ELECTROMAGNETIC FIELDS OF VERY LOW-FREQUENCY BAND EFFECT ON MICROBIAL GROWTH AND VIRUSES REPLICATION

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Electromagnetic fields (EMF) effects on the living organisms today takes very important place among the studied problems in biology, medicine and allied subjects.

The life on the Earth was arising against a background of complicated electromagnetic radiation (EMR). In the process of evolution the living organisms were strongly influenced by electric, magnetic and electromagnetic fields. Much information was obtained about geomagnetic fields (GMF) effect on microorganisms [1–4]. Metabolism and microbial cells growth activated under the effect of constant magnetic field [5, 6].

At the same time, in spite of multiple researches in different countries, basic experiments about the effect of electromagnetic field on the biological objects are conducted in the next ranges: constant and low-frequency fields (up to meter range), very high frequency (VHF) (meter, decimeter and centimeter waves), extremely high — EHF (millimetric waves), and also submillimetric waves.

There is a lot of researches of influencing EMR of millimetric range of low (non-thermal) intensity (EHF-radiation) on microorganisms are known now [7–9]. The electromagnetic fields of all frequency ranges influence the living organisms to a varying extent. There is described the following effect on some physiological processes and properties of microorganisms: cell division, morphological signs, speed of growth, biomass yield, enzymatic activity etc. [7–13]. Some researchers revealed a high sensitivity of different microorganisms to weak fields [13, 14], but reliable results are absent.

The electromagnetic signal has an effect on the biological objects. Biochemical processes can both accelerate and slow down at different frequencies, which influences the growth of microbial colonies. At definite frequencies their growth is fully inhibited, up to death of colonies, in other cases – it is activated. This process is hardly managed because of a number of factors (signal frequency, temperature condition, microbial growth phase, etc.) [7–9, 12].

Local and global distribution of polyantibiotic resistant agents of nosocomial and opportunistic infections, including Methicillin-Resistant *S. aureus* (MRSA) and coagulase negative Staphylococci (MR-CNS), is a serious problem of modern medicine [15]. In this connection it is actual that new potential targets of Staphylococcus cells can be found for the antimicrobial therapy [12, 15].

The search for new strategic approaches in order to effect on microorganisms is actual and expedient.

Most researches were directed on studying nonthermal (informative) effect of EHF on the biological objects. A signal of a definite frequency was used in such situation. It is established that influence of electromagnetic fields of less power had an informative character of action [10].

In public media there were no researches on effect of own spectra of electromagnetic fields in the range of very low frequency band on microbes and viruses. The researches like that became possible with appearance of new class of devices allowing to registrate and reproduce weak electromagnetic fields of extremely low power in very low frequency band. The hardware and software spectral correction complex “SCS-BARS” is one of such devices. It differs from other ones, in accordance with the concepts of Federal Service of Russia for the Oversight of Public Health and Social Development according to results of Federal State Institution “VNIIIMT” examination No 14/E-10-031/2-005 of December, 10, 2010 year, in that registration of electromagnetic vibrations in the device is carried out in low frequency band (less than 30 kHz). This property sets it apart from many other known devices, which register in EHF range (30–80 GHz) [15, 16].

Examination of particularly nonthermal (informative) action of very low frequency waves on the biological objects – infectious agents of both microbial and viral nature with the purpose of their replication inhibiting is of some interest.

Therefore the aim of this research was studying influence of own spectra of electromagnetic fields of extremely low power in the very low frequency band on microbial growth and viruses replication.

We set the following tasks:

1. To examine the effect of different electromagnetic spectra (S-markers of database of "SCS-BARS" program and microorganisms autospectrum) under different modes of action on *Staphylococcus aureus ATSS 25923, Staphylococcus aureus 2781, Escherichia coli ATSS 25922* strains.

2. To examine possibility of extremely low power electromagnetic fields effect on influenza A/Hong Kong/1/68(H3N2) virus replication *in vitro*.

3. To examine possibility of influence on hepatitis C virus replication in human by recorded electromagnetic field of virus.

