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Genetic Peculiarities of Blood Group Distribution in Infants Born to Mothers With 0(I) Rh(+) Blood Group in Bukovyna Region

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Abstract

The article presents the analysis of distribution of the group (according to AB0 system) and rhesus characteristics (according to anti-D system) in infants born to mothers with 0(I) Rh(+) blood group in Chernivtsi region (Ukraine) during the period of 2013-2014. The relationship of the umbilical bilirubin level and hemolytic disease of the newborn (HDN) (erythroblastosis fetalis), the necessity of its careful monitoring during the first day of life of a newborn, especially in case a baby belongs to a certain risk group, has been analyzed. The issue concerning probable HDN occurrence from mothers with A(II) Rh(+) blood group which is dominant in this region has been touched upon. The tasks for further work in this direction of ecopathophysiology in the areas of gene penetration have been assigned.

Keywords: hemolytic disease of the newborn (HDN), jaundice form, blood group system: AB0 and Rh-factor, bilirubin concentration, alloimmunization, gene penetration.

Introduction

«Alloimmunization with erythrocyte antigens is a global population problem» depending on many factors, and gene geographic region first of all [1]. According to the data presented by A.E.Skudnytskyi the index of alloimmunization of the population in every certain area is constant. At the same time, genogeographic junctions, the areas of gene penetration serve as a source of unusual antibodies increasing the index of alloimmunization of a certain region on the whole [1].

Pregnancy as one of the main ways of alloimmunization is the major natural sensitizing factor of the population. Realization of immune conflict in AB0 system in mothers with 0(I) Rh(+) blood group and possible occurrence of HDN is a consequence of this process, and jaundice form is its most frequent manifestation [2].

Group erythrocyte antigens are divided into three large categories: 30 systems, 6 collections, two series. All of them are coded by allele or adjacent genes and do not depend on the antigens from other systems. In AB0 system blood groups possess distinct area conformities. For example,

A(II) group has two maxima – in Europe and South-Eastern Asia [3, 4]. This is the most spread blood group in Ukraine dominating over 0(I) group.

AB0 incompatibility of mother and fetus have been found to affect the activity of antibodies. It is an interesting fact that antibodies anti-A and anti-B of Ig G class together with blood serum are present in the first portion of colostrum of practically all recently confined women, it is cause by their formation in 95,7-98,0 % cases. Presence of these antibodies is a physiological phenomenon which is not connected with incompatibility of mother and fetus by the antigens of Ig M class of AB0 system [5].

Recently a tendency to transplacental alloimmunization has been outlined when a reverse synthesis of Ig G antibodies into Ig M antibodies becomes possible, and vice versa, Ig M into Ig G in re-immunized individuals [2]. Transplacental transfer of the antibodies of producing cells is hardly a rare phenomenon – it can explain their high frequency (19,5%) among male carriers [1].

An important feature of the Ukrainian population is a vast anthropological and cultural-ethnographic diversity of local territorial-ethnic regions [6]. It is highly relevant to the region of Northern Bukovyna which together with sub-Carpathian and trans-Carpathian lands were isolated from the rest of Ukrainian territory for a long time. First, these regions were annexed to already formed part of Soviet Ukraine during the World War II [7]. Second, modern increasing migration flows of Caucasian race (peoples of Northern and Central Europe, Armenian and Alpine groups) as well as cross population flows from Eastern Ukraine and Western part of Russia characterize the region of Northern Bukovyna as a polymorphic population area of gene penetration.

In recent years a growing tendency of newborns with the signs of hyperbilirubinemia of hemolytic character by AB0 system is indicative of a probable change of the sensitizing index by this sign.

In previous works we have examined certain peculiarities of HDN depending on the blood group of a newborn, level of umbilical bilirubin, and maternal blood group characteristics [8, 9].

The results of distribution of blood groups in the infants born to mothers with 0(I) Rh(+) in 2013 induced an interest to analogical examination of distribution of these determinants in 2014 with their further comparative analysis.

Materials and methods

Examination of maternal and umbilical blood (examination of infantile blood was conducted in case blood transfusion became necessary) to detect blood group by AB0 and Rh-factor (anti-D) systems was conducted on the base of the laboratory of Anaesthetic Department with beds for intensive care units (ICU), Municipal Clinical Maternity Home № 1, the town of Chernivtsi, Ukraine.

Examinations to detect blood group and Rh-factor were performed according to the Order № 164, the Ministry of Public Health of Ukraine dated 05.07.1999 «Instruction to Detect Blood Groups by AB0 system and Rhesus» [10].

Belonging to blood group of patients was detected by agglutination reaction by means of reagents: standard erythrocytes and monoclonal antibodies (coliclones anti-A, anti-B, anti-AB).

Standard erythrocytes were prepared in the laboratory of Chernivtsi Regional Center of Blood Service according to the instruction on taking and registration of blood received from donors in small doses to prepare standard erythrocytes.

Monoclonal antibodies were used – erythrotest-coliclones produced in Russian Federation, Moscow, "Hematolog" certified on the territory of Ukraine. Rh-factor was detected by means of anti-Rh0(D) Ig M reagent of the same firm.

The series and expiry dates were indicated on all the reagents.

The level of general bilirubin and its fractions as one of the main biochemical prognostic criteria for the development of HDN was detected in the umbilical and infantile blood.

Detection of bilirubin and its fractions was performed by means of the unified technique with Endrashyk's method with the set of reagents produced by "Filicit-Diagnostica" (Dnepropetrovsk, Ukraine). Photoelectrocolorimeter KFK-2 was use to measure optic density of solutions.

The study was conducted during the period of 2013-2014.

Results and discussion

It is ABO immunization is an often cause of severe hyperbilirubinemia [11]. According to medical protocols of advising newborns born to mothers with O(I) blood group and negative Rh-factor, irrespective of blood group, umbilical blood should be examined to find group and Rhesus characteristics as well as the concentration of general bilirubin.

The results of comparative analysis of the distribution of group and Rhesus determinants in infants born to mothers with O(I) Rh(+) blood group during two years of the study are presented in Table 1. According to genetic laws the embryo inherits genetic information from both parents to the same extent. Analysis of the results showed maximally possible reflection of maternal group signs.

Table 1: Comparative distribution of blood characteristics in infants born to mothers with O(I) Rh(+) blood group by ABO system during 2013-2014 (on the base of Maternity Home № 1, Chernivtsi)

	O(I)		**A(II)**		**B(III)**	
	Rh(+)	**Rh(-)**	**Rh(+)**	**Rh(-)**	**Rh(+)**	**Rh(-)**
2013 (n=569)	55,89% (n=318)	4,92% (n=28)	24,78% (n=141)	3,34% (n=19)	8,96% (n=51)	2,11% (n=12)
2014 (n=553)	48,10% (n=266)	6,51% (n=36)	28,57% (n=158)	2,17% (n=12)	13,20% (n=73)	1,45% (n=8)

Note: n – number of individuals for every category

In nature crossing of organisms with different blood groups results in negative consequences and even death of such species. In human population sensitization on the ground of this incompatibility is solved with pharmacotherapy, the number of individuals with A(II), B(III) AB(IV) blood groups dominate over O(I). According to one of the theories initially there was only one O(I) blood group – the rest appeared in the result of expansion of residential areas and adaptation to new ecological conditions [4]. L.Segurel et al. [12] state that blood groups, A and B, are not the result of convergence, they are characterized by general origin among distant relative species and are preserved under the influence of factors of selective stabilizing selection during millions of years. ABO was the first human gene which displayed polymorphism, that is, this gene possesses not only usual dominant and recessive alleles, but two dominant alleles A and B, and the third allele – O – recessive [12].

In the majority of cases occurrence of HDN is natural in case of combination of the maternal phenotype O(I) with A(II) and B(III) groups of the fetus. On the other hand, dominant belonging of the majority of the population in Bukovyna region to A(II) blood group results in the process of continuous competition between O(I) and A(II) blood group of newborns. Both genes A and B are dominant concerning O, but they lose their dominance towards each other.

According to our study in spite of dominance of A and B genes, children born to mothers with O(I) Rh(+) blood group approximately in half of the cases inherited group and Rhesus characteristics identical to maternal ones which is the evidence of minimal risk of isoimmunization. This fact is indicative of a positive genetic mechanism of natural selection by these characteristics. In case only group belonging is considered the number of newborns with O(I) blood group was 60,8% and 54,61% in 2013 and 2014 respectively, which was reliably more than a half of the cases of distribution from general amount. The range difference in 6,19% was just the difference of competitive genes: A(II) and B(III) blood groups and dominant Rh(+), when the studies of two years were compared.

Why are we talking about HDN increase in recent years where the process of isoimmunization plays “first fiddle”?

Let us consider ranges of umbilical bilirubin concentration level as a primary index in HDN screening (Table 2).

Table 2: Comparative distribution of umbilical bilirubin concentration (micromole/L) in infants born to mothers with 0(I) Rh(+) blood group in 2013-2014. (on the base of the Maternity Home, Chernivtsi)

	0(I)		**A(II)**		**B(III)**	
	Rh(+)	**Rh(-)**	**Rh(+)**	**Rh(-)**	**Rh(+)**	**Rh(-)**
2013 (M±m)	34,0 ± 0,71 (n=318)	36,70±0,71 (n=28)	38,70±1,32 (n=141)	40,9 ± 2,30 (n=19)	39,8 ± 1,66 (n=51)	35,7 ± 2,17 (n=12)
2014 (M±m)	35,0 ± 0,48 (n=260)*	36,8 ± 1,46 (n=34)*	40,7 ± 0,99 (n=158)	38,1 ± 3,81 (n=12)	40,3 ± 1,50 (n=71)*	37,4 ± 3,19 (n=8)

Note: * – the number of bilirubin definition differs from the number of group definition due to hemolysis of the serum;
M – mean arithmetic value of bilirubin concentration and its standard error m=(σ√n).

The data presented in Table 2 show that levels of umbilical bilirubin in all the groups of newborns are in rather narrow ranges: 35-40 micromole/L. The use of Kraskel-Wallis criterion to conduct statistical analysis was indicative of a reliable influence of both group and Rhesus factors upon the value of umbilical bilirubin ($p<0,01$) in 2014: in comparison of all the six possible group and Rhesus combinations between themselves as well as in comparison with 0(I) A(II) B(III) blood groups irrespective of Rhesus distribution [13].

The use of another criterion – U-Wilkinson's criterion – to compare infants with 0(I) Rh(+) blood group with five other variants of possible group distributions concerning bilirubin level, showed reliable differences only for diametrically opposite groups: A(II) Rh(–) ($p<0,05$) and B(III) Rh(–) ($p <0,01$) [13].

As a rule, HDN development occurs during the first 12-24 hours of baby's life, the process is characterized by rather quick course: it is confirmed by average statistical levels of bilirubin in the umbilical blood in the moment of birth and pathological ones on the second-third days of life. There may be a number of mechanisms of development of such pathology, but hemolytic conflict remains a primary one and not only by AB0 system [14].

It is noteworthy that neonatologists notice inconsiderable yellowness of the skin, at the same time, the concentration values of general bilirubin on the second-third days were higher than 270,0 micromole/L, with normal average statistical value within the range of 32,0-36,0 micromole/L in the umbilical blood of nine infants with A(II) Rh(+) blood group in 2014 and five in 2013. General number of hyperbilirubinemia cases in this group of the study is presented in the Table 3.

According to literary data severe HDN is considered with general bilirubin concentration higher than 340 micromole/L during the first 28 days of life. The priority in reaching critical values belong to infants B(III) Rh(+) blood group: in 2013 two children showed the concentrations higher than this limit on the second-third days of their lives. In 2014 only one baby was with analogical group characteristics and critical level of bilirubin in the first days of life. These signs are characterized by inconsiderable frequency of features concerning the number of newborns in the region studied.

Table 3: The number of hyperbilirubinemia in infants born to mothers with 0(I) Rh(+) blood group by AB0 system during 2013-2014. (on the base of the Maternity Home, Chernivtsi)

	0(I)		A(II)		B(III)	
	Rh(+)	Rh(-)	Rh(+)	Rh(-)	Rh(+)	Rh(-)
2013 (n=569)	6	---	32	7	10	---
2014 (n=553)	7	2	43	3	13	4

Note: n – the number of individuals for every category

According to literary data general bilirubin level in the blood higher than 86,0 micromole/L is considered to be critical in the first 24 hours of life [14].

There were 34 such babies from the general group (2014), but real number of them is unknown as monitoring of bilirubin level with normal value of its concentration is conducted only on the second-fifth days in case HDN develops or any other pathological process against the ground of HDN. It is the first 24-36 hours of life when attention should be drawn to the monitoring of general bilirubin detection in babies with A(II) and B(III) blood groups against the ground of general anamnesis of the mother.

Cephalocaudal development of HDN should be considered here as the CNS is its target. Every fourth-fifth infant with A(II) blood group from general number of such babies is at risk of hyperbilirubinemia. On the other hand, critical concentrations of general bilirubin are purely individual characteristics: 340 micromole/L may be critical for some individuals, and for others – 250 micromole/L.

