Electrophoretical mobility of nuclei in buccal cells of cystic fibrosis patients

Minarowska A.[1*], Litwiejko-Pietryńczyk E.[2], Minarowski Ł.[3], Trochimowicz L.[1], Sierżantowicz R.[1], Dzięcioł J.[2], Chyczewska E.[3]

[1] Department of Surgical Nursery, Medical University of Bialystok, Poland
[2] Department of Human Anatomy, Medical University of Bialystok, Poland
[3] Department of Lung Diseases and Tuberculosis, Medical University of Bialystok, Poland

ABSTRACT

Introduction: Cystic fibrosis (CF) is inherited, metabolic, multisystem disease with various clinical symptoms. In the airways neutrophilic inflammation and increased levels of neutrophil elastase (NE) are observed even in young children. The method of electrophoretic mobility of cell nuclei (EMN) is a way of assessment cell maturity. Observed distorted nuclei mobility is mostly dependent on chromatin distribution and remodeling.
Purpose: To evaluate the EMN index in buccal cells collected from CF patients.
Materials and methods: The study was conducted in the group of 15 CF patients aged 5-26 years.
Results: In the healthy subjects we have observed that the lowest EMN index values were reach around 2 year of life (7.6 ± 3.1) and in the late senescence (8.5 ± 2.5) with the peak values around 16-21 years (61.4 ± 2.5). The results differed significantly from the healthy controls.
Conclusion: EMN index in CF buccal cells may be a simple method to quickly assess the chromatin remodeling.
Key words: cystic fibrosis, children, electrophoretic mobility of cell nuclei

***Correspondening author:**
Alina Minarowska
Department of Surgical Nursery,
Medical University of Białystok
Szpitalna Str. 37, 15-274 Bialystok, Poland
Tel./Fax: +48856865078
e-mail: minar@mp.pl

Received: 5.06.2013
Accepted: 30.06.2013
Progress in Health Sciences
Vol. 3(1) 2013 pp 61-64

INTRODUCTION

Cystic fibrosis (CF) is inherited, metabolic, multisystem disease with various clinical symptoms [1]. The cause of the disease is a mutation of *CFTR* gene, which encodes CFTR protein - an intracellular regulator of ion transport across apical part of a bronchial epithelium. Pancreatic and liver insufficiency in CF often disturbs proper energetic balance and results in malnutrition [2, 3]. In the airways neutrophilic inflammation and increased levels of neutrophil elastase (NE) are observed even in young children. Senescence is a multistep process requiring the expression of both p21 and p16. p16 up-regulation is a key event in the terminal stages of growth arrest in senescence, which may explain why p16 but not p21 is commonly mutated in immortal cells and human tumors [4]. Recently new data has been published on increased expression of senescence makers in CF airway epithelial cells. High load of NE in the CF airway triggers epithelial senescence by upregulating expression of p16, which inhibits cyclin-dependent kinase 4 (CDK4) [5]. The method of electrophoretic mobility of cell nuclei (EMN) is alternative way of assessment cell maturity. EMN is based on evaluation of mobile cell nuclei percentage in alternating electric field. The share of cells with increased nuclei mobility grows until complete maturity of the organism. After reaching the peak of organism maturity, EMN begins to drop regularly [6]. The EMN method is easy to perform a collection of the cells is almost non-invasive even in young children, therefore the aim of our study was to evaluate the EMN index in buccal cells collected from CF patients.

MATERIAL AND METHODS

Patients. The study was conducted in the group of 15 CF patients aged 5-26 years. The results were compared to normal EMN values assessed in a group of 330 healthy subjects aged 40 weeks-80 years old acquired in the previous study [6]. The results were assessed in the sub-groups divided according to age.

Cells. The evaluation of Electrophoretical Mobility of Nuclei (EMN) was carried out in buccal epithelium cells. The material for examination was taken in the morning with the non-invasive, painless method, from the oral epithelium of the left bucca at the level of upper molar teeth. Immediately, after collection, the samples were placed in test-tubes in 0. 09%% NaCl solution.