Material and methods

Objects of research are *Staphylococcus aureus ATSS 25923, Staphylococcus aureus 2781, Escherichia coli ATSS 25922* strains, influenza A/Hong Kong/1/68 (H3N2) virus and patients with hepatitis C virus.

We studied effect on the objects with electromagnetic fields of extremely low power in the range of frequencies from 20 Hz about 20 KHz, that corresponds to extremely low (ELF), super low (SLF), infra-low (ILF) and very low (VLF) ranges of very low frequency waves.

Electromagnetic signal of extremely low power of examined object in the frequency range from 20 Hz about 20 kHz was recorded by the complex of spectral correction "SCS-BARS". Then with the help of the original mathematic device it was analyzed and transformed into an electronic S-marker, containing informative description of the examined object [15, 16]

Companding of electromagnetic signals in the hardware and software complex of spectral correction "SCS-BARS" was carried out as follows: at first the signal is pro-

cessed on the nonlinear element of the analog scheme with the logarithmic characteristics, and then is delivered to a 8 bit analog-to-digital converter (ADC); ADC is a digitized signal, it has logarithmically located levels of quantization; the signal is digitized by ADC, and then is processed by algorithm, for example, giving data through the table of correspondence of every level of signal after ADC and 8 bit code, determining the logarithm function. For solving a similar task in device "SCS-BARS" there is used signals wavelet-transform, which is spectral analysis generalization, a typical representative of such transformation is the classic Fourier transformation. With S-marker reproduction, a digital signal was transformed into the analog one.

For conducting the experiment a daily culture of microbes was carried out. As an object for influence there were used dilution of microbial cultures in the final concentration 10^9 microbial cells/ml on the Mueller-Hinton agar in volume of 25–30 ml.

With the device "SCS-BARS" there were conducted different types of influence depending on the purpose of experiment. Experiments with influence on the microbial culture in the liquid bouillon by the spectra of the S-markers data-base program were conducted on strains *Staphylococcus aureus ATSS 25923 "SCS-BARS"*, recorded before, and objects autospectra, recorded right before the effect. A single influence by S-markers spectra was conducted during 30 min, autospectrum in the inversed mode during 5, 15 and 30 min. *Staphylococcus aureus 2781* strains were influenced by autospectrum in the inversed mode during 5 and 15 min, *Escherichia coli ATSS 25922* strain — during 15 min.

Then the samples were placed in 5 test tubes, the initial optical density of microbial suspension (by 3 measuring) was measured and test tubes were placed in thermostat. Control samples were not processed by signal. After incubation of control and experimental samples during 24, 48 or 72 hrs at 37 ° there were determined presence and growth of microorganisms by the change of optical density, which registered by the Densi-la-Meter device. The device works on principle of measuring a change of intensity of the light flux, passing through bacterial suspension solution. The measured data are interpreted in turbidity units by McFarland. The device allows to measure turbidity of solutions in the wide range (from 0.0 up to 15) by McFarland.

For the studying influence of electromagnetic fields of extremely low power on influenza A/Hong Kong/1/68(H3N2) virus replication *in vitro* there were used the generally accepted methods of influenza virus accumulation on the infective embryo and determination of antiviral activity for influenza viruses on the chorionallantoic membrane (ChAM) tissue culture [17, 18].

For accumulation of virus there were used 9-11-day embryos infected in volume of 0.2 ml in amniotic and allantoic cavity. After that the embryos were incubated in a thermostat at 37 °C for 48 hrs. Allantoic fluid was sterile separated, the presence of virus in the hemagglutination inhibition test (HAIT) was determined, using 1% suspension of chicken red blood cells. Virus identification was conducted with hemagglutination inhibition test with the foregone antibodies. The samples of allantoic virus fluid was aliquoted, frozen and kept at -20 °C. Before the test the fluid was unfrozen and diluted up to 10^{-3} in the glucose-gelatin medium. With the device "SCS-BARS" they influenced with the autospectral fields of object, recorded right before influence on virus replication in the inversed mode during 30 min. Then the material was titrated from 10^{-3} to 10^{-9} and by tenfold dilution ChAM fragments were infected, located in the polystyrene panels. After thermostating at 37 °C the presence of virus was determined by HAIT results in 8, 24 and 48 hrs. The control is carried out similar to the test without treatment by the hardware-software complex «SCS-BARS".