Why do we have the right to speak about blood group distribution in newborns of the given region?

The matter is that Municipal Clinical Maternity Home № 1 gives medical aid not only to urban residents but to the population of district centers as well: Hertsa, Zastavna, Kitsman, Novoselytsia, Sokyriany and Khotyn districts occupying a wide area of Northern Bukovyna (six out of eleven districts).

The data presented report on the two-year study during 2013-2014. Naturally, to get a wider characteristic of distributions the study should be conducted during five years. They will present more comprehensive manifestation of dominant distribution and variations of group and Rhesus characteristics in newborns of this group in a temporal factor.

Naturally a question arises concerning genetic distribution of the determinants studied in babies born to mothers with negative Rh-factor. In addition, a tendency of HDN development has been noticed in infants born to mothers with A(II) Rh(+) blood group (15 babies in 2014), whose umbilical blood was not taken to detect group and Rhesus characteristics and who were not examined additionally as they were in satisfactory condition at birth.

Conclusion

HDN occurrence in infants born to mothers with 0(I) Rh(+) blood group is of certain priority due to rather big number of the pregnant with the above characteristics.

In spite of inconsiderable variations of general bilirubin in umbilical blood between groups, the development of HDN has individual characteristics. Bilirubin metabolism, its synthesis, metabolic processes and excretion are under genetic control. For example, polymorphism in glutathione-S-transferase gene (GTS) can promote the increase of general serum bilirubin level.

The infants with A(II) blood group born to mothers with 0(I) Rh(+) blood group, are most often at risk of HDN development due to its prevailing distribution in Bukovyna region.

Future studies can develop in several directions and the following ones may have priority:

- genogeographic distribution of infants born to mothers with 0(I) blood group and negative Rh-factor irrespective of the blood group;

- markers of HDN development in the pregnant (compete typology of blood group with the use of up-to-date gel-type technologies, examination of acute phase proteins belonging to α_1-, α_2- and β-globulin fractions);

- circadian distribution of bilirubin level concerning time of the day and time of the infant's life;

- formation of HDN at the expense of immune and non-immune factors.

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Генетические особенности распределения групп крови у новорожденных от матерей С 0(I) Rh(+) в Буковинском регионе

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Аннотация. В статье проведен анализ распределения групповых (за системой АВ0) и резусных характеристик (за системой анти-D) у новорожденных от матерей с 0(I) Rh(+) группой крови в Черновицком регионе (Украина), на протяжении 2013–2014 годов. Проанализирована взаимосвязь уровня пуповинного билирубина и гемолитической болезни новорожденных (ГБН), необходимость тщательного его мониторинга в течение первых суток жизни новорожденного, особенно в случае принадлежности ребенка к определенным группам риска. Затронут вопрос о возможном проявлении ГБН от матерей со А(II) Rh(+) группой крови, доминантной в исследуемом регионе. Определены задачи для дальнейшей работы в данном направлении экопатофизиологии в зонах генопенетрации.

Ключевые слова: гемолитическая болезнь новорожденных (ГБН), желтушная форма, системы групп крови: АВ0 и Rh-фактор, концентрация билирубина, аллоиммунизация, генопенетрация.

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Evaluation of Possible Methods and Approaches for Registering of Non-Ionizing Radiation Emitted from the Human Body

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Abstract

This paper presents the results of evaluation of possible biophysical methods and approaches for registering of various non-ionizing radiation (NIR) wave types of the human body in the optic and electromagnetic range. Various types of NIR (electromagnetic waves, infrared radiation, thermo radiation, bioluminiscence) emitted from the human body were reviewed. In particular the results on the spontaneous biophoton emission and the delayed luminescence from the human body were submitted along with infrared thermography (IRT) results. It was shown that 1 cm^2 of skin generally emits ~85 photons for 1 s. The intensity of biophoton emission ranges from 10^{-19} to 10^{-16} W/cm^2 (approx. ~1–1000 photons·cm^{-2}·s^{-1}). The specific bioluminiscence emission from part of the human thumb was detected as a spectrum of various colours with the method of Colour coronal spectral analysis on a device with an electrode made of polyethylene terephthalate (PET hostaphan) with applied electric voltage 15 kV, electric impulse duration 10 μs, and electric current frequency 15 kHz. It was established that photons corresponding to a red color emission of visible

electromagnetic spectrum have energy at 1,82 eV. The orange color of visible electromagnetic spectrum has energy at 2,05 eV, yellow – 2,14 eV, blue-green (cyan) – 2,43 eV, blue – 2,64 eV, and violet – 3,03 eV. The reliable result measurement norm was at E ≥ 2,53 eV, while the spectral range of the emission was within λ = 380–495±5 nm and λ = 570–750±5 nm. Also were estimated some important physical characteristics (energy of hydrogen bonds, wetting angle, surface tension) of water by the methods of non-equilibrium energy (NES) and differential non-equilibrium energy (DNES) spectrum of water, that helps understand in general how electromagnetic radiation interacts with water and establish the structural characteristics of water.

Keywords: electromagnetic waves, thermo-infrared radiation, bioluminescence, colour coronal spectral analysis, NES, DNES.

Introduction

All living organisms have a cellular therefore, a molecular organized structure. The living processes inside of them run on a cellular and a molecular level. Bioelectrical activity is one of the very important physical parameters of living organisms [1]. Bioelectric potentials generated by various cells are widely used in medical diagnostics [2] and are recorded as electrocardiogram, electromyogram, electroencephalogram, etc. It was proved that the human body and tissues emanate weak electromagnetic waves, the electric voltage of which is denoted as resting potential, action potential, omega-potential etc. [3]. Between the outer surface of the cell membrane and the inner contents of the cell there is always the electric potential difference which is created because of different concentrations of K^+, Na+ and Cl- inside and outside of the cell and their different permeability through the cell membrane [4]. Their value in the human body varries at ~50–80 mV and is defined by the galvanic contact of a voltmeter input with an object that indicates on the galvanic type of their source [5]. When being excited a living cell changes the membrane electric potential due to changes in membrane permeability and active ion movement through the membrane. In cells of excitable tissues (muscle, nervous), these processes can occur within a very short time intervals (milliseconds) and are called “current action” potential. Its magnitude makes up ~120 mV.

Electromagnetic fields refer to non-ionizing radiation (NIR), i.e. the radiative energy that, instead of producing charged ions when passing through matter, has sufficient energy only for excitation. Nevertheless it is known to cause biological effects [6]. The NIR spectrum is divided into two main regions, optical radiations and electromagnetic fields. The optical spectrum can be further sub-divided into ultraviolet, visible, and infra-red. The electromagnetic fields are further divided into radiofrequency (microwave, very high frequency and low frequency radio wave). NIR encompass the long wavelength (> 100 nm) and low photon energy (<12,4 eV) portion of the electromagnetic spectrum, from 1 Hz to $3 \cdot 10^{15}$ Hz.

As a result of research carried out in the 1990-s and subsequent years, it was established the property of animal and plant tissues to generate relatively strong transient NIR electric fields due to mechanical stresses and temperature changes in biological structure [7]. These electric fields are mainly due to the piezoelectric and pyroelectric voltage electric polarization of natural biological structures. Owing to cell metabolism, electric dipoles (polar and ionized molecules) involved in polarization of biostructures are continuously destroyed and restored, i.e. this is a non-equilibrium polarization. Such type of non-equilibrium electric polarization is known as a main characteristic of electrets [8]. Electrets include dielectric insulators and semiconductors, which under certain conditions, i.e. under the influence of a strong electrostatic field or ionizing radiation, light and other factors acquire property to generate an external electric field, existing for a long time (days, years) and slowly diminishes because the destruction of their substance by polarization [9]. Along with the electromagnetic field electrets generate specific elecric currents produced by heating – thermally stimulated current (TSC) [10]. Electrets belong to the non-galvanic type of electrical sources, which tend to a strong electric field (up to 10^6 V/m) and the infinitesimal electric current (~10^{-14} A/mm^2). By analogy with the physical fields the electric field emitted from the human body on its physical characteristics resembles the electric field generated by electrets. The electrets play an important role in functioning of many biological structures as they themselves possess electret properties. The bioelectret fild registered on the surface of the human body basically are generated by the basal cells of the epidermis. Dermis cells adjacent to the bottom layer of basal cells are surrounded by a conductive interstitial fluid, which electric voltage while grounding of the human

body is close to zero (so called ground potential). This interstitial fluid screens off electromagnetic fields of underlying tissues. With the average thickness of the epidermis (~0,1 mm) and the maximum value of electric voltage (~30,0 V), the electric field strength can reach significant values at ~300000 V/m [11]. The strength of the electric field is quiet sufficient for its influence on the biological processes in cells and surrounding tissues, including the synthesis of proteins and nucleic acids [12]. This electric field along with the field of transmembrane assymetry of ions concentrated at inside and outside of the membrane (~10^5 V/cm^2) can participate in the cooperative effects in cell membrane structures [13]. Thus, owing to the bioelectret condition of certain subcellular structures in the cell and its surroundings is generated slowly oscillating electric field that is strong enough to influence the biological processes. This field and the electric field due to the piezoelectric voltage and intramembrane electric field formes the total electromagnetic field of the cell and its supracellular structures.

It is known that the human skin emanates electromagnetic waves in close ultraviolet range, optic range and also in close infrared range. Infrared thermal bioradiation is found in the middle infrared range at wavelengths from 8 to 14 μm. At wavelength of 9,7 μm infrared bioradiation has its maximum value at t = 36,6 ^{o}C. At this temperature the skin emission is closest to the emission of absolute black body (ABB) being at the same temperature. Infrared emission penetrates the skin surface at a depth of ~0,1 mm, and is reflected in accordance with the physical laws of reflection of the visible part of the electromagnetic spectrum. Evidently, radiation energy influences tissues while being absorbed by them. Yu.V. Gulyaev and E.E. Godik [14] determined that the threshold of skin sensitivity for infrared radiation compiled ~10^{-14} W/cm^2. When thermal influence is applied to the point of threshold skin sensitivity, there is developed a physiological reaction toward the thermal current. The intensity of the radiated thermal current generated by skin makes up ~$2{,}6 \cdot 10^{-2}$ W/cm^2.

The second component of electromagnetic waves is bioluminescence. It is supposed that biophotons, or ultraweak photon emissions of biological objects, are weak electromagnetic waves in the optical range of the spectrum [15]. The typical observed emission of biological tissues in the visible and ultraviolet frequencies ranges from 10^{-19} to 10^{-16} W/cm^2 (~1–1000 photons$\cdot$cm$^{-2}\cdot$sec^{-1}) [16]. This light intensity is much weaker than that one to be seen in the perceptually visible and well-studied spectrum of normal bioluminescence detectable above the background of thermal radiation emitted by tissues at their normal temperature [17].

Bioelectric emission from parts of the human body as thumbs can be easily detected with the method of Color coronal spectral analysis under applying gas electrical discharge of high voltage and friquency developed by I. Ignatov [18]. This method has big scientific and practical prospects in biophysics and medical diagnostics. Its advantages include safety, sterility, clarity and interpretability of the data obtained, ease of storage and subsequent computer data processing, the ability to monitor the development of processes in time, comparing the structural, functional and temporal processes etc.

The purpose of this research was studying of possible biophysical methods and approaches for registering various NIR wave's types emitted from the human body (electromagnetic waves, infrared radiation, thermoradiation) and methods of their visualization by different technique including magnetography, infrared thermography, chemiluminescence and coronal gas discharge spectral analysis.

Material and methods

Infrared thermography (IRT)

The research was made with using infrared thermography (IRT) method according to M. Marinov. The range of the infrared thermal-imaging camera was in the middle infrared range from 9 μm to 14 μm. The temperature range was from 24,0 ^{o}C to 38,0 ^{o}C. The first camera was of Inframetrics/FLIR ThermaCam PM 290 wave type. FLIR ThermaCam PM 290, FLIR 390, Inframetrics PM 250 and Inframetrics PM 350 thermal infrared cameras were of FLIR short wave type, handheld, Focal Plane Array cameras that are capable of temperature measurement. These cameras stored images on a PCMCIA Card, and the images were further analyzed using one of several available FLIR software packages (Thermogram 95, FLIR Reporter 2000 Software, Researcher 2000). The second camera (D.I.T.I.) was a totally non-invasive clinical imaging camera

for detecting and monitoring a number of diseases and physical injuries, by revealing the thermal abnormalities present in the human body's patterns. It was used as a tool for diagnosis and prognosis, as well as monitoring therapy progress; the type of this device was TB 04 K.

Registration of electromagnetic fields

The registration of electromagnetic fields was used with super conductive detectors based on Joseffson junctions – divice made by sandwiching a thin layer of insulating nonsuperconducting material between two layers of superconducting cooper pairs (S-I-S).This allows the registering of magnetic fields 10^{10} times weaker than the Earth's magnetic field. The study of electric field nearly the human body was done using a standard Faraday cage formed by conducting material (aluminium foil) blocks external static and non-static electric fields by channeling electricity through the conducting material, providing constant voltage on all sides of the enclosure.