EMN assay. When individual cells were isolated, the nuclei were observed with a light microscope (Olympus BX40) magnification 360x in the environment of alternating electric field (20-30 V; 0.1 m A; 1-2 Hz) generated by Biotest apparatus. In each group, we estimated the number of cells with mobile nuclei per one hundred cells observed (EMN-index).

Statistical analysis. The data were analyzed using Statistica 7 (StatSoft, Cracow, Poland). All results were checked for normality and expressed as mean ±SD in case of normal distribution or median in case of other-than-normal distribution. For variable with normal distribution student t-test was used to test the differences between the groups, in other cases U Mann-Whitney test was used. Statistical significance was achieved if $p \leq 0.05$.

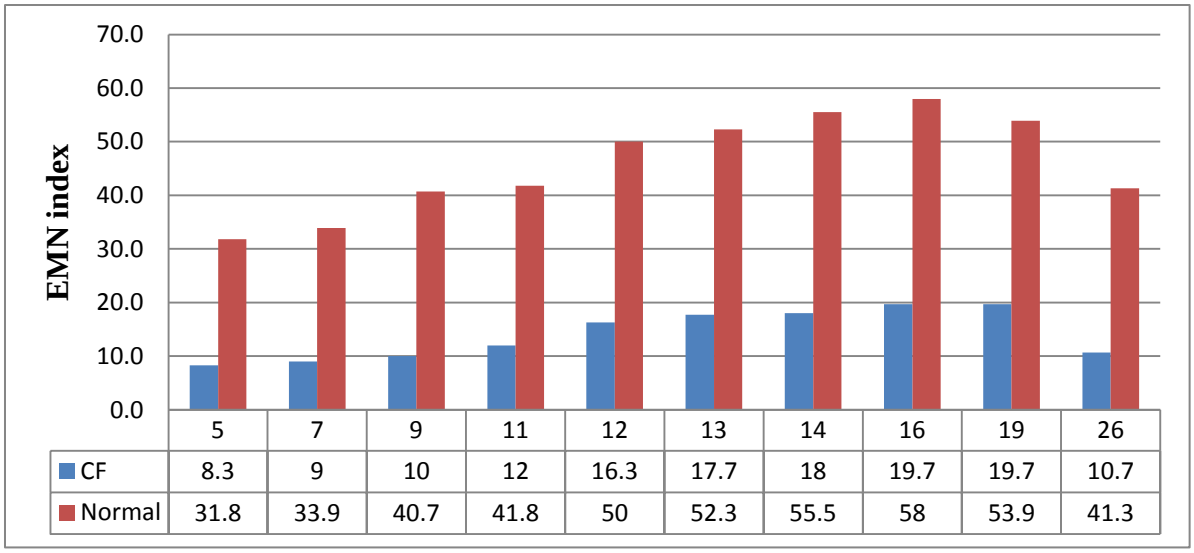
Ethical issues. In accordance with the Helsinki 2nd Declaration, each patient was informed verbally and in writing for the purpose of the study and methods used. All patients gave their written consent. The study was approved by the Bioethics Committee of medical University of Bialystok, Poland.

RESULTS

The results of EMN index assessment are presented in Fig. 1. In the healthy subjects, we have observed that the lowest EMN index values were reach around two year of life (7.6 ± 3.1) and in the late senescence (8.5 ± 2.5) with the peak values around 16-21 years (61.4 ± 2.5) [6]. In the CF patients, the EMN index as low as 8.3 ± 3.51 was observed in the five year of life with the highest values of 19.7 ± 1.37 around 16-19 year of life. In each age sub-group, the CF EMN index results differed significantly from the normal values.

DISCUSSION

Human biological age does not always correspond to the chronological age. Patients with cystic fibrosis (CF) are plagued by recurrent infections and neutrophilic inflammation in their airways that begin at an early age and result in a decline in lung function. There are very high concentrations (μM) of neutrophil elastase (NE), a serine protease, in the airways of patients with CF. NE, in concert with increased levels of chemokines present in the bronchoalveolar lavage fluid of patients with CF, can lead to airway epithelial injury [7]. In CF patients, neutophil elastase (NE) treatment triggers cell cycle arrest. Cell cycle arrest can lead to senescence, a complete loss of replicative capacity. Importantly, senescent cells can be pro-inflammatory and would perpetuate CF chronic inflammation [5].