The 50% tissue invective dose calculation — TID_{50} was made by Kerber–Ashmarin formula

Quantitation of hepatitis C virus was conducted by the method of the polymerase chain reaction (PCR) "Real-Time" hepatitis virus RNA. RNA virus PCR fragments were selected and an original S-marker was created by the above-described method. The patient was effected ones a week in the inversed mode during 30 mines. In the process of exposure quantitation of hepatitis C virus was conducted with one month interval and after the end of exposure – in two months. Antiviral medical treatment was not conducted at the test period.

Statistical data were processed by the computer programs IBM SPSS Statistics 20 and Microsoft Excel 2007.

Results and their discussion

Effect of different electromagnetic spectra under different modes on *Staphylococcus aureus ATSS 25923, Staphylococcus aureus 2781, Escherichia coli ATSS 25922* strains.

The test results (table 1) revealed the same type reaction of microorganisms on the effects.

Table 1

Optical density of medium in experiment and control

Test No	Microbes	Method of influence	Duration of influence	Term after influence (hrs), optical density units		
				initial	24 hrs	48 hrs
1	***2***	***3***	***4***	***5***	***6***	***7***
1	*St. aureus ATSS 25923*	marker St. aureus ATSS 25923	control	0,44±0,01	4.76±0.04 *	6.53±0.05 *
			inv. 30 min	0.40±0.02	4.19±0.02 *	5.58±0.02 *
2	*St. aureus ATSS 25923*	autospectrum	control	0.44±0.01	4.05±0.05 *	5.35±0.12 *
			inv. 30 min	0.42±0.01	3.55±0.06 *	4.56±0.04 *
3	*St. aureus ATSS 25923*	autospectrum	control	0.42±0.01	4.31±0.03 *	4.53±0.06 *
			inv. 5 min	0.40±0.01	3.60±0.04 **	3.82±0.08 **
			inv. 15 min	0.42±0.01	3.45±0.04 **	3.66±0.07 **
1	***2***	***3***	***4***	***5***	***6***	***7***
4	*St. aureus 2781*	autospectrum	control	0.46±0.01	3.27±0.01 *	4.79±0.03 *
			inv. 15 min	0.50±0.02	2.93±0.02 *	4.41±0.06 *
5	*E. coli ATSS 25922*	autospectrum	control	0.62±0.01	2.87±0.03 *	3.22±0.05 ***
			inv. 15 min	0.64±0.01	2.68±0.02 *	3.06±0.03 ***

	* – reliable distinctions between the control and experiment p<0,001
	** – reliable distinctions between the experiment 5 and 15 min p<0,001
	*** – reliable distinctions between the control and experiment – p<0,05

The greatest distinctions in microbial growth were registered in 24 hrs after the exposure (fig. 1), and by the point of 48 hrs the difference in microbial number between the control and experiment decreased during 15 min.

The average indices of optical density of control samples of *Staphylococcus aureus ATSS 25923* and *Staphylococcus aureus 2781* in 24 and 48 hrs were lower than the control at average by 0.57±0.10 and 0.75±0.13 optical density units (ODU) by the McFarland scale correspondently. It corresponds to $4.1\pm0.1\times10^8$ colony-forming units, ml (CFU/ml) and $4.7\pm0.1\times10^8$ CFU/ml correspondently.

This difference was more pronounced at *Staphylococcus aureus ATSS 25923* in 48 hrs at exposure during 30 min irrespectively of exposure spectrum type (Fig. 1). The difference between the control and experiment averaged 0.87±0.12 EOP or $5.1\pm0.1\times10^8$ CFU/ml.

The Escherichia coli ATSS 25922 response to autospectral fields exposure during 15 min was similar to staphylococcal one, but less pronounced (fig. 1).

Average indices of optical density of control samples in 24 and 48 hrs were lower than the control at average by 0.19±0.10 and 0.16±0.10 ODU, that is $0.57\pm0.02\times10^8$ and $0.48\pm0.05\times10^8$ CFU/ml correspondently.