Bioluminescence detection

The measurement was made with using photoemission detector with photomultiplier (EMI9558QA selected type) in impervious to the light dark room wherein the light was minimized about ~5 cps. This was 4 times lower than the noise of the cooled photomultiplier – 20 cps. The photomultiplier was connected through an amplifier and other intermediate devices with potentiometer recorder or a personal computer. The detection of biophoton emission was performed within time interval with real count rate 3 cps, for 30 min, with reliability according to *t*-criterion of Student at $p < 0,05$. The registration was performed from area of the skin with average diameter 7 cm within time interval of 100 ms or 1 s. The calculation of biophoton emission was measured in count/100 ms/count. The irradiation time of the 150 W-tungsten lamp was 5 s. Within 100 ms after switching off the external lamp the first measurement of the delayed luminescence was recorded; after then were recorded 256 units of the delayed luminescence. The delayed luminescence of biological objects and tissues in terms of coherent states was detected within time intervals of 100 ms. The relaxation function was of 25,6 units.

Colour coronal gas discharge spectral analysis

Experiments were caried out by using selective high-frequency electric discharge (SHFED) on a device with the electrode made of polyethylene terephthalate (PET, hostafan) with an electric voltage on the electrode 15 kV, electric impulse duration 10 μs, and electric current frequency 15 kHz. The electrode of the device was made of hostafan, and was filled up with electro-conductive fluid. The spectral range of the emission was in the range at λ = 380–495±5 nm and λ = 570–750±5 nm. The measurements were measured in electronvolts (eV). Detection of gas disharge glowing was conducted in a dark room equipped with a red filter. On the electrode put a photosensitive paper or color film. The object under study (human thumb) was placed on top of a sheet of photo paper or color film. Between the object and the electrode were generated impulses of the electric voltage 15 kV and electric current frequency – 15–24 kHz; on the reverse side of the electrode was applied the transparent electrically conductive thin copper coating. Under these conditions in the thin contact gas space between the studied object and electrode was generated gas electric discharge in the form of characteristic glow around the object – a corona gas electric discharge in the range at λ = 280–760 nm, illuminates a color photo or a photographic film on which was judged about the bioelectric properties of the studied object. Along with the visible range, for this method were obtained color spectra in UV and IR range. Evaluation of the characteristic parameters of snapshots was based on the analysis of images treated by standard software package. Statistical processing of the experimental data was performed using the statistical package STATISTISA 6 using Student's *t*-criterion (at $p < 0,05$).

NES and DNES experiments on interaction of electromagnetic field with water

The research was made with the method of non-equilibrium spectrum (NES) and differential non-equilibrium spectrum (DNES). The device measures the angle of evaporation of water drops from 72 ° to 0 °. As the main estimation criterion was used the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water's samples. The spectrum of water was measured in the range of energy of hydrogen bonds 0,08–0,387 eV or λ = 8,9–13,8 μm with using a specially designed computer program.

Results and Discussion

Electric fields

The electric field surrounding the human body with frequency $\nu = 1 \cdot 10^3$ Hz is created by electrochemical processes in the organism and is modulated by the rhythm of internal organs [19]. The spatial distribution of the electric field around the body reflects the teamwork of the different organs and systems in the organism. There are also electric fields, which are generated by accumulation of triboelectric (caused by friction) charge on the epidermis, which depends on epidermal electric resistance and varies from 10^9 to 10^{11} Ω/cm^2. Radiothermal emission is being detected in the centimetre and decimetre range of the spectrum. This type of emission is connected with the temperature and the biorhythms of the internal organs, and is being absorbed by surface layer of skin at depth from 5 cm to 10 cm [20]. Long persistent electric field nearby the human body can be detected with using an electrometer voltmeter after neutralizing electric charges on the skin caused by triboelectric charges. The electric strength of this field is undergoing slow oscillations, and most patients exert its value within the range of 100–1000 V/m at a distance of 5–10 cm from the body. People in a state of clinical death usually have the electric field strength's value reduced to 10–20 V/m after 2–3 hours of cardiac arrest. Intensity vector of the detected electric field is found to be normal to the surface of the skin, and the electric voltage is inversely proportional to the distance. On the skin surface the electric voltage of the field (the difference of its electric potential with respect to ground potential) reaches essential values of ~10000 mV or more, i.e., is about 1000 times greater than the source electric voltage of the electric unit above the bioelectric potentials. This allows us to characterize the electric field detected nearby the human body as relatively strong electric field emitted from living tissues. Its electric voltage was measured by electrometric methods, indicating on non-galvanic type of its source.

If the physical basis of the generation of a relatively strong electric field in the human tissue is non-equilibrium electric polarization of the substance due to metabolic processes, the electric field strength should depend on these processes. As noted above, this dependence is actually observed: inhibition of tissue metabolism due to hypoxia during cardiac arrest was accompanied by drop in the electric field strength. This relationship is confirmed in experiments on animals [21]. For example, in rats inhibition of metabolism of the tissue due to cardiac arrest (death of the animal) or by general anesthesia is accompanied by a significant drop in the electric field strength [22].

Electric fields depend on the magnitude of the electric voltage and the distance from the source. Generally, the electric voltages are stable and remain the same; however electric fields are easily perturbed and distorted by many surounding objects.

Relatively strong electric field investigated in humans and animals is being formed evidently by skin's biostructures, since the electric fields of the underlying tissues are largely shielded by conductive interstitial fluid [23]. The greatest contribution to the detected electric field makes the basal cells of the epidermis – the top layer of the skin. Electric polarization vector of these cells is normal to the surface of the skin, i.e., coincides with the electric voltage's vector field, and yet it is inherent in the metabolism intensity, conditioning the generation of the electric field.

Magnetic fields

Magnetic field of a living organism can be caused by three following reasons. First of all, it is ion channels arising from electrical activity of cell membranes (primarily muscle and nervous cells). Another source of magnetic fields is tiny ferromagnetic particles, trapped or specially introduced into the human body. These two sources create their own magnetic fields. In addition, at imposition of external magnetic field there appears inhomogeneity of the magnetic susceptibility of different organs and tissues distorting the external magnetic field. The magnetic field in the last two cases is not accompanied by the appearance of the electric field, so the study of the behavior of magnetic particles in the human body and the magnetic properties of various organs are applicable only with using of magnetometric methods. Biocurrents on the contrary except for the magnetic fields create the distribution of electric potentials on a body's surface. Registration of these electric potentials has long been used in research and clinical diagnostics – in electrocardiography, electroencephalography etc. It would seem that their magnetic counterparts, i.e. magnetocardiography and magnetoencephalography recording the signals from the same electrical

processes in the body, will give almost the same information about the studied organs. However, as follows from the theory of electromagnetism, the structure of the electric current source in the electric conductive medium (the body) and the heterogeneity of the medium have significantly different impact on the distribution of magnetic and electric fields: some types of bioelectric activity manifest themselves primarily in the electric field, giving a weak magnetic signal, while the others – on the contrary create rather strong magnetic signal [24]. Therefore, there are many biophysical processes which observation is preferable by using of magnetographic methods.

Magnetography does not require the direct contact with the investigated object, i.e., it allows carry out measurements over a bandage or other obstructions. It is not only practically useful for diagnostics, but is fundamental advantage over electrical methods towards data recording, as the attachment of the electrodes on the skin can be a source of slowly varying contact electric potentials. There are no such spurious noises while using magnetographic methods, therefore, magnetography allows, in particular, reliably explore slowly occurring processes (with the characteristic time of tens of minutes).

Magnetic fields rapidly diminish with distance from the source of the activity, as they are caused by relatively strong currents running in the body, while the surface potentials are determined mainly by the weaker and "smeared" electric currents in the skin. Therefore, magnetography is more convenient for accurate determination (localization) of bioelectric activity parts on the human body. And finally, the magnetic field vector is characterized as not only by the absolute value but also by the direction, which also may provide additional useful information. However, it should not be assumed that the electricity and magnetographic methods compete with each other. On the contrary, it is their combination that gives the most complete information about the processes being investigated. But for each of the individual methods, there are practical areas wherein the use of any one of them is preferable.

Water is the main substance of all living organisms and the magnetic field exerts a certain influence on water. This influence is a complex multivariate influence, which the magnetic field exerts on dissolved in water metal cations (Fe^{2+}, Fe^{3+}) and the structure of the hydrates and water associates [25]. Experimentally was proved that the magnetic field acts much weaker on still unmoved water, because water has conductivity; as water moves in the electromagnetic field it is generated a small electric current.

The research performed with superconductive detectors based on Joseffson junctions shows that magnetic fields around the human body are in the range from 1 to 100 Hz. The magnetic activity of the brain for example makes up $\sim 30 \cdot 10^{15}$ $T/Hz^{1/2}$. The magnetometric system has a sensitivity of $10 \cdot 10^{15}$ $T/Hz^{1/2}$ in the range of 1 to 100 Hz [26].

Chemiluminescence

Chemiluminescence denotes luminescence accompanying chemical reactions, detected in the near infrared, the optical and the near UV-range of the electromagnetic spectrum. When chemiluminescence takes place in living organisms, the phenomenon is called bioluminescence. The term is generally used for higher luminance ATP-dependent luciferin/luciferase systems observed in *Lampyridae* lightning bugs [27]. Although there bacterial, latia and dinoflagellate luciferin, and coelenterazine found in some bacteria, freshwater snails, dinoflagellates, radiolarians, shrimp, squid and deep-sea fish species [28].

Chemiluminescence is observed in reactions accompanied by allocation of large amounts of energy, such as the reaction of combining of two radicals, or in reactions involving peroxides, e.g. peroxide oxidation of lipids [29]. Peculiar ("ultra-weak") glow of cells and tissues of animals and mammals is caused by free radical reactions: lipid radicals and oxygen, and nitrogen oxides – compounds that play an essential role in cell metabolism, and under certain conditions in the development of a number of pathological conditions.

It is suggested that the major radicals responsible for the light emission are excited triplet carbonyl and excited singlet oxygen, and that these radicals arise through the decomposition of hydroperoxides formed in the process of lipid oxidation [30]. The process of lipid peroxidation (LPO) is an important cause of the accumulation of cellular defects caused by radicals. LPO main substrate is polyunsaturated fatty acids in composition of cell membranes and lipoproteins. Their attack by oxygen radicals leads to the formation of hydrophobic radicals, interacting with each other [31]. Many different mechanisms have been suggested for the oxidative lipid fragmentation

that produces biologically active aldehydes as 4-hydroxynon-2-enal (HNE), oxononanoyl phosphatidylcholine (ON-PC) from linoleic acid (LA) esters, or HNE and oxovaleroyl phosphatidylcholine (OV-PC) [32].

Processes of life are almost always accompanied by a very weak radiation, which is sometimes called ultra-low illumination or radiation of cells and tissues. Some organisms possess the ability to emit bright light at photon fluxes below about 10^4 photons·cm^{-2}·s^{-1}, visible to the naked eye, this phenomenon is denoted "bioluminescence". In biochemical systems, i.e. in tissue's homogenates, cell suspensions or cell organelles, mixtures of enzymes and substrates, chemiluminescence in most cases has an extremely low intensity, and requires particularly sensitive equipment for its detection and measurement [33]. Some substances – enhancers, have the ability to essentially enhance the chemiluminescence, sometimes many thousands of times (activated, or enhanced chemiluminescence). In addition, weak luminescence is accompanied by the formation of free radicals under the action of a number of physical factors on the object: at ionizing radiation is observed radiochimoluminescence, at ultraviolet or visible light illumination – photochimoluminescence, at passing an electric current – electroluminescence, with ultrasound – sonoluminescence, under the influence of friction forces – triboluminescence.

Chemiluminescence differs from fluorescence in that the electronic excited state is derived from the product of a chemical reaction rather than the more typical way of creating electronic excited states, namely adsorbtion. It photomemical reactions, in which light is used to drive an endothermic chemical reaction, light is generated from a chemically exothermic reaction.

At present time it is known quite a lot of chemical reactions involving the formation of luminescence glow. In most cases they are generally quite a complex processes having many intermediate stages, but the basic processes leading to luminescence glowing in general are similar. They include the separation and transfer of charged particles (electrons and free radicals), the electron transfer (redox reactions) at one of the higher energy levels with the formation of the reaction product in an electron-excited state and further releasing of a photon in the transition of the molecule to the low excited electronic ground state with a lower energy level (luminescence). Theoretically, in this process on each molecule of the reactant should be allocated one photon.

Chemiluminescence accompanies many chemical reactions (ozonation and fluorination reactions, the oxidation of phosphorus and complex organic substances, lipids) and has an impulse mode; the signals of this process usually are very week. Thus, the human skin dissociates few photons per 1 sec. with emission power level ~10 mW/cm^2 [34].

