to allocate the latent signs of environment as reference, as well as a cognitive disinhibition that increased the associative range and split of the personal representations with formation of judgments in accordance with the convergent type—has formed a unique ability to create sustainable mental constructs non-associated with reactive behavior, combining image and meaning (projected into the external environment by the individual) in the form of artistic images. The subsequent transformation into independent symbolic forms, perceived as the external objects, forms a need in bilateral relations, initiating an adaptive behavior that balances the relationships in the "subject-a symbol" system. The results of the described process are advantages and then domination in intra- and interspecific competition in conditions of uncertainty, as well as improvements in the quality and expectancy of life for HS. As a criterion of creativity, with the potential ability for quantitative measurement, we propose the value of deviation of creative oscillations ("proposal" of a creative individual) from the boundaries between stereotypes: for the cognition sphere, a deviation from the border between recognizable and unrecognizable; for the social sphere, between acceptable and unacceptable.

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International Journal of Biomedicine 6(4) (2016) 303-304
doi: 10.21103/Article6(4)_CC1

CURRENT CONCEPTS
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How Advances in Technology Improve HIV/AIDS Care

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Abstract

In the U.S., the number of individuals aged 50 and older who are living with HIV has increased, leading to a phenomenon called the graying of the HIV/AIDS epidemic. Advances in treating HIV have brought about a large growing population of seniors with HIV who are simultaneously facing social, psychological, and physical challenges correlated with the aging process. The stigma against HIV/AIDS has been linked to poor health, depression, and loneliness. In a recent study, about 39.1% of HIV/AIDS patients showed symptoms of major depression (C. Grov et al, 2010). Consequently, to reduce lasting effects of major depressive symptoms, there is a vital need for service providers to employ innovative efforts to confront the stigma and psychosocial and physical health problems that are characteristic of an older HIV/AIDS population. The new technological approaches to healthcare delivery have resulted in faster, more accurate diagnosis and monitoring, in more sophisticated coordination across regions and agencies, and in sophisticated risk-checking procedures. New healthcare technology that can help the AIDS/HIV patient is called Health Information Technology, a basic element of Health Relationship Management Services (HRMS), which is a new approach to healthcare. HRMS can assist individuals with HIV/AIDS in managing not only their physical, but also their mental health. (**Int J Biomed. 2016; 6(4):303-304.)**

Key Words: HIV/AIDS • IoT• Health Relationship Management Services • Remote Health Monitoring

Introduction

With all of the advances in medical technology, is there anything that can help the AIDS/HIV patient? AIDS is a chronic disease that is typified by suppression of an individual's immune system. The clinical materialization of AIDS is characterized by the presence of opportunistic infections, dementia, wasting, and AIDS-related cancers. The number of U.S. individuals aged 50 and older who are living with HIV has increased, leading to a phenomenon that has been described as the *graying* of the HIV/AIDS epidemic (Gorman, 2006; Shah & Mildvan, 2006). Advances in treating HIV have brought about a large growing population of seniors with HIV who are simultaneously facing social, psychological, and physical challenges correlated with the aging process. In addition, the HIV/AIDS-related stigma has been linked to health, depression and loneliness. A recent study found that 39.1% of HIV/AIDS patients showed symptoms of major depression.[1] Consequently, to reduce lasting effects of major depressive symptoms, there is a vital need for service providers to employ innovative efforts to confront the stigma and psychosocial and physical health problems that are characteristic of an older AIDS/HIV population.[1]

However, the question remains, "Is there any healthcare technology that can help the AIDS/HIV patient?" The answer is, yes, and it is called Health Information Technology, a basic element of HRMS,[2] which is a new approach to health care. Information is essential to high quality health care; therefore, it is important to have nationwide, reciprocal, interchangeable health information available wherever and whenever it is needed, in private, secure electronic form.[3] The largest population of beneficiaries of this innovative system is chronically ill individuals, especially HIV/AIDS patients.

