Cohort Clinical and Microbiological Study of Young Patients Infected with Seasonal Influenza Subtypes A/H3N2 (Victoria, Pert strains) and B Viruses in Ukraine: Pathophysiology Reaction of Large Intestinal Cavity Microbiota

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Abstract

Introduction: The ecologic intestinal system fulfills a myriad of functions. There is continuous interaction and communication between the flora and the gut-associated lymphatic tissue determining many aspects of host immunity and metabolism. The digestive tract's immune system is often referred to as gut-associated lymphoid tissue and works to protect the body from invasion. However, it is well-established that influenza virus much more easier attached to mucous membranes of upper respiratory tract in condition of decreased functions of local immunity, which probably accompanies with mucosa-associated lymphoid tissue.

Objectives: This article discusses about pathophysiology reaction of large intestinal cavity microbiota by the means of determination its species composition and populational level with evaluation of dysbiotic abnormalities level in young Caucasian patients infected with influenza A and influenza B in Ukraine.

Study design: Cohort variant of observational study enrolled 109 young Caucasian race persons were born since 1986 till 1995 in western Ukraine (Eastern Europe, Chernivtsi region, traditionally called Bukovina) which were infected with influenza A and B during 2011-2012 autumn-winter-spring seasons. Fresh samples of stool, vein blood, and nasopharyngeal swabs were investigated by means of serological reactions, PCR, bacteriological and mycological methods. Statistical analysis performed due to “Biostat” PC programme with evaluation of average error and P-value with purpose to appreciate confident valuable changes between two investigated groups.

Findings: In 51 (46.8%) patients A/H3N2/Pert/16/2009 virus was diagnosed, in 44 (40.4%) patients – A/H3N2/Victoria/361/2011 subtype and in 14 (12.8%) young patients causative agent
of influenza had determined virus B/Visconsin/1/2010 by molecular biologic assay – PCR (polymerase chain reaction).

Alterations in influenza A and influenza B infected patients the intestinal micoflora pronounced and included increased bacterial density and a higher incidence of opportunistic flora accompanied by decreased normal microflora activity. The level *Bifidobacterium* declined to $5.12\pm0.08$ vs $9.17\pm0.19$ lg CFU/g, *Lactobacillus* – to $6.14\pm0.08$ vs $8.51\pm0.24$ lg CFU/g respectively. Meanwhile the complete elimination of indigenous *Enterococci* simultaneously with appearance of *haemolytic enterococci* strains in high level – $8.66\pm0.06$ lg CFU/g as well as *peptostreptococci* ($8.90\pm0.08$ lg CFU/g) noticed.

The values of *Candida* fungi increased in five times, anaerobic *Clostridium* – twice, *Staphylococcus* – 1.8 times, *coli bacilli* in 1.2 times respectively.

**Conclusion:** Young Caucasian patients were borne in period of 1986-1995 and infected with A/H3N2/Pert/16/2009, A/H3N2/Victoria/361/2011 and B/Visconsin/1/2010 in 2011-2012 cold seasons had manifested typically moderate and mild clinical form of influenza accompanied with dysbiotic alterations of large intestinal microbiota. Since first days of influenza onset the clinical course in patients presented objective and laboratory data of that, logically explained as the pathophysiologic reaction by means of interaction between virus-induced immune dysfunction and mucosa-associated lymphoid tissue. Degree of alteration in microbiota of large intestine cavity depends on the type of virus: III degree in 76.5% persons infected A/H3N2/Pert/16/2009 in comparison with 54.5% persons infected with A/H3N2/Victoria/361/2011. II degree of abnormalities was determined in 17.6% patients (Pert strain) vs 34.1% patients (Victoria strain). Minimal intestinal abnormalities observed in 5.9% patients with A/H3N2/Pert/16/2009 influenza, in 11.4% – A/H3N2/Victoria/361/2011 and in absolutely all infected with B/Visconsin/1/2010 influenza virus.