Luminescence of cells and tissues are accompanied by three types of reactions:

- Reactions with active oxygen;
- Chain reactions of lipid peroxidation;
- Reactions involving nitric oxide ($NO^{\cdot}$).

The more lipid radicals contain the system, e.g. the more energetically occures the chain reaction of lipid oxidation, the higher the intensity of chemiluminescence accompanying the reaction of radicals. Substances reacting with free radicals and thereby inhibiting the chain lipid oxidation (so-called antioxidants) simultaneously inhibit chemiluminescence. That inhibition of chemiluminescence by cells and tissues by such antioxidants as tocopherol (vitamin E), indicating that chemiluminescence is stipulated by lipid oxidation chain reactions. On the other hand, studying the impact of various natural and synthetic compounds on the time (kinetics) of chemiluminescence, it can be judged on the ability of these substances to protect our body from the harmful effects of free radicals and thereby select candidates to certain medications.

Chemiluminescent methods are used for recording of ultra-weak light wave accopmanying from the chemical and biochemical reactions involving the formation of free radicals. They do not require special laboratory conditions and special material preparation for analysis and characterised by high sensitivity, reliability, meet the requirements for rapid methods of express research. Chemiluminescent methods are widely used in biomedical diagnostics for studying of the molecular basis of physiological processes in biological systems and general mechanisms of development of pathological conditions.

Bioluminescence (biophoton) emission

The term biophoton emission means a photon of non-thermal origin spontaneous emission in the visible and UV-spectrum emitted from all biological objects and tissues and covered over a

wide range of wavelengths, from 200 to 800 nm. Biophoton emission was discovered by the Soviet biologist Alexander Gurwitsch (USSR) who was among the first discovered that in the process of cell mitogenesis cells emited ultra-weak UV waves, named mitogenetic or Gurwitsch rays [35]. The biophoton emission evidently is linked to the endogenous production of excited states within the biological system. As a possible carrier of biophotone emission A. Gurvitsh offered chromatin – the complex of DNA with special proteins that during eukaryotic cell division formes chromosomes – thread-like structures inside the cell's nucleus, consisting of DNA, RNA and proteins. Cells that do not contain chromatin do not possess the ability to emit biophotones. It was found that if the other surrounding cells were under mitogenetic rays, the mitosis was being increased, i.e. stimulated the cell growth [36]. It was also demonstrated that mitogenetic radiation can not only stimulate, but also inhibit the cell growth. Further F.A. Popp developed a biophoton theory to explain their possible biological role and the ways in which they may control biochemical processes, growth, cell differentiation etc. [37]

For many years the existence of Gurwitsch rays has been under the question because of the very contradictory experimental results for their detection. The situation has changed for the better with the development of more sensitive resolution technical devices for their precise detection [38].

The contemporary photon-emission detectors used for the photon's detection are divided into two classes: photodetectors, or photon detectors and temperature detectors. In photodetectors photons absorbed by the material of the detector at interaction with electrons change the electrical characteristics of the detector, which is reflected in the measured electrical signal. In thermodetectors the absorption of photons leads to an increase of the temperature and temperature characteristics of the detector. Thus, in the pyroelectric detector is being measured the change in internal electron polarization; in bolometers – electrical resistance, etc. The photodetectors have, as a rule, better sensitivity and more widespread, whereas temperature detectors are used mainly for standard measurements.

Photodetectors are divided into three classes: photoemission, semiconductor and superconducting detectors. These types of photodetectors may be used in hybrid devices; photoemission electrons may be recorded when they are exposed to a semiconductor CCD (EBCCD type detectors and ICSD).

In photoemission detectors incident photon knocks an electron from the surface of the photocathode, which is accelerated under electric field in vacuum and moves to the anode when an electric potential. The resulting electric current of the detector is proportional to the number of photoelectrons, i.e. the intensity of the incident radiation. The effectiveness of photoemission and spectral energy characteristics of such a detector are defined by the working surface of the photocathode. Advantages of such photodetectors are: high sensitivity and convenience; disadvantages: low quantum efficiency, the spectral dependence of the detector response and the dependence of its efficiency on surface cleanliness.

In semiconductor detectors, photons are absorbed within the volume of the semiconductor material, creating a pair of "electron-hole" and the corresponding conductivity. This class of detectors uses an internal photoelectric effect, in which the photon energy must be large enough to overcome the photoelectron band gap to be moved to the conduction band. In photodiodes, Schottky diodes and metal-insulator-semiconductors to overcome the potential barrier the external electric field is applied. External detector photocurrent is proportional to the number of detected photons. Advantages of such photodetectors: a wide operating range, linearity, high quantum efficiency, wide dynamic range, large image matrices; disadvantages – aging effects by UV radiation.

Superconducting photon detectors – are temperature detectors based on the change in the physical state of matter at increased internal energy of the material due to the absorption of UV photons.

To photodetectors usually put forward the following demands:

• Low sensitivity to visible light (solar-blind). Outside the Earth's atmosphere per one photon in the range of 100–200 nm coresponds ~104–106 photons in the visible and infrared ranges. This ratio is increased on the surface of the Earth. Therefore, the use of transmissive UV-filters with attenuation of signal on 10^{-4}–10^{-3} level is not enough, if the detector has good sensitivity in the visible and/or infrared ranges;

• High detection quantum efficiency (DQE). In optical systems, the UV range, where are large losses in transmission, this value may differ from the quantum efficiency of the photocathode, or CCD. As a result, the use of special filters and windows to block of visible light DQE of photoemission detectors decreases by an order from ~50 % to ~2–5 %;

• Wide local dynamic range: a maximum value of the ratio of flux in a given point of the detector to a minimum signal level composed ~3 units of the noise signal. At the integration over the detector area is obtained integral dynamic range of the detector. This option is especially important for sensitive photomultiplier detectors and the development of high-speed position-sensitive detectors;

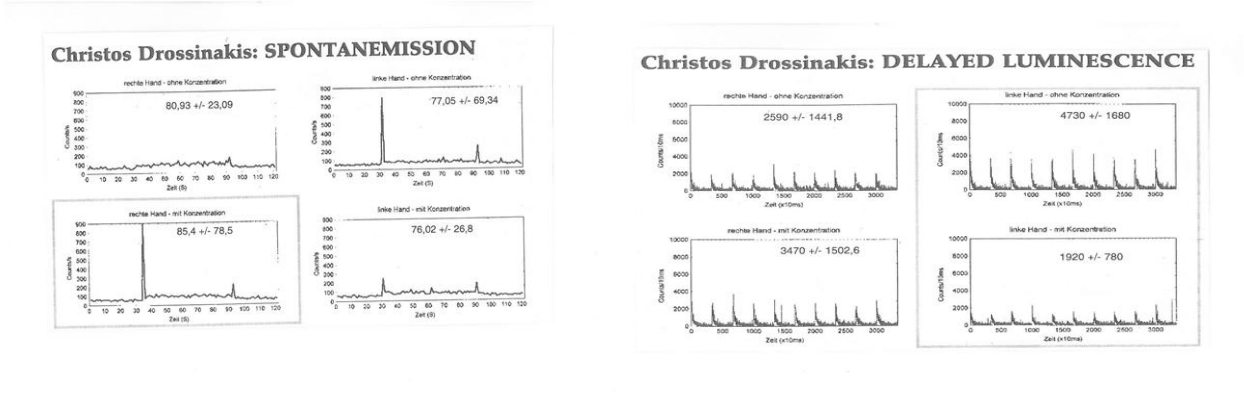
• Low level of background noise and signal that determines the practical sensitivity of the device and the image contrast.

It shold be mentioned that biophotonic emition is measured to be much weaker compared to other types of radiation. Biophotons according to recent studies have a wavelength from λ = 200 nm to λ = 800 nm (0,2–0,8 μm). They are usually being observed in the close UV range (from 300 nm to 400 nm) and visible optic range (from λ = 0,38 μm to λ = 0,75 μm) with frequencies ranges from 10^{-19} to 10^{-16} W/cm^2 (approx. ~1–1000 photons·cm^{-2}·sec^{-1}). This light intensity is much weaker than that one may be seen in the perceptually visible and normal bioluminescence, but is detectable above the background of thermal or infrared (λ = 0,74–2,5 μm) radiation emitted by tissues at their normal temperature.

F.A. Popp proposed that this type of light is not radiated in a dispersed way as daylight, but quiet coherently maintaining order in the flow oscillations, stability and continuity of the phase difference of the amplitude of the total wave, i.e. it might be both semi-periodic and coherent [39]. It is known that coherence is an ideal property of waves that enables stationary (i.e. temporally and spatially constant) interference. More generally, coherence describes all properties of the correlation between physical quantities of a single wave, or between several waves or wave packets. This may signify that biophotones in the light's beam vibrating simultaneously, like in a laser beam that can indicate on information characteristics of the signal.

The photon emission in its turn is weaker than normal bioluminescence because during that process are being emanated individual photons. The "delayed luminescence" is connected with hyperbolic relaxation of biological objects that is a characteristic active response of coherent states. In recent years has been found the evidence that the light has a high degree of coherence because of its photon count statistics, the spectral distribution and unstable decay behavior after exposure to light illumination, and its transparency through optically thick materials. Moreover, DNA is apparently an important source of biophoton emission, since conformational changes of DNA induced with 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide *in vivo* are clearly reflected by changes of photon emission in cells [40]. The physical properties of the emission are described, using the DNA molecule as an exciplex ultraviolet laser system, where a stable state can be reached far from thermal equilibrium at threshold [41].

One of us, Ch. Drossinakis has performed the scrutinized studying of luminescence emission in over 30 scientific laboratories, and part of them on the topic of bioluminescence. He used special photoemission detector for detection of strongly expressed biophotone radiation emitted from the human body. These results are shown on Figure 1. The registration was performed from area of the skin with average diameter 7 cm within time interval of 100 ms or 1 s. The calculation of biophoton emission was measured in count/100 ms/count. The measurement was made using EMI9558QA photoemission detector in impervious to the light dark room wherein the light was minimized about ~5 cps. The photomultiplier was connected through an amplifier and other intermediate devices with potentiometer recorder or a personal computer. The detection of biophoton emission was performed within time interval with real count rate 3 cps, for 30 min, with reliability according to *t*-criterion of Student at $p < 0,05$. The irradiation time of 150 W-tungsten lamp was 5 s. Within 100 ms after switching off the external lamp the first measurement of delayed luminescence was recorded; after then were recorded 256 units of the delayed luminescence. The delayed luminescence of biological objects and tissues in terms of coherent states was detected within time intervals of 100 ms. The relaxation function was of 25,6 units. These dates are the object of further scrutinized analysis.



a) *b)*

Figure 1. The results of Ch. Drossinakis on research of biophoton emission of the human body with using EMI9558QA photoemission detector: figures represent the graphs with different intensities; left graphs – left hand, right graphs – right hand; the calculation of biophoton emission was measured in count/100 ms/count; the reading interval – 100 ms; the irradiation time – 5 s; the average diameter of the skin area – 7 cm; axis X – time; axis Y – counts/cm²: *a*) – spontaneous emission; *b*) – delayed luminescence

In experiments with bioluminescence was established that 1 cm² of human skin generally emits ~85 photones for 1 s. The intensity of biophoton emission ranges from 10^{-19} to 10^{-16} W/cm² (approx. ~1–1000 photons·cm^{-2}·s^{-1}) and depends on a number of conditions as intensity of biochemical processes, metabolism, temperature etc. In both cases the intensive peaks were observed in diagrams. The result of Ch. Drossinakis demonstrates ~900 biophotons from 1 cm² of the skin for 1 s. Bioluminescence may be used in clinical diagnostics. However there still has not been reliable proof that this method has consistent results for medical diagnostics.

Coronal gas discharge spectral analysis in analyzing electromagnetic fields

Coronal gas discharge effect is indicated by the glow corona electrical discharge (flooding, crown, streamer) on the surface of objects being placed in the alternating electric field of high frequency (10–150 kHz) and electric voltage (5–30 kV) [42]. In this process in the ionization zone develops the gas corona discharge sliding on dielectric surface, occurring in a nonuniform electric field near the electrode with a small radius of curvature.

In the thin air layer with thickness of ~10–100 μm between the studied object and the electrode are developed the following processes:

1) Excitation, polarization and ionization by electric field of high frequency the main components of air – the molecules of nitrogen (78 % N_2), oxygen (21 % O_2) and carbon dioxide (0,046 % CO_2). In the result of this is formed an ionized gas, i.e. gas with separated electrons having negative charges, creating a conductive medium as plasma;

2) Formation of a weak electric current in the form of free electrons separated from molecules of N_2, O_2 and CO_2, which generate gas discharge between the studied object and the electrode. The form of gas discharge glowing, its density and surface brightness distribution is determined mainly by electromagnetic properties of the object;

3) The transition of electrons from the lower to higher energy levels and back again, during which there appears a discrete quantum of light radiation in the form of photon radiation. The transition energy of electrons depends on the external electric field and the electronic state of the studied object. Therefore, in different areas surrounding the electric field, the electrons receive different energy impulses, i.e. “skipping” at different energy levels which results in emission of photons with different wavelengths (frequencies) and the energy, coloring the contour of the glow in various spectral colors.