	5	7	9	11	12	13	14	16	19	26
CF	8.3	9	10	12	16.3	17.7	18	19.7	19.7	10.7
Normal	31.8	33.9	40.7	41.8	50	52.3	55.5	58	53.9	41.3

Figure 1. Dependency of EMN index form age in CF cells (blue bars) and healthy controls (red bars). The results in each age sub-group reach the statistical significance ($p<0.05$).

The senescence theory in chronic inflammatory lung diseases like emphysema can link the pathological features of chronic inflammation with the molecular mechanism [8]. In EMN method, electric field exposure induced an increase in electrophoretic mobility of cell nuclei (EMN index), decreased numbers of heterochromatin granules near the inner membrane of cell nucleus, and induced cell membrane damage; but cell viability was conserved. Nuclear and cell membrane properties varied with electric field strength and age of the donors. The data obtained are interpreted as evidence of electric field-induced activation of the functional state of nuclei [9]. Observed distorted nuclei mobility is mostly dependent on chromatin distribution. Chromatin conformation changes mediated by covalent histone modifications and ATP-dependent chromatin remodelers are essential for the efficient repair of DNA double-strand break (DSB), which is highly conserved across species. The chromatin remodeling and DSB repair pathways are critical for physiological V(D)J recombination, CSR, meiosis, etc. Defects in these processes are associated with pathological changes during aging [10].

CONCLUSIONS

EMN index in CF buccal cells may be a simple method to quickly assess the chromatin remodeling. Further studies should be conducted in this field to confirm these observations.

Conflicts of interest: none declared.

REFERENCES

1. Sands D, Nowakowska A, Piotrowski R, Zybert K, Milanowski A. Postępowanie diagnostyczne w mukowiscydozie. Przegl Pediatr. 2003 Jul-Sep; 33(3):198-203.
2. Ball SD, Kertesz D, Moyer-Mileur LJ. Dietary supplement use is prevalent among children with a chronic illness. J Am Diet Assoc. 2005 Jan; 105(1):78-84.
3. Barra E, Socha J, Teissyere M, Teisseyre M, Celińska-Cedro D, Stolarczyk A, Pertkiewicz J, Kowalska M, Skoczeń M, Wawer Z. Zaburzenia trawienia tłuszczów u dzieci z mukowiscydozą z przewlekłym zapaleniem trzustki. Ped Współcz. 1999 Jan; 1(2-3):165-7.
4. Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC. Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc Natl Acad Sci U S A. 1996 Nov 26; 93(24):13742-7.
5. Fischer BM, Wong JK, Degan S, Kummarapurugu AB, Zheng S, Haridass P, Voynow JA. Increased expression of senescence markers in cystic fibrosis airways. Am J Physiol Lung Cell Mol Physiol. 2013 Mar 15; 304(6):L394-400.
6. Litwiejko-Pietryńczyk E. Variability of electrophoretical mobility of nuclei in human ontogenesis. Rocz Akad Med Bial. 2001 Jan; 46:225-230.
7. Brennan S, Sly PD, Gangell CL, Sturges N, Winfield K, Wikstrom M, Gard S, Upham JW; AREST CF. Alveolar macrophages and CC

chemokines are increased in children with cystic fibrosis. Eur Respir J. 2009 Sep; 34(3):655-61.
8. Aoshiba K, Nagai A. Senescence hypothesis for the pathogenetic mechanism of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2009 Dec 1; 6(7):596-601.
9. Shckorbatov YG, Shakhbazov VG, Rudenko AO. Modification of electrokinetic properties of nuclei in human buccal epithelial cells by electric fields. Bioelectromagnetics. 2001 Feb; 22(2):106-11.
10. Liu B, Yip RKh, Zhou Z. Chromatin remodeling, DNA damage repair and aging. Curr Genomics. 2012 Nov; 13(7):533-47.