**Key words:** influenza A, influenza B, dysbiosis, young patients

**Background**

Influenza virus has the ability to evade host immune surveillance through rapid viral genetic drift and re-assortment; therefore, it remains a continuous public health threat.\(^1\) Epidemic season at Ukraine 2011-2012 was characterized by moderate intensity; totally, 12.3% of population got infected with acute respiratory viral diseases, including influenza. During the last years through the world the viruses A/H1N1, two strains of A/H3N2 (Pert & Victoria), were wide spreading, including Ukraine.\(^2\) This infectious disease often complicated with different pathologic states (respiratory distress syndrome, pulmonary edema, hemorrhages, bacterial pneumonia, etc), a death cases are not rare.\(^3,4\) Clinical course of influenza in each individual patient depend on the state of systemic immunity. There is growing evidence supporting an important role for human gut bacteria in mucosal immunity;\(^5\) interactions at the level of both intestinal and colonic epithelial cells, dendrite cells, and T & B immune cells have been documented.\(^6,7\) Recent explorations of the human gut microbiota suggest that perturbations of microbial communities may increase predisposition to different diseases.\(^8,9,10\) Microbiota – is the totality of microbes, presenting a certain biotope and environmental interactions in a particular environment.\(^11\) Some consider it a "newly-discovered organ" since its existence was not generally recognized until the late 1990's and it is understood to potentially have overwhelming impact on human health.\(^12\)
Hypotheses

Increasing evidence indicates that the complex microbial ecosystem of the human intestine plays a critical role in protecting the host against disease.\textsuperscript{13,14} Influenza accompanied with general virusemia, endotoxicosis that lead to immune dysfunction, firstly to non-specific anti-infectious immune system abnormalities.\textsuperscript{15} Pathophysiologic reaction of large intestinal microbiota in young Caucasian patients infected with seasonal influenza viruses Victoria and Pert strains and influenza B virus is not determined yet, that inspired us to establish the main quantitative and qualitative characteristics of large intestine microflora.

Aim of research

To establish the state of microbiota of large intestine cavity in Caucasian patients infected with influenza A (Victoria and Pert strains) and influenza B viruses.

Method

Cohort clinical prospective study of 109 patients aged 18-25 had conducted in 2011-2012 (average 21.5 years old) infected mostly with seasonal influenza viruses. Gender allocation included 62 (56.9 \%) females and 47 (43.1 \%) – males. Investigated persons were belonging to Caucasian race. All enrolled persons having the same high risk to get influenza virus because of student activity based on the epidemiologic data being upon same exposure during communication and overcrowding. One hundred and nine young patients with clinical features and laboratory findings (acute onset with hyperthermia more 38.5°C, scleritis, intoxication syndrome, etc., positive epidemiologic data) were investigated during October-March 2011-2012 at the Dept. of Respiratory Infections in the Regional Clinical Hospital, Chernivtsi (South Western region of Ukraine, Eastern Europe).

Exclusion criteria: recent treatment with antibiotics, antimicrobial medications, and anti-inflammatory drugs because of their possible impact onto the intestinal microbiota.\textsuperscript{16}

Research material (fresh stool samples) had delivered to Microbiological Clinical Laboratory of Regional Clinical Hospital (Chernivtsi, Ukraine) with purpose to evaluate a species composition and populational level of large intestinal cavity microflora. Material probes had weighed on sterile wax paper; put it into the sterile porcelain mortar; add isotonic solution in the tenfold volume, carefully grind to get of homogenous mass in dilution \(10^{-1}\). From the homogenate the row of tenfold serial dilution on the base of isotonic solution from \(10^{-2}\) to \(10^{-10}\) have been prepared. Every time it was used a new sterile pipette. From each tube row by sterile micropipette had taken 0.1 ml of solution and applied it to the corresponding solid nutrient medium optimal for each kind of microbe, where by means of sterile glass spatula was seeding the "lawn" on Petri dish sectors.

Cultures of facultative anaerobic and aerobic bacteria had cultured in an incubator (37° C) for 24-48 hours. Obligate anaerobic bacteria had grown in the stationary anaerostat "CO2-Incubator
T-125" during 5-7 days, sometimes up to 14 days. Then received single-type colonies had studied for each genus of the microbes, from the colonies there had obtained pure cultures of obligate and facultative anaerobic and aerobic microorganisms. Pure culture identified by genus (species) by morphological, tinctorial, cultural and biochemical properties. The identification of isolated microorganisms was done by Bergey’s Manual of Systematic Bacteriology.\(^{17}\)

Mathematic, statistical analysis of the results was performed by the method of variation statistics with the definition of average value, average error, and probability of possible error by statistical Student’s t-test by means of Biostat\(^\circledR\) PC program (USA).

**Results**

In 51 (46.8%) patients A/H3N2/Pert/16/2009 virus was diagnosed, in 44 (40.4%) patients – A/H3N2/Victoria/361/2011 subtype and in 14 (12.8%) young patients causative agent of influenza had determined virus B/Visconsin/1/2010 by molecular biologic assay – PCR (polymerase chain reaction).