Processes outlined above form the total gas electric effect, allows study the electrical properties of the object at its interaction with an external electromagnetic field [43]. It was shown

that the electrical conductivity of the object has almost no effect on the formation of the electric images, which mostly depends on the dielectric constant [44].

There is a relationship (1) of the electric discharge per unit area of the recording medium on the following parameters:

$$\sigma = a - [U_p \frac{(d_2 + \delta)}{\delta \cdot d_2} \varepsilon_0 \frac{(d_2 + \delta)}{\delta \cdot d_2}], \quad (1)$$

where: $\delta = d_1/\varepsilon_1 + d_3/\varepsilon_3$

α – *slope rate of electrical pulse;*

U_p – *breakdown voltage of the air layer between the sobject and the recording medium;*

d_1 – *the width of the object;*

d_2 – *width of the zone of influence of the electromagnetic field;*

d_3 – *width of the recording medium;*

ε_0 – *dielectric permittivity of the air (ε_o = 1,00057 F/m);*

ε_1 – *dielectric permittivity of the studied object;*

ε_3 – *dielectric permittivity of the medium.*

To calculate the breakdown voltage of the air layer is used this formula:

$U_p = 312 + 6{,}2d_2$ *(2)*

As a result of mathematical transformations is obtained a quadratic equation describing the width of the air layer:

$$6{,}2d_2^2 - (\alpha T - 6{,}2\delta - 312)d_2 + 312\delta = 0 \quad (3)$$

The equation (3) reduces to the standard quadratic equation:

$$ax^2 + bx + c = 0 \quad (4)$$

where $a = 6{,}2$; $b = \alpha T - 6{,}2\delta - 312$; $c = 312\delta$

This quadratic equation (4) has to solutions:

$$x_{1,2} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (5)$$

Accordingly:

$$d_{1,2} = \frac{-[a \cdot T - 6{,}2\delta - 312] \pm \sqrt{[a \cdot T - 6{,}2\delta - 312)]^2 - 77{,}376\delta}}{12{,}4} \quad (6)$$

The above equations allow calculate maximum and minimum width of the air layer for the occurrence of elecric discharge under which is being formed the electrical image of the studied object.

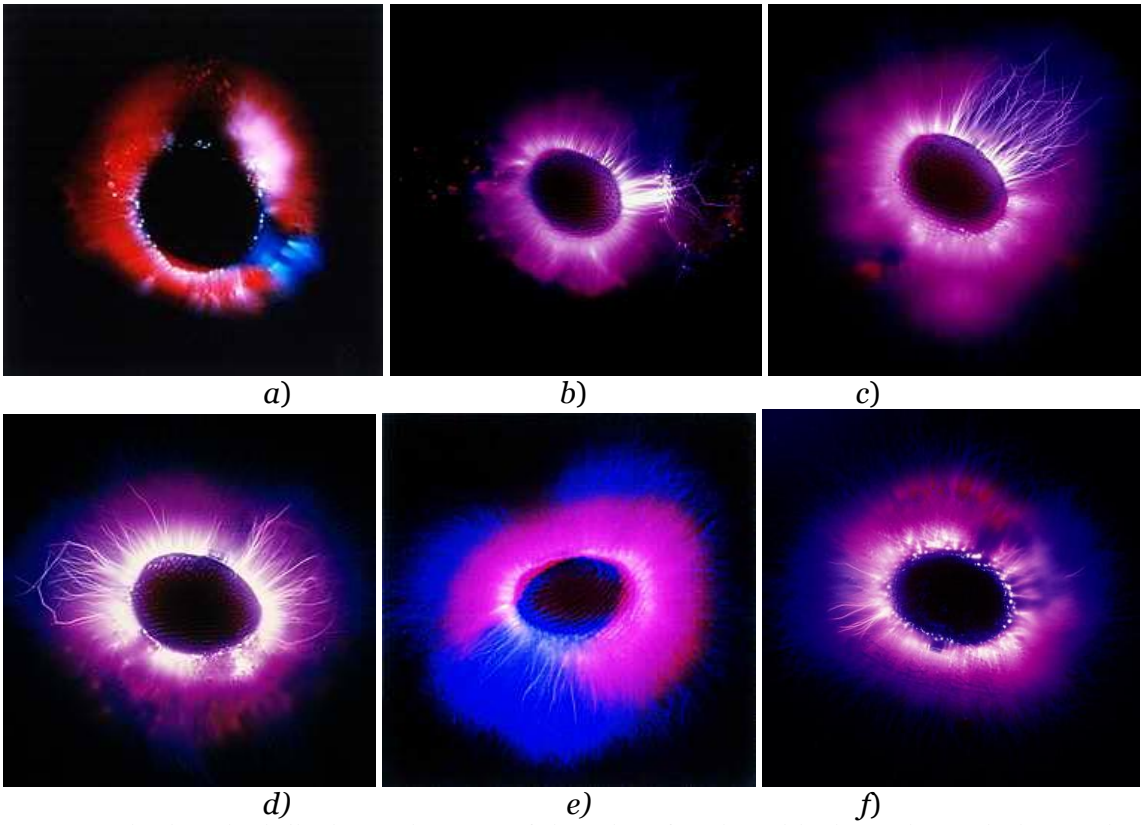
Gas discharge characteristics for various biological objects vary in character and light intensity, size of contour glow and color spectrum and depend both on its own electromagnetic radiation and the dielectric constant of the object. The intensity depends on the electric voltage applied on the electrode.

Studies have shown that the contours of gas discharge electric glow at 12 kHz and 15 kHz are homogeneous in their structure. The contour at kHz is 55 % of the contour at 15 kHz and at 24 kHz – only 15 % of the contour at 15 kHz that is important for further analysis and identification of images. The incidence of bioelectrical activity of the body reducing the intensity of gas discharge glow. Pathology in the organism and surrounding tissues also alter the bioelectric activity and the shape and color of gas discharge glow, which is determined mainly by energy of photon emission at the transition of electrons from higher energy levels to the lower ones when being excited by the external electric field. Thus, for red colour of the electromagnetic spectrum this energy compiles 1,82 eV, for orange color – 2,05 eV, yellow – 2,14 eV, blue-green (cyan) – 2,43 eV, blue – 2,64 eV, and violet – 3,03 eV. The reliable result norm is at $E \geq 2{,}53$ eV. The spectral range of the photon

emission for different colors is within λ = 380–495±5 nm and λ = 570–750±5 nm. The photons, corresponding to the emission with green color in the visible electromagnetic spectrum, are not being detected under those experimental conditions. Thus, the more predominant in the color spectrum yellow, orange, blue, blue-green and purple colors, the more pronounced is gas discharge glow and bioelectric properties of the object. According to the data obtained, the incidence of bioelectrical activity of the body reducing the intensity of gas disharge glow.

Studies carried out by A. Antonov and I. Ignatov on 1120 patients showed that the overall drop in the bioelectric activity of the body, as well as pathology in organism alter the bioelectric activity and reduce the apparent size of the gas disharge electric glow. This dependence is observed for many disorders, although there are not statistical reliable results that this method can be applied in medical diagnostics.

Figure 2 shows the results on bioelectrical discharge images of thumbs of various biotherapists detected on color photofilm under coronal gas electric discharge conditions. The research area was from part of the thumb contacted with transparent electrode. The norm of energy of photon emission compiles 2,54 eV. If the value is over than 2,54 eV this is an indicator of normal bioelectrical status. Some people with high energy status possess the values of photon emission over 2,90 eV. The high values of this parameter are possible with practicing of yoga, sport etc. The emission less than 2,53 eV is characteristic for people with low bioelectrical status. These results are interesting from scientific point of view, because they may provide brilliant prospects for further using this method for clinical studies.



a) *b)* *c)*

d) *e)* *f)*

Figure 2. Bioelectrical discharge images of thumbs of various biotherapists relative to the bioelectrical discharge image of a normal person (according to I. Ignatov): the snapshots were obtained using colour coronal spectral analysis on a device with the polyethylene PET-electrode with an electric voltage on the electrode 15 kV, electric impulse duration 10 μs, and electric current frequency 15 kHz; *a*) – normal bioelectrical discharge of the human body (1,94 eV); *b*) – bioelectrical image of Laura Weigman (3,00 eV); *c*) – bioelectrical image of Pascal Bosinger (2,95 eV); *d*) – bioelectrical image of Enrico Bauer (3,03 eV); *e*) – bioelectrical image of Lieselotte Eder (2,84 eV); *f*) – bioelectrical image of Elisabeth Caggiano (3,97 eV)

NES and DNES analysis of water samples while interacting with electromagnetic fields

Water seems to be a good model system for studying the interaction with electromagnetic fields and structural research. The recent data indicated that water is a complex associated non-equilibrium liquid consisting of associative groups (clusters) containing from 3 to 50 individual H_2O molecules [45]. These associates can be described as unstable groups (dimers, trimers, tetramers, pentamers, hexamers etc.) in which individual H_2O molecules are linked by van der Waals forces, dipole-dipole and other charge-transfer interactions, including hydrogen bonding. At room temperature, the degree of association of H_2O molecules may vary from 2 to 21 units.

The measurements were performed with using NES and DNES methods. It was established experimentally that the process of evaporation of water drops, the wetting angle ($<\theta$) decreases discreetly to zero, and the diameter of water drop basis is only slightly altered, that is a new physical effect [46]. Based on this effect, by means of measurement of the wetting angle within equal intervals of time is determined the function of distribution of H_2O molecules according to the value of $f(\theta)$. The distribution function is denoted as the energy spectrum of the water state. A theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules. The hydrogen bonding results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H_2O molecule; the structure of hydrogen bonding may be defined as $O\cdots H^{\delta+}-O^{\delta-}$.

For calculation of the function f(E) represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2} \qquad (7)$$

where b = *14,33 eV*$^{-1}$

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = \arccos(-1 - 14{,}33E) \qquad (8)$$

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used. The energy of hydrogen bonds measured by NES is determined as E = -0,1067±0,0011 eV.

The difference $\Delta f(E)$ = f (samples of water) – f (control sample of water) – is called the "differential non-equilibrium energy spectrum of water" (DNES).

Thus, DNES spectrum is an indicator of structural changes of water as a result of various external factors. Figure 3 shows NES-spectrum of deionized water that was used as a model system for studying the interaction of electromagnetic field with water. On the X-axis are given three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is shown the energy distribution function f(E) of H_2O molecules measured in eV^{-1}. It was shown that the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle IR-range almost covers NES-spectrum of water. Arrows A and B designate the energy of hydrogen bonds among H_2O molecules. Arrow C designates the energy at which the human body behaves itself as absolute black body (ABB) at optimum temperature 36,6 ^{o}C and adsorbs the thermal radiation. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle IR-range.

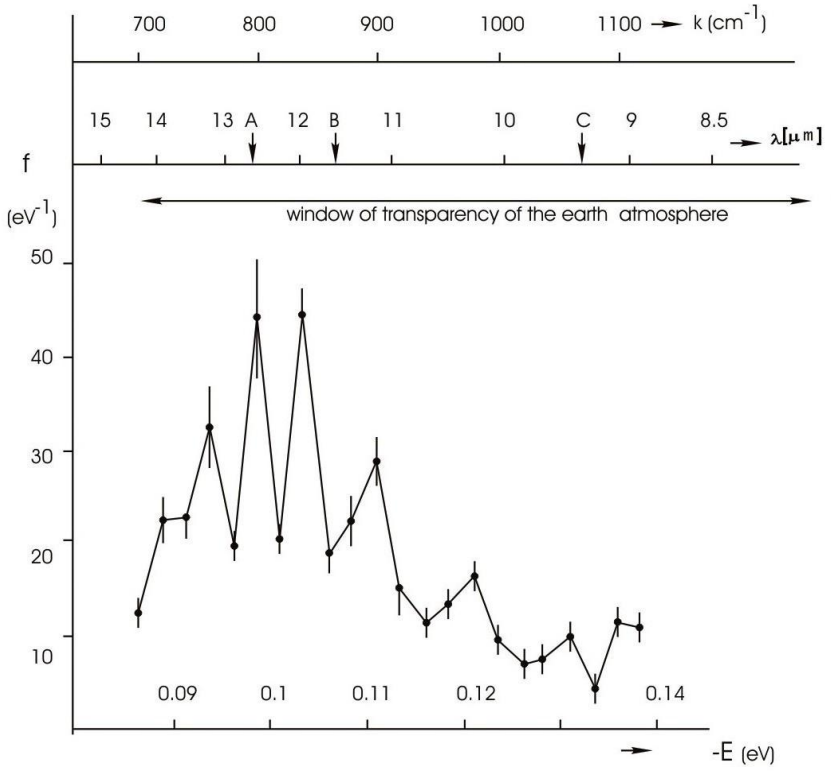


Figure 3. Non-equilibrium energy spectrum (NES) of water as a result of measurement for 1 year: on the X-axis is shown the average energy of H…O-bonds between H_2O measured in eV; on the Y-axis is shown the energy distribution function f(E) of H_2O molecules measured in eV^{-1}; λ – wavelength (μm), *k* – wave number, (cm^{-1}).