Reliable distinction was revealed in all the tests between the control and experiment (see table 1).

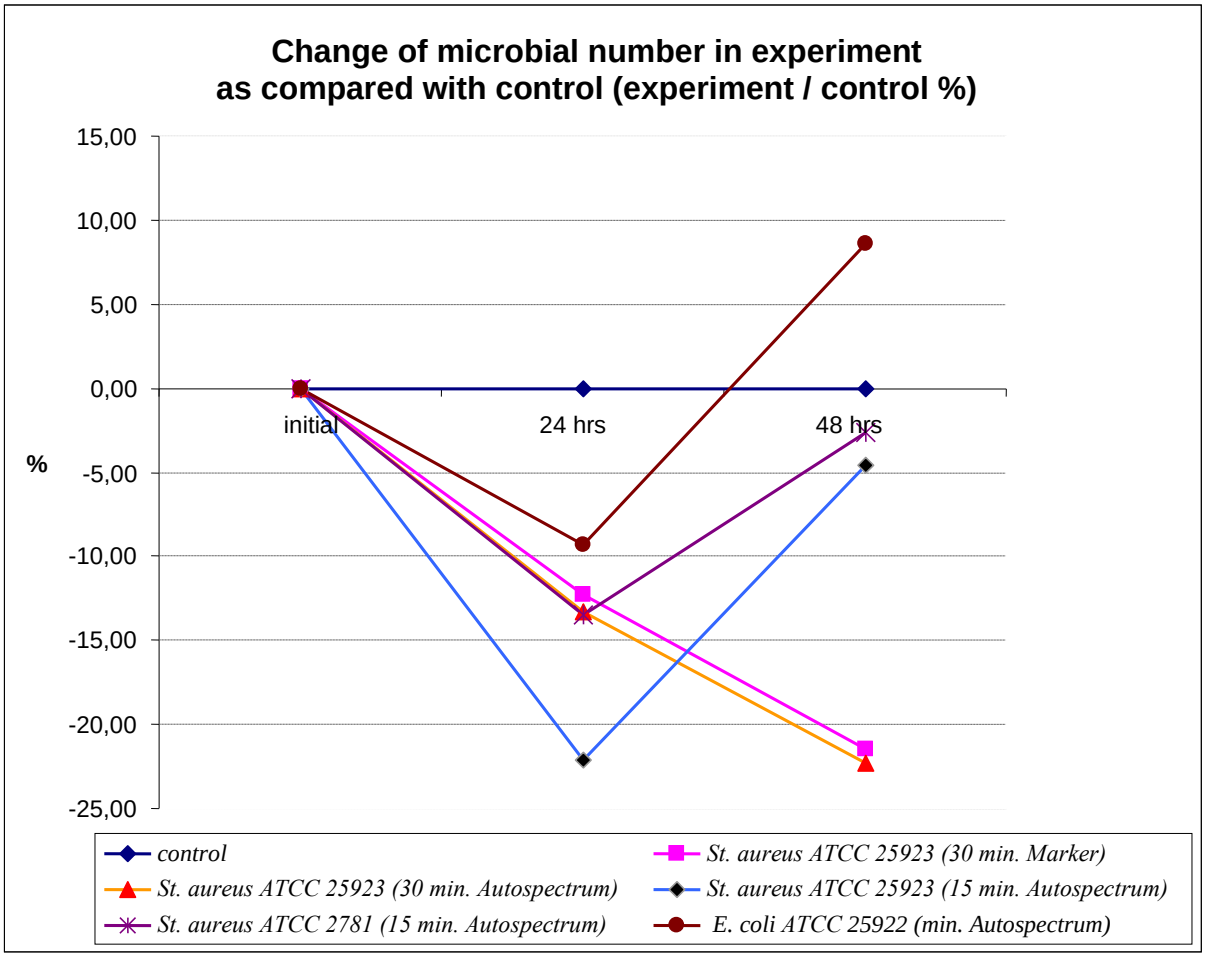

Fig. 1. Dynamics of microbial growth under experiment
as compared with the control

The research established that electromagnetic fields of extremely low power generated by "SCS-BARS" as autospectral and S-markers spectra of the similar object exert an impact of microbial growth at very low frequency band. The application of these fields spectra at inversed mode leads to inhibition of microbial growth, which depends on term of exposure.