Individuals who are living with AIDS or HIV have health problems in addition to adhering to a complicated medication regime. To ensure that all caregivers and nurses are getting the complete picture for these chronically ill patients, it is important to be able to collect AIDS/HIV patient information. Information Technology (IT) and eHealth technologies and resources for HIV/AIDS patients are rapidly changing due to the influx of new media, such as interactive internet communication that allows them to create, alter, and share

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content using simple, inexpensive, or free tools. All that is needed is a computer or mobile device with Internet access.[4] HRMS is an innovative technical approach to delivering health care. By using a remote health monitoring system as part of HRMS, an individual's personal health data are collected and sent immediately to the cloud for restructuring into actionable information that can be acted on when needed.

HRMS can assist individuals with HIV/AIDS in managing not only their physical, but also their mental health. Patients can join online networks, comment on blogs, and receive medication reminders on their mobile phones. Such health IT tools can connect HIV/AIDS with other HIV/AIDS patients and experts, friends, family, and caregivers to share their medical information and experiences and connect with others who are in similar positions to share their health stories and obtain emotional and social support.

The most ideal method of making information available at the right time at the right place is through electronic systems, which HRMS can provide,[2] to convert traditional paper records into electronic form. This Electronic Health Record (EHR) information can be easily transmitted, displayed in a variety of ways, and be combined with information from many sources, which is sent to caregivers and care-friends. A patient can keep his/her own personal health records, either on a personal computer, on Google or Microsoft, or even on a hospital computer. The main idea is that the patient maintains and controls personal health data, and the more health issues a chronically ill individual has, the more crucial it is that this information be made available.[3]

Advances in technology, such as remote health monitoring systems and HRMS, are being used to improve healthcare service delivery and make it possible to help providers and patients conduct 24/7 health monitoring. An estimated 70% of U.S. doctors already utilize some aspect of new medical (or health) technology; however, only about one quarter subscribe to a sophisticated, multifunctional system. Ideally, a comprehensive IT system should empower patients, advance healthcare delivery, and transform patient data into life-saving research.[4]

Healthcare technology has come a long way, and many innovations can now benefit AIDS and HIV patients. The new technological approaches to healthcare delivery have resulted in faster, more accurate diagnosis and monitoring, in more sophisticated coordination across regions and agencies, and in sophisticated risk-checking procedures. Over 70 million people worldwide have been diagnosed with HIV. To lower healthcare costs, it is necessary to improve the care of patients who suffer from HIV/AIDS disease and help them remain in good health and out of emergency rooms and hospitals, where patients often contract infections, resulting in millions of dollars in healthcare costs. Remote monitoring of health can improve healthcare delivery and improve patient involvement and adherence. Many HIV/AIDS patients find themselves isolated and lonely, which can lead to dementia.[1] To avoid this, HRMS provides a patient platform wherein HIV/AIDS patients can join online networks to interact with others on social network sites, share personal health stories, offer commentaries, watch videos or news, and discuss issues of relevance to people with similar experiences. Health-related online communities, such as HRMS, offer social and emotional support by connecting with others who are experiencing similar challenges to exchange information, while at the same time keeping their privacy. Facebook and MySpace allow people to connect by creating a personal page or group. Blogs, which are frequently updated websites that combine images and text with links to other websites, allow HIV/AIDS patients to participate in discussions on HIV/AIDS issues, share personal health stories and experiences, ask questions, and share opinions and other information. HRMS and certain blogs encourage dialogue by allowing people to leave comments and receive HIV policy information, news media, health news, and HIV/AIDS research updates.[4]

Cellular phones and other wireless devices make viewing, sending, messaging, and receiving information easy. The benefits of HRMS, coupled with mobile health assistance, enable AIDS/HIV patients to set up medical and medication reminders, and receive alerts for these reminders via email or text messages (Short Message Service, SMS) (HRSA, 2016). Mobile health applications (apps) on iPhones or other smartphones support healthcare management, and through a weight tracker, HIV/AIDS patients can set weight gain or loss goals, which are especially important for individuals dealing with this illness.

Remote health monitoring devices, such as the one used in HRMS, are used to track and store health metrics, which can be used to manage and monitor a wide range of health conditions. Some devices can collect an individual's blood pressure, glucose, and oxygen and send the data by phone or internet to a provider or to the cloud for analysis to support patient engagement and physician-patient communication.

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