Another important physical parameter was calculated with using NES and DNES methods – the average energy ($\Delta E_{H...O}$) of H…O-bonds between H_2O compiled –0,1067±0,0011 eV. The most remarkable peculiarity of H…O-bond consists in its relatively low strength; it is 5–10 times weaker than chemical covalent bond. In respect of energy hydrogen bond has an intermediate position between covalent bonds and intermolecular van der Waals forces, based on dipole-dipole interactions, holding the neutral molecules together in gasses or liquefied or solidified gasses. Hydrogen bonding produces interatomic distances shorter than the sum of van der Waals radii, and usually involves a limited number of interaction partners. These characteristics become more substantial when acceptors bind H atoms from more electronegative donors. Hydrogen bonds hold H_2O molecules on 15 % closer than if water was a simple liquid with van der Waals interactions. The hydrogen bond energy compiles 5–10 kcal/mole, while the energy of covalent O–H-bonds in H_2O molecule – 109 kcal/mole. With fluctuations of water temperature the average energy of hydrogen H…O-bonds in H_2O molecule associates changes. That is why hydrogen bonds in liquid state are relatively weak and unstable: it is thought that they can easily form and disappear as the result of temperature fluctuations. The next conclusion that can be drawn from our research is that there is the distribution of energies among individual H_2O molecules.

DNES-temperature-dependent experiments on heat exchange from the surface of the human body

Further we performed two types of temperature-dependent experiments on heat exchange from the surface of the human body by the DNES-method. In first experiment we studied heat exchange when the temperature of the human body was higher than the temperature of the surrounding environment (curve 1a and 1b on Fig. 4). In second experiment there was heat exchange when the temperature of the human body was lower than that of the surrounding environment (curve 2a and 2b on Fig. 4). In both experiments it was detected a local maximum at λ = 9,7 μm on curve 1 and curve 2 (Fig. 4). This local maximum corresponds to the maximal level of heat emission from the surface of the human body and lays within the “transparency window” of

Earth atmosphere to electromagnetic radiation in the mid IR-range of the electromagnetic spectrum. In this range, the electromagnetic radiation emitted by the earth in the surrounding space is being absorbed by the Earth atmosphere. There is a statistical difference between the results of heat emission from the surface of the human body to the surrounding environment and back to the human body according to the *t*-criterion of Student at $p < 0{,}01$. The local maximum on curve 1a is detected at 7,3 eV^{-1}, while the local maximum on curve 2a – at 2,4 eV^{-1} (Fig. 4).

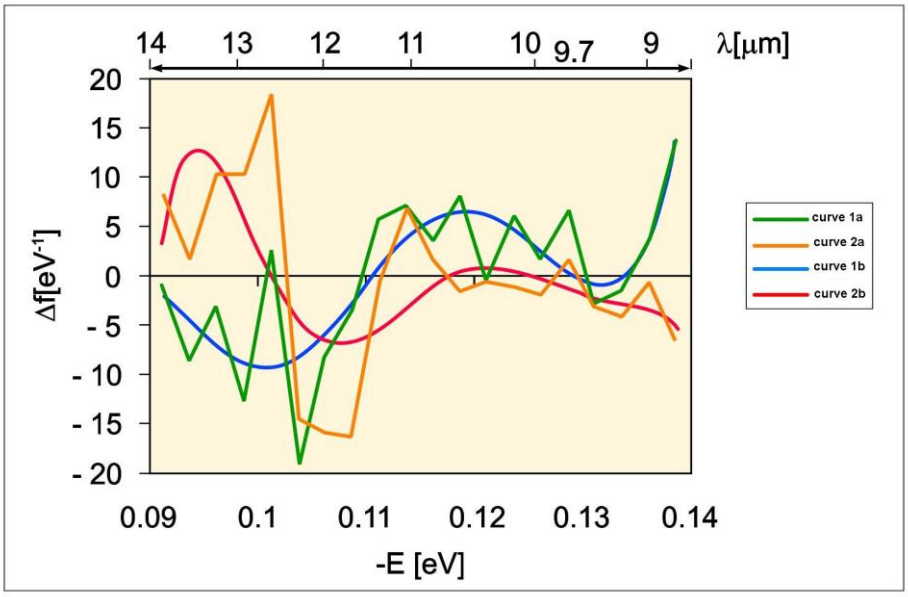


Figure 4. Differential non-equilibrium energy spectrum (DNES) reflecting the heat exchange of the human body with surrounding environment: curve 1a – normal heat exchange at normal temperature; curve 1b – heat exchange at the temperature of the human body higher than the temperature of the surrounding environment; curve 2a – normal heat exchange; curve 2b – heat exchange at the temperature of the human body lower than the temperature of the surrounding environment; on the X-axis is shown the average energy of H...O-bonds between H_2O measured in eV; on the Y-axis is shown the energy distribution function *f(E)* of H_2O molecules measured in eV^{-1}; λ – wavelength, μm.

Infrared *thermography (IRT)*

The human body as a biological body having temperature in the range from 31 °C to 42 °C, therefore being preferably a source of infrared radiation that percieves by organism as thermoradiation. The main part of this radiation predominantly falls on human skin with a long wavelength range from λ = 4 μm to λ = 50 μm. Maximum of spectral density covers the range approx. ~10 μm i.e. the long wavelength IR range. Specifically the infrared radiation of the human skin can be distributed as follows: radiation with a wavelength of up to 5 μm covers 1 %, the radiation with a wavelength of 5 μm to 9 μm – 20 %, the radiation with a wavelength of 9 μm to 16 μm – 30 % and at a long wavelength – 41 %.

The physical essence of the thermal radiation consists in the presence of charged particles (electrons and ions), which are in random motion and have the properties of electrical or magnetic polarity. Infrared radiation is emitted or absorbed by excited atoms or ions when they change their rotational-vibrational movements. Electromagnetic waves propagate throughout the body and reach the surface, passing through the skin and partly emitted into the environment. The intensity of these processes is proportional to the body temperature. That is why any heated body having temperature above absolute zero (273 °K) emits electromagnetic waves in a broad frequency spectrum. Because particle motion is random, they generate different wavelengths. The wavelength of infrared radiation emitted by the body depends on the heating temperature: the higher the temperature, the shorter is the wavelength and therefore the higher the emission intensity.

Studies have shown that in the long wavelength infrared region (8–14 μm) the human skin radiates as a black body, regardless of age, degree of pigmentation and other features. Therefore,

the emissivity of the human skin can be considered equal to 1 absolute unit. In practice, it is proved that the difference between the emission characteristics of the human skin and blackbody still exist, however, it is small and depends essentially on the influence of the surrounding background.

The limit of effective temperature measurement is equal to the thickness of the emitting layer (skin layer) and is defined as a distance at which electromagnetic waves propagate from the object's surface before the layer in which the intensity decreases in ~2,5 times. Under equal conditions the greater is the wavelength, the greater is the depth, which can detect the temperature perturbations. The maximum intensity of thermal radiation at normal ambient temperature is located in infrared rage of the spectrum (wavelength ~10 μm at t = 36,6 ^{o}C). The threshold of the skin sensitivity toward the thermal radiation according to Yu.V. Gulyaev and E.E. Godik compiles ~10^{-14} W/cm^2 [47]. This led to the feasibility of establishing IR thermal imaging (thermography) for the study of the temperature anomalies. However, the measurement of the thermal radiation of the human body in the IR range gives the true temperature for only the top layer of skin with thickness of ~1 mm; after that the thermal radiation is reflected back into the environment. The temperature of the underlying tissues and organs can be judged indirectly when the temperature changes are "projected" on the skin.

Infrared thermography is a scientific method for registering the thermogram – infrared image showing the distribution pattern of infrared waves emitted from the objects. Thermographic cameras detect radiation in the infrared range of the electromagnetic spectrum (approx. ~0,9–14,0 μm), and on its basis are obtained thermographic images (thermograms) allowing to determine the locations of patterns having different temperatures. Thermograms, therefore are actually visual displays of the amount of infrared energy emitted, transmitted, and reflected from the surface of the object. Since infrared radiation is emitted by all objects with the temperature according to Planck's formula for black body radiation, thermography allows "see" the environment with or without visible illumination. The intensity of the thermal radiation of the body increases with the temperature, therefore thermography allows to see the temperature distribution on the surface of the body. As a result warm objects are seen better on the cooler environment background; mammals and warm-blooded animals are better visible on the environment. That is why the thermography may find many diagnostic applications and is often being used for breast diagnostics, tumour detection etc.

Most thermographic cameras use CCD and CMOS image sensors having most of their spectral sensitivity in the visible light wavelength range. The most commonly used is a matrix of indium antimonide (InSb), gallium arsenide (GaAs), mercury telluride (HgTe), indium (In) and cadmium (Cd). The latest technology allows use the inexpensive uncooled microbolometer sensors. Their resolution is varried from 160×120 pixels or 320×240 up to 768×1024 pixels in the most advanced camera's models. Often the thermogram reveals temperature variations so clearly that a photograph is not necessary for further analysis. Usually a block of the focal planes of thermo imager can detect radiation in the medium (3 μm to 5 μm) and long (8 μm to 15 μm) infrared wave band, designated as MWIR and LWIR corresponding to two infrared windows with high coeficient of transmittance. Improperly selected temperature range on the surface of the objects, indicates a potential problem.

It should be noted that the intensity of the thermal radiation of the human body in the microwave (MW) range is much smaller on magnitude than in the infrared part of the spectrum. In particular at a wavelength of 17 cm the intensity is less in ~10 times, so the heat reception of signals in this range of the spectrum requires equipment with higher sensitivity. However, the advantage of this method is that the measurement range and the depth of radiation penetration is much greater, therefore it is possible to obtain data on the temperature parameters of the internal organs and structures of the human body, but the resolution is significantly reduced, therefore it is impossible to obtain reliable thermal image of the study area. *Infrared* thermography registered a thermal infrared radiation emitted by the capillary network of the skin, which is used in medicine for thermovisual diagnostics. The closer an ailing organ is to the skin, the more accurate the diagnosis based on a thermal signal.

Today it seems to be an established fact that some biotherapists possess the ability to increase the temperature of the treated area of the human body. Figure 5 shows the thermovisual result of the temperature difference between the initial (t = 35,6 ^{o}C) and the final skin section

temperature (t = 37,3 °C) of the patient before and after the treatment of the biotherapist. It was calculated that the temperature of the skin part was increased after the treatment on 1,7 °C.

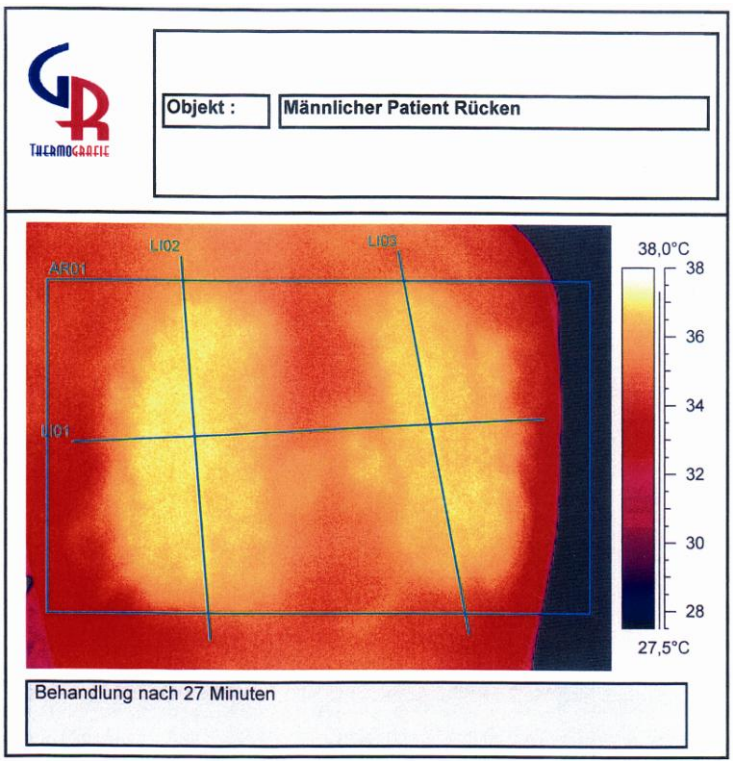
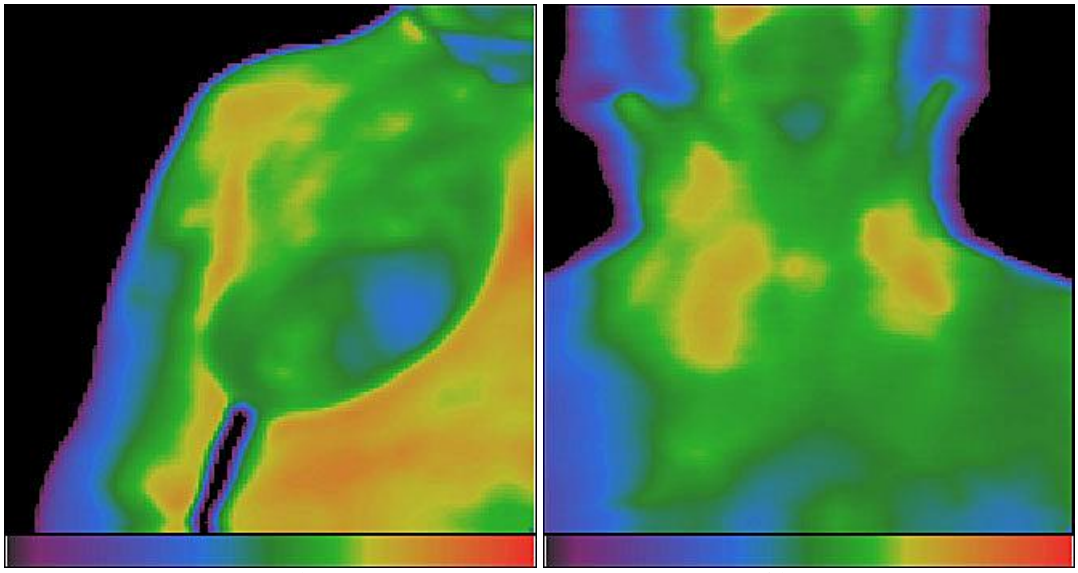