Influenza caused by A/H3N2/Pert/16/2009 and A/H3N2/Victoria/361/2011 in investigated young patients characterized mostly by a moderate severity course. Influenza caused by B type virus had mild severity course. Of the various niches under investigation, the human gut houses the most complex and abundant microbial community and is an arena for important host-microbial interactions that have both local and systemic impact.

Constantly from the cavity of large intestine of practically healthy volunteers, it was isolated *bifidobacteria, lactobacteria, bacteroides, E.coli, enterococci, staphylococci, Proteus and peptococci* (totally 10 taxonomic groups).

Considered the variety of species composition presence of pathogenic *Escherichia* (enterotoxigenic and enteropathogenic strains – *E.coli Hly*\(^+\), *O55:K59*; opportunistic pathogenic peptostreptococci, bacteria of *Proteus, Citrobacter, Enterobacter, Hafnia, Serratia* genus, *haemolytical enterococci*, *staphylococci*, yeast-like fungi of *Candida* genus) had determined, that totally represented 19 taxonomic groups.

Thereafter, all young infected patients’ noticed the expressed fatigue, episodes of diarrhea, transient abdominal discomfort. The detailed microbiological analysis proved the elimination of dominant physiologic important microorganisms of *Eubacteria, Bifidobacteria* genera (in few cases) and *Enterococci* microbes. The contamination of the large intestinal cavity by means of enterotoxigenic and enteropathogenic coli bacilli and opportunistic pathogenic *Enterobacteria*, that testified the possibility of further bacterial translocation to the peritoneum or other internal organs.

On the background of acute viral infectious disease with expressed intoxicative syndrome the cavity of large intestine became contaminated with different opportunistic microbes of next genera persisted in great quantity. *E.coli Hly*\(^+\) – 8.75±0.02 lg CFU/g, *E.coli O55:K59 strain* – 8.68±0.03 lg CFU/g, *Citrobacter* and *Hafnia* – 8.73±0.03 lg CFU/g, *Enterobacter* and *Serratia* – 8.68±0.03 lg CFU/g respectively, that proved the high sensitivity of microbiota system to the exogenous infectious agents. It believed that intimate interactions between gut stable microbiota
and immune homeostasis could exist and being imbalanced under influenza viruses caused immune dysfunction.

The well known is the fact of evaluation of microbiota disorders based upon the populational level of indigenous autochthonous and allochthonous microbiota representers. Thus, in large intestinal cavity in infected patients with influenza A and influenza B was contaminated with high populational level of *Enterococcus haemolyticus* 8.66±0.06 lg CFU/g in 14 (12.8 %) cases, yeast-like fungi of *Candida* genus 5.7±0.05 lg CFU/g in 19 (17.43%) investigated patients.

**Discussion**

Probably virusemia with dissemination of agents, in pathogenesis of influenza infection consequently lead to circulatory disorders, fever, amplification of fibrinolysis with hemorrhagic effects, neurotoxin actions of viral proteins and metabolites, immune suppression and allergy had provided the feedback reflected in imbalance of gut microbiota.

These interactions influence gut barrier and defense mechanisms that include antimicrobial peptide and secretory IgA synthesis. The functional effects of commensal bacteria on CD4+ cells differentiation have led to the emerging concept that microbiota composition determines CD8+ cell balance, immune responsiveness, and homeostasis. The last is assumed to be the important reason in the development of secondary bacterial post-influenza complications (pneumonia, sinusitis, otitis, meningitis by means of translocation or proper activation of opportunistic microbiota), which should be kept on the alert. According to new paradigm in medicine, therapeutic approach should include probiotics, recently this scientific direction is widely discovered. It could be suggested to study the application of probiotics based upon indigenous microbiome in patients with influenza (pandemic, seasonal, epidemic strains of viruses).

**Conclusion**

In most (95.3%) young Caucasian patients with influenza A/H3N2/Victoria/361/2011, A/H3N2/Pert/16/2009 and B/Visconsin/1/2010 the abnormal correlation between dominant and opportunistic microbiota parts became evidenced with the next structure: mild alterations – in 17 (15.5±1.5%) persons, moderate – in 24 (22.0±2.0%) cases, deep severe – in 63 (57.8±4.7%) infected patients.

More expressed alterations recognized in 76.5% patients infected with A/H3N2/Pert/16/2009 subtype of influenza virus. Further research will be dedicated to determination of correlation between degree of immune dysfunction and the level of intestinal microbiota abnormalities. Finally the possibility of correction by means of bacterial preparations contained obligate gut microflora could be recognized.

**Conflict of Interest:** None.
References

1. Pandemic influenza preparedness framework for the sharing of influenza viruses and access to vaccines and other benefits. WHO Library Cataloguing-in-Publication Data, 2011.