Effect of electromagnetic fields of extremely low power on influenza A/Hong Kong/1/68 (H3N2) virus replication *in vitro*.

The exposure of the autospectral electromagnetic signal of extremely low power on the extracellular virus, which leads to decrease of A/Goncong/1/68 strain on the tissue culture ChAM by 0.25 lg TID_{50} in 8 and 48 hrs in comparison with the control unprocessed sample. The difference between control and experimental samples made out 1.0 lg TID_{50} in 24 hrs after the beginning of experiment, which corresponds to decrease factor of 10. So, the obtained results prove that a single exposure of the elec-

tromagnetic signal of extremely low power at very low frequency band by autospectrum of viral material results in inhibiting influenza A/Goncong/1/68 virus replication on the tissue culture ChAM.

So, one can expect the similar impact on the virus being in an organism.

Effects on hepatitis C virus replication in a human

Owing to reliable results obtained at the above-described experiments, researches on studying a possibility of effects on hepatitis C virus replication in a human organism have been initiated. The research included 6 patients with RNA HCV detected in blood plasma (at the moment of writing the article). The "viral load" was registered from 3.1×10^3 to 7.6×10^6 IU/ml. A general tendency of variability of the revealed RNA HCV in blood plasma is given in table 2 and figure 2.

Table 2

Dynamics of RNA HCV volume in plasma of blood (lg)

	initial	**1 months**	**2 month**	**3 month**
lg RNA HCV	5.80±0.52	6.25±0.41	5.26±0.94	4.48±1.26
p	<0.001	<0.005	<0.005	<0.05

An increase of RNA HCV PCR-fragments by 1.15×10^6 IU/ml was detected within the first month of conducting research, and then this index was falling down to the 3rd month up to 3.0×10^4 IU/ml with initial values 6.3×10^5 IU/ml or by 1.32 lg.

The HCV S-marker and RNA HCV volume change according to the spectral-correlation test data during the first two months was differently directed (fig. 3).