Figure 5. Thermovisual snapshot of the result of bioinfluence of Ch. Drossinakis on the skin back section of a patient suffering from radiculitis after 27 min of bioinfluence: different colors show the temperature area of the human body corresponding to different temperatures from 27,5 °C to 38,0 °C.

In this connection there should be noted two important empirical thermography results obtained by M. Marinov [48], which allow carry out the medical diagnostics of various human organs and monitor their condition and malfunction by this method. Figure 6a shows the thermography snapshot of a patient having the benign tumour growth in the mammary gland, which has highest temperature than the surrounding tissues lower temperature than the surrounding tissues at 0,54 °C. Figure 6b shows a patient having hyperfunction of thyroid glands, which has highest temperature than the surrounding tissues lower temperature than the surrounding tissues at 0,76 °C. The middle value on the scale is 36,6 °C. In the left side of the scale there are temperatures less than 36,6 °C. In the right side of the scale there are temperatures more than 36,6 °C.



a) b)

Figure 6. Thermovisual snapshots of the human body by IRT method (Marinov & Ignatov, 2008): the spectral range of the infrared thermal-imaging camera was in the middle infrared range from 9 μm to 14 μm; the temperature range – from 24,0 °C to 38,0 °C; different colors show the temperature regions of the body corresponding to different temperatures: *a*) – patient having the benign tumour growth in the mammary gland; *b*) – patient having hyperfunction of thyroid glands.

Conclusion

In frames of this research various types of NIR radiation (electromagnetic waves, infrared radiation, thermo radiation, bioluminiscence) emitted from the human body were studied and carefully scrutinized. It was established taht the intensity of biophoton emission of the human body ranges from 10^{-19} to 10^{-16} W/cm^2 (approx. ~1–1000 photons·cm^{-2}·s^{-1}). The specific photon emission from part of the human thumb was detected as a spectrum of various colours with the method of Colour coronal spectral analysis on a device with an electrode made of PET (hostaphan) with applied electric voltage 15 kV, electric impulse duration 10 μs, and electric current frequency 15 kHz. The photons corresponding to a red color emission of visible electromagnetic spectrum have energy at 1,82 eV. The orange color of visible electromagnetic spectrum has energy at 2,05 eV, yellow – 2,14 eV, blue-green (cyan) – 2,43 eV, blue – 2,64 eV, and violet – 3,03 eV. The approaches and methods for detecting of various types of radiation employed in this research as magnetography, infrared thermography, chemiluminescence and coronal gas discharge spectral analysis may find further application in many branches of applied science and medical diagnostics, while other methods as NES and DNES may be applied for studying the interaction of electromagnetic fields with water and structural studies.

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Разработка возможных методов и подходов регистрации неионизирующих излучений, испускаемых человеческим телом

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Аннотация. В статье представлены результаты оценки возможных биофизических методов и подходов для регистрации различных неионизирующих излучений (NIR), испускаемых человеческим телом в оптическом и электромагнитном диапазоне. Рассмотрены различные виды NIR-излучений (электромагнитные волны, инфракрасное излучение, тепловое излучение, биолюминесценция), испускаемые человеческим телом. В частности, показаны результаты по спонтанному биофотонному излучению и замедленной люминесценции человеческого тела и данные инфракрасной термографии (ИКТ). Показано, что 1 см² кожи человека, в среднем испускает ~85 фотонов в 1 сек. Интенсивность биофотонного излучения в среднем варьирует от 10^{-19} to 10^{-16} W/cm² (~1–1000 фотонов·см$^{-2}$·с$^{-1}$). Специфическое биолюминисцентное излучение органов человеческого тела регистрировалось в виде спектра различных цветов методом цветного коронного спектрального анализа на устройстве с электродом из полиэтилентерефталата (ПЭТ, хостафан) с напряжением 15 кВ, длительность электрического импульса 10 мс, и частотой электрического тока 15 кГц. Установлено, что фотоны, соответствующие видимому излучению красного цвета электромагнитного спектра имеют энергию 1,82 еВ. Оранжевый цвет электромагнитного спектра имеет энергию 2,05 эВ, желтый – 2,14 эВ, сине-зеленый (голубой) – 2,43 эВ, синий – 2,64 эВ, а фиолетовый – 3,03 эВ. Уровень достоверности полученных результатов находится в пределах E≥2,53 эВ, а спектральный диапазон излучения – в пределах длин волн λ = 380–495±5 нм и λ = 570–750±5 нм. Также определены некоторые важные физические характеристики (энергия водородных связей, угол смачивания, величина поверхностного натяжения) воды методами неравновесного энергетического (НЭС) и дифференциального неравновесного энергетического (ДНЭС) спектра воды, что способствует пониманию, как электромагнитное излучение взаимодействует с водой и установить структурные характеристики воды.

Ключевые слова: электромагнитные поля, инфрактрасная термография, биолюминисценция, цветной коронный спектральный анализ, НЭС, ДНЭС.

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Research Influence Biological Active Agents in the Course of Regulation of Functional Activity of Platelets and System of a Haemostasis

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Abstract

It is shown that the flavonoid pulikarin suppresses activity of an adenylate cyclase and reduces level intracellular [Ca^{2+}], perhaps its effect is connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages. Perhaps, oppression of fluorescence of membrane-bound Ca^{2+} is connected with inhibition of a pulikarin of release of calcium from intracellular depots. The inhibiting effect of a pulikarin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

Keywords: platelet, aggregation, forskolin, verapamil, pulikarin.

Introduction

Now the intracellular alarm system is the most popular model both for studying of the mechanism of action of biological active agent, and for screening of new medicines. It allows estimating influence of the studied substances on membrane receptors, Ca^{2+}-and Na^{+} exchange, and also the enzymes participating in synthesis and destruction of secondary intermediaries [1].

Definition of time of bleeding belongs to screening tests of studying of function of platelets. For the first time, this method was described to Dyuk in 1910, then improved by Ivy in 1941. The method can be regarded as the most ancient way of research of function of platelets and consists in definition of time from the moment of drawing a standard wound for skin before the termination of an effluence of blood. The analysis allows suspecting thrombocytopathiae of various

genesis, Ville brand's illness and violations the proagregant of properties of a vascular wall. Lack of lengthening of time of bleeding not always allows excluding hemostasia pathology (at violations of average degree of expressiveness less) [2].

One of the objects on which these researches are conducted, is platelets. These cages represent the excitable population of blood corpuscle, responsible for processes of coagulation, reparation of a vascular wall, deposition and transport of biological active agent, implementation of immune reactions of an organism [3].

Platelets represent highly specialized nuclear-free blood cells participating in many processes proceeding in an organism: in regeneration of the damaged fabrics, development of inflammatory, immune and allergic reactions. However their main function providing primary haemostasis, the important protective reaction preventing big blood loss at damage of vessels.

The blood platelets haemostasis is carried out by means of adhesion, swelling and formation of shoots of platelets, their aggregation and secretion, a retraction of a clot, a spasm of small vessels and formation of white blood platelets blood clot in microcirculation vessels. Participation of platelets in a haemostasis is defined by also angio trophic function and procoagulant properties (in the course of activation negatively loaded phospholipids move on an external membrane of platelets and are involved in the cascade of folding of plasma factors).

The methods of research existing today allow studying almost each stage of participation of platelets in the course of a blood formation.

Research of functional activity of platelets actually for definition of the reasons of different types of bleeding and thrombosis, implementation of selection of specific methods of prevention and treatment, the prevention of postoperative bleedings and thromboembolic; solutions of problems of habitual not incubation of pregnancy at an anti-phospholipid syndrome, the thrombophilia, the coagulopathy; control of efficiency and safety of therapy by anti-modular preparations. The developed research of aggregation functions of platelets is conducted for an assessment of safety of platelets when performing plasmas and a citeferez, hemosorption, use of cardiopulmonary bypasses and a haemodialysis, at storage the platelet of transfusion environments [4-7].

At activation from platelets over ten chemicals - aggregation inductors are released. One of them comes out of storage granules, others are synthesized at activation. Search and the characteristic of the new connections which are selectively contacting with receptors of a plasmatic membrane of platelets will give the chance of pharmacological regulation of functional activity of platelets [8].

Materials and methods

Animals and Ethics statement: This study was carried out in the Laboratory Electrophysiology of Institute of Bioorganic Chemistry of Academy Sciences of the Republic of Uzbekistan on physically fit, adult, albino rats in both sexes (female and male) obtained from the vivarium of the Laboratory of Pharmacology. Animals had been fed with standard food and water in the vivarium. In all experiments albino rats weighing 200 – 250 g were used (n = 18). During the experiments, while working with experimental animals, International principles of the Helsinki Declaration and the rules of human attitudes towards animals were completely followed.

Solvents and chemicals: Platelets allocated with a centrifugation method at 1500 rpm, within 15 min., for sedimentation of erythrocytes. The plasma enriched with platelets was centrifuged repeatedly within 10 min. at 3 thousand rpm. A deposit of platelets of a suspended in 5 ml of the environment containing *150 mm of NaCl, 2,7 mm of KCl, 0,37 mm of NaH_2PO_4, 1 mm of $MgCl_2$, 1 mm of $CaCl_2$, 5 mm glucose, 10 mm of HEPES-NaOH, pH 6,55, 50 of piece/ml of heparin, 0,35% of serumal albumine and 0,15 mg/ml of an apyrase*. Aggregation of platelets was registered on Born's method [9]. As inductors of aggregation of platelets used ADP (2 microns), adrenaline (5 microns) and thrombin (0,5 pieces/ml) (Sigma).

For an assessment of influence of the studied connections on the level of intracellular calcium used a fluorescent method of registration according to Tsien [10].

Data analysis. The statistical importance of distinctions between controlled and skilled values was defined for a number of data, using the pair t-test where controlled and skilled values are taken together, and the unpaired t-test if they are taken separately. Value P<0, 05 indicated

statistically significant distinctions. The received results are statistically processed on Origin 6.1 (Origin Lab Corporation, the USA).

Results and discussion

At Institute of the Chemistry of Plant Substances, Academy of Sciences Republic of Uzbekistan, researches on studying of pharmacological properties of vegetable substance of a pulikarin were conducted. Pulikarin is one of perspective connections possessing potential tire-tread and anti-toxic action. However at what level their action is realized and what their molecular mechanisms remain not studied. In this regard, in experiments action of a flavonoid of a pulikarin on system of a haemostasis of a blood of rats was investigated. In the real work influence of a flavonoid of the pulikarin (allocated from plants of *P.salviifolia* which represents *6,3 '-digidroksi-3,5,7,4 '-tetrametoksiflavon*) on system of a fibrillation was studied.

Pulikarin in concentration of 60 microns in plasma rich and poor in platelets didn't cause fibrillation of plasma in vitro. But at research of influence of a pulikarin on thrombin and thrombin is more similar effects of poisons of snakes (*Vipera lebetina, Echis multisquamatus and Akqistrodon halys*) is revealed that pulikarin more dose dependent reduces influence of thrombin and these poisons (0,01g/ml) on process of a blood clot formation and of a fibrinous clot in the plasma rich with platelets. If to consider that antitromb action of a pulikarin is shown more in plasma rich with platelets, perhaps, its action is connected with secretion inhibition from platelets of activators of a fibrillation (a thromboxane of A_2, ions of Ca^{2+}, the factor of activation of platelets (FAP), fibrinogen and many others).