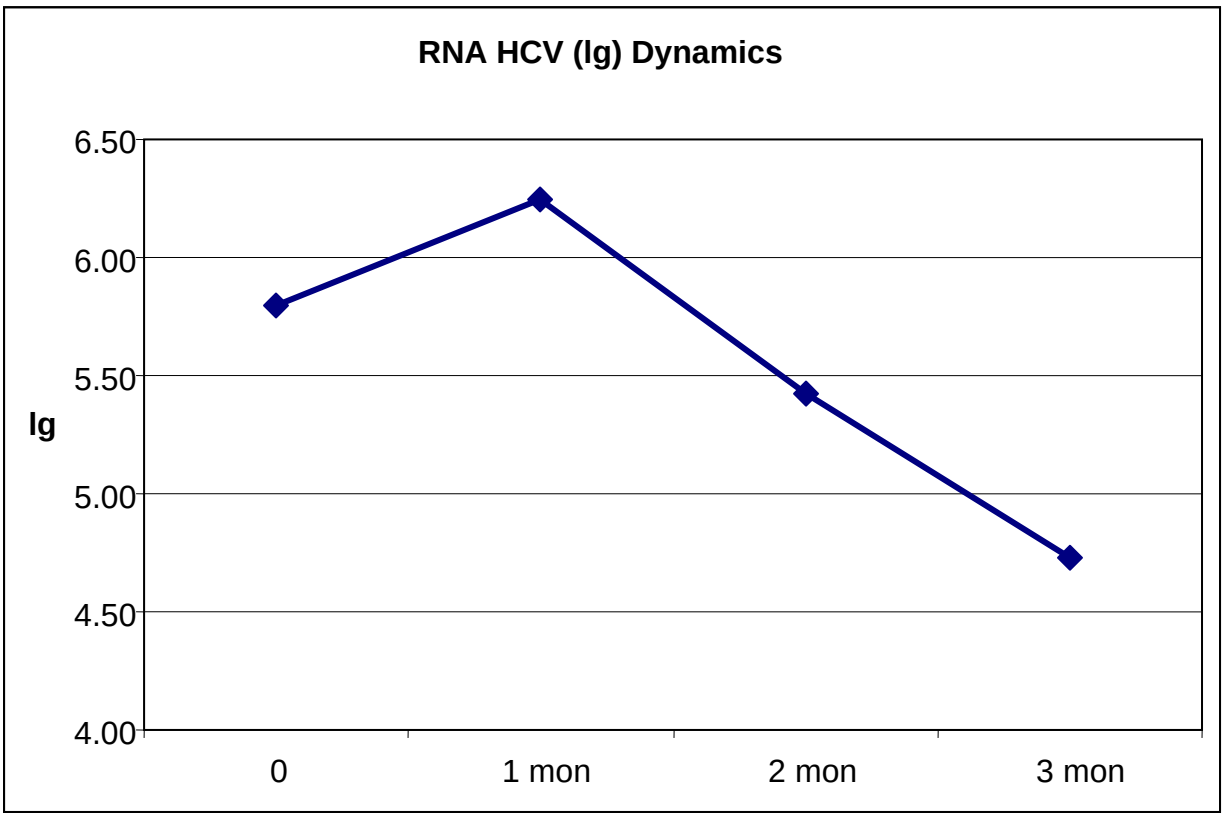

Fig 2. The RNA HCV volume detected in blood plasma
of RNA HCV patients during S-markers spectra exposure (lg)

Among 6 observed patients, 1 patient stopped participation in the experiment, because his analysis results did not reveal viral load in 2 and 4 months. At the beginning of supervision this index made up 3.1×10^3 IU/ml or 1.24×10^4 RNA copies/ml.

One can suppose that the influence on object at inversed mode (own field antiphase) recorded by spectral field characteristics of the same object results in destruction of informative field of object and DNA/RNA structure. It leads to failure of reproduction mechanisms.

Many authors, conducting researches on microorganisms under the range of EHF, registered influence on morphological signs, change of cell hydratation, enzymatic activity [7–13].

A rise of activity of bacteria antioxidant defense enzymes under the action of EHF EMR is associated with initiation of definite mechanisms of biochemical reactions under the action of resonance frequencies waves [20]. In opinion of these authors, exposure of EHF electromagnetic fields of extremely low power has an informative character of action.

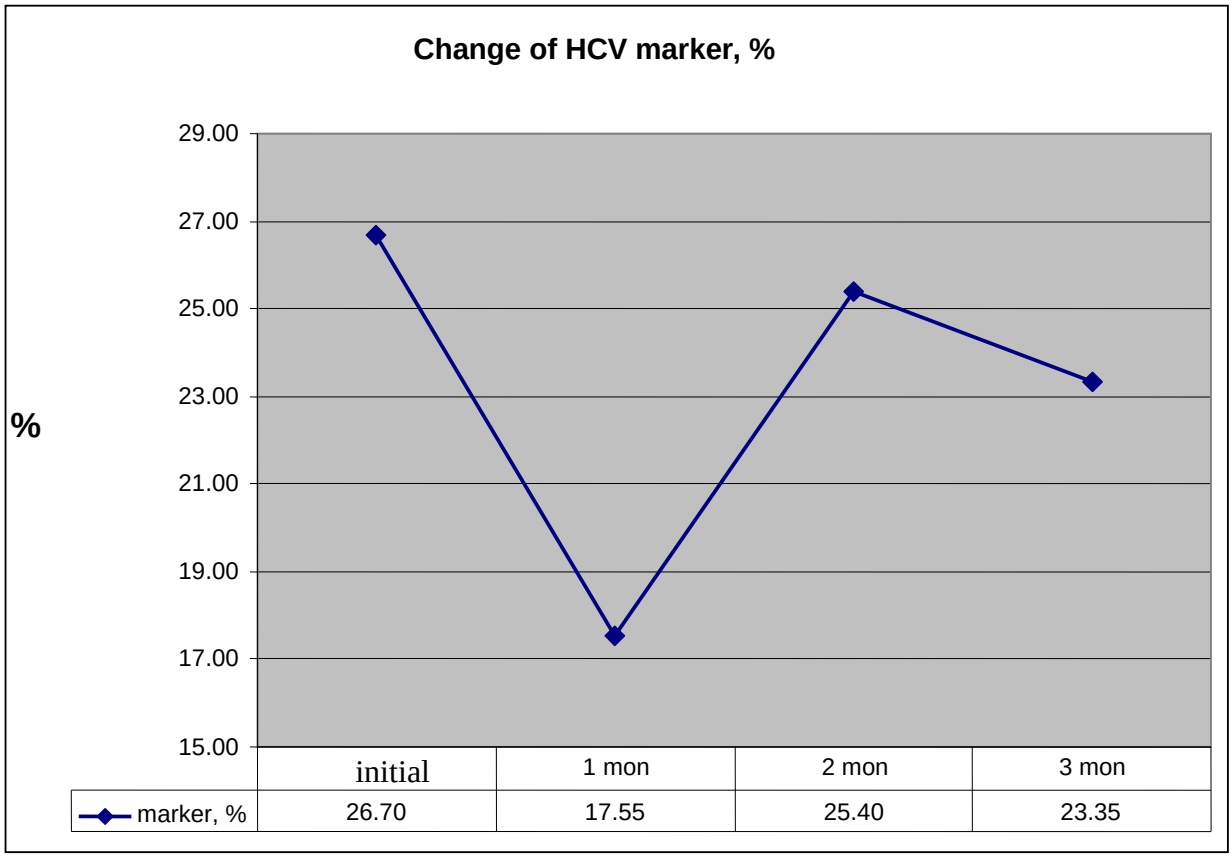

Fig. 3. The HCV S-markers change according to the data of spectral-correlation test

There are definite “frequency-amplitude windows” — inside of their range there is detected a bioobject reaction, and the similar response is absent outside. So, the most informative value has frequency of influence, and the amplitude determines only the organism response realization mechanism [21].

The bioeffective frequencies are revealed experimentally and explained by a possible resonance between the external EMF and own vibrations parameters [22].

DNA/RNA – like any polar molecules, are very sensitive to frequencies. Every DNA molecule has its resonance frequency. In final analysis, the more primitive a living substance, the lower its resonance frequency [23]. Every living cell has DNA molecules, so the cells of the same type have similar DNA with the identical resonance frequency. When the cells are affected by resonance frequency of a proper power, they disintegrate. For example *Paramecium caudatum* disintegration takes place under exposure of frequency 1150 Hz [23], at the same time some other authors deny this effect and similar ones considering them impossible [24].

Peculiarities of resonance impacts can be explained by the results of theoretical and experimental researches directed by P. P. Garyaev. According to authors' data, DNA destruction took place under exposure in laser optical radiation spectra. In opinion of authors, the fundamental principle of biological systems' code hierarchy is infrastructure of extracellular matrixes (ECM) of cytomembrane, cytosceleton and nucleus. Information exchange along the physical channels of nonlinear acoustic and electromagnetic vibrations takes place between them in the epigenetic mode [25, 26].

Obviously, the inversed mode of exposure in our experiments causes change of object's own field and information content of the object.

The obtained data demonstrate necessity of further researches in this sphere.

Besides, generators and acceptors of informative waves inside of bioobjects are different liquid-crystalline structures and intracellular water with an ability to form fractal structures [34]. Obviously, these structures break down under the action of definite frequencies, which results in microbial or viral death.

This conception can be a foundation for the alternative choice for antibacterial and antiviral therapy by nonpharmacological means.

Results

1. A single exposure of electromagnetic autospectral fields of extremely low power at very low frequency band on studied microbial strains and influenza virus inhibits their growth/replication *in vitro*.

2. Electromagnetic autospectral fields of extremely low power at very low frequency band affects virus replication in human.

3. The obtained results prove a necessity of conducting further researches both for studying mechanisms of electromagnetic fields of extremely low power at very low frequency band action on causative agents, and for development of new methods of infection therapy.

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