In preliminary researches influence of the pulikarin on functional activity of platelets isn't revealed, however pulikarin dose dependent inhibited thrombin, adrenaline and ADP-induced aggregation of platelets. Thus the most inhibiting property of a pulikarin was observed at ADP-induced aggregations (fig. 1.) pulikarin in concentration 50mkm caused 50% suppression ADP-induced of aggregation of platelets. Further increase of concentration of a flavonoid of a pulikarin to 80mkm and 100mkm led to almost full inhibition of aggregation of platelets.

Having contacted with specific receptors on a membrane of a platelet, ADP creates favorable conditions for reception of fibrinogen on a surface of platelets that the glycoprotein of receptors of IIb/IIIa leads to activation [11]. ADF is the natural inductor of aggregation of platelets in the blood course, collects in dense granules of platelets and is allocated in the course of primary haemostasis. Working through purinoreceptor, ADF activates platelets owing to sharp increase level the intracellular calcium coming to cytosol as from extracellular space as a result of stimulation of activity of a phospholipase of (PLC), and also from intracellular depots, owing to start of a phosphoinositol way. Stimulation of Ca-dependent of enzymes leads to change of a form of platelets, the subsequent aggregation and secretion of biologically active agents from granules in extracellular space [12].

Adrenaline activates process of aggregation of platelets, causing stimulation α2– adrenergic receptors. Thus there is an oppression of an adenylate cyclase, reduction of the sAMF level and change of the maintenance of intracellular Ca^{2+}. Also adrenaline promotes activation of other agonist therefore, can lead to intra vascular aggregation of platelets [13].

It is known that ADP leads to sharp increase in intracellular concentration [Ca^{2+}], and this increase is carried out as due to activation of an adenylate cyclase, and release from intracellular storages.

To check whether it is connected the inhibiting action of a pulikarin of aggregation of platelets with oppression of activation of an adenylate cyclase, and release from intracellular storages, its action a verapamil background (a blocker of calcic channels) and a forskolin (the adenylate cyclase activator) is investigated.

Thus it is revealed that against a background verapamil and a forskolin in the concentration, for 50% reducing ADP-induced aggregation of platelets, the inhibiting effect of a flavonoid of a pulikarin amplified.

The received results show that the inhibiting effect of a pulikarin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

With the purpose of specification of some mechanisms of antiagregant action of a flavonoid of a pulikarin, its influence on the level of intracellular and membrane-bound Ca^{2+} with use of

fluorescent probes Fura-2/AM and chlortetracycline (CTC) was investigated. To define, whether action of a pulikarin on a gain of cytoplasmatic concentration of Ca^{2+} is based, the induced ADP, experiment was made at presence and without physiological concentration of Ca^{2+}.

In control at presence and without physiological concentration of Ca^{2+} the fluorescence gain Fura-2/AM and CTC induced by ADP is revealed.

At research of action of a pulikarin on fluorescence gain Fura-2/AM induced by ADP for lack of extracellular Ca^{2+} it is revealed that pulikarin dose dependent release of Ca^{2+} from intracellular depots oppresses. Thus full suppression of a gain of cytoplasmatic concentration of Ca^{2+} wasn't observed (fig. 1).

At the same time against a background a pulikarin, in the presence of extracellular Ca^{2+} the fluorescence Fura-2/AM induced by ADP was much more, than in lack of extracellular Ca^{2+} that says that pulikarin oppresses only release of Ca^{2+} from intracellular depots (fig. 1).

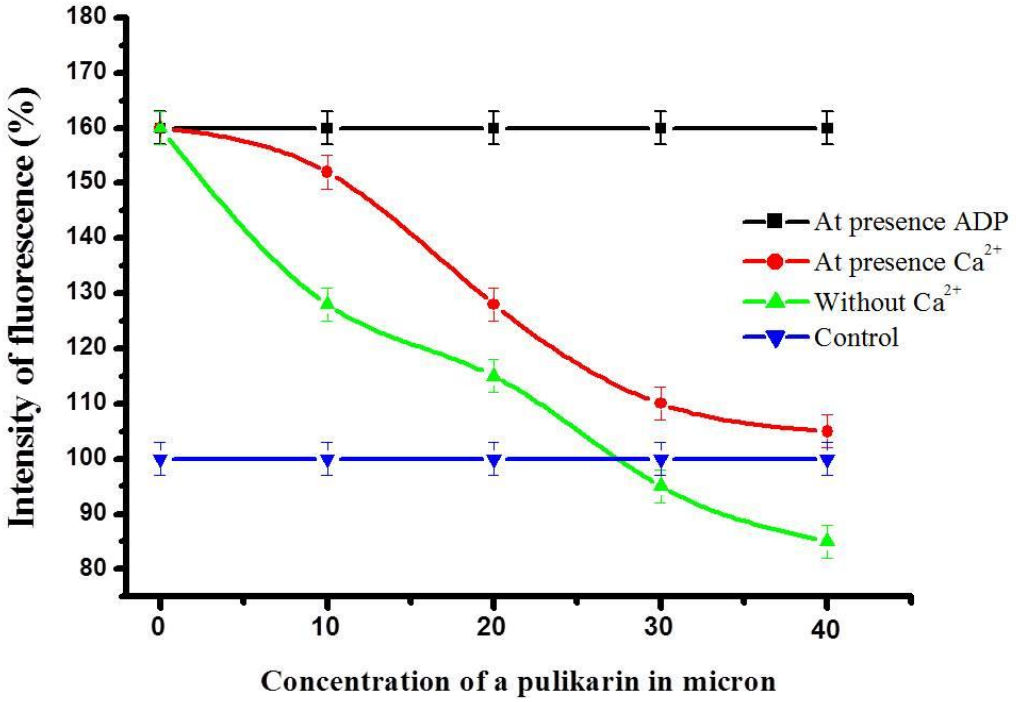

Fig. 1. A fluorescence gain at ADP-induced aggregations under the influence of a pulikarin. Reliability indicator: P<0,05

These assumptions are confirmed in researches of action of a pulikarin against a background blocker of Ca^{2+} verapamil. Against a background verapamil pulikarin the gain of level of intracellular Ca^{2+}, the induced ADP (fig. 2) slightly oppressed.

At linkng of ADP with the corresponding receptors on a membrane of platelets, intermediate connections which stimulate release of calcium from depot are formed.

In researches of action of a pulikarin against a background a forskolin, it is revealed that pulikarin dose dependent strengthened the inhibiting action of a forskolin on ADP-induced increase of intracellular calcium (fig. 2). These results show that the flavonoid pulikarin suppresses activity of an adenylate cyclase and reduces level intracellular [Ca^{2+}], perhaps its effect is connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages.

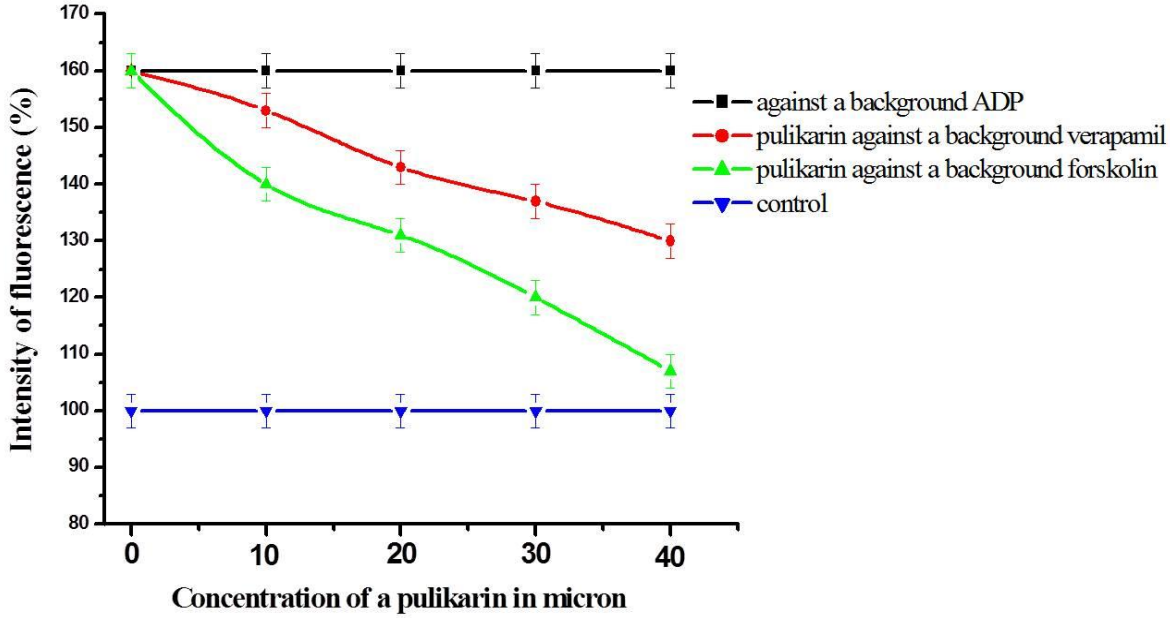

Fig. 2. A fluorescence gain at ADP-induced aggregations under the influence of a pulikarin, against a background blockers of verapamil and a forskolin. Reliability indicator: P<0,05

In a case with use of fluorescent probes of CTC, against a background a pulikarin considerable oppression of fluorescence of membrane-bound Ca^{2+} in lack of physiological concentration of Ca^{2+} was also observed.

Conclusion

Received results show that flavonoid pulikarin suppresses activity of an adenylate cyclase and reduces level intracellular [Ca^{2+}], perhaps its effect is connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages. Perhaps, oppression of fluorescence of membrane-bound Ca^{2+} is connected with inhibition of a pulikarin of release of calcium from intracellular depots. The received results show that the inhibiting effect of a pulikarin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

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Исследование влияние биологические активные вещества в процессе регуляции функциональной активности тромбоцитов и систему гемостаза

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Аннотация. Показано, что флавоноид пуликарин подавляет активность аденилатциклазы и снижает уровень внутриклеточного [Ca^{2+}], возможно его эффект связан с ингибированием прироста цитоплазматического Ca^{2+} как за счет его входа снаружи, так и высвобождения из внутриклеточных хранилищ. Возможно, угнетение флуоресценции мембраносвязанного Ca^{2+}, связано с ингибированием пуликарина высвобождения кальция из внутриклеточных депо. Ингибирующий эффект пуликарина на АДФ-индуцированную агрегацию тромбоцитов связан с угнетением прироста цитоплазматической концентрации Ca^{2+} из депо тромбоцитов.

Ключевые слова: тромбоцит, агрегация, форсколин, верапамил, пуликарин.

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Influence of Environmental Factors on the Population Health: Regional Approach for the Medical-Ecological Analysis (for Example, the Sumy Region of Ukraine)

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Abstract

Today is still insufficiently developed the methods of associative analysis of the medical, ecological and demographic parameters that would make it possible to determine the nature and extent of the influence of environmental factors on the population health, establish the basic laws of territorial differentiation of human diseases and to identify ways to optimize the environment. Identifying of such influence and its evaluation is the primary task of our study. The correlations between the environmental pollution of and level of morbidity prevalence of population of the Sumy region we obtained by the mathematical methods. In the analysis, we obtained the 143 pairs for which the correlation is significant at the level ≤0.05 and 50 pairs – for which the correlation is significant at the level ≤0.01, reflecting the most closely dependencies between prevalence of morbidity and the state of the environment. In the integrated form, the quality of drinking water is the most important factor, which closely correlated with the prevalence of diseases among the population of Sumy region. The influence of the ecological state of the air, including the gamma-background level, chemical and radioactive contamination of soils and crop production are also important, although less markedly.

Keywords: medical-ecological analysis, environmental pollution, correlation, prevalence of diseases, Sumy region.

Introduction

Medical and environmental research is relevant scientific field, which develops on the interface of ecology and medicine. Due to the state of the environment, health problems are occupying a special place in scientific investigations. Now there is an active search of criteria of optimal interaction between society and the environment, which will provide the necessary quality of living conditions. Nowadays methods of associative analysis of the medical, ecological and demographic parameters which will make it possible to determine the nature and extent of the influence of environmental factors on the human body, establish the basic laws of territorial differentiation of human diseases and to identify ways of optimizing the environment are still insufficiently developed. Therefore, the primary task is the identifying of such influence and evaluation of it.

All medical-ecological problems irrespective of reasons that caused them, are inextricably linked to a particular territory. Their sharpness depends on the structure of environmental management and presences of appropriate natural or social factors on the territory, especially the demographic situation and the level of medical care. Uneven distribution of these factors causes differences in the magnitude of anthropogenic load which is based on the national, regional and local levels.

The regional level is the most relevant and dynamic. On this level the most fully medical and environmental problems of the natural-social interaction are manifested.

All regions, that have a high density of population, developed mining and manufacturing industries, especially if the latter are based on resource - and energy-intensive technologies have to face with problems of the impact of the environment on human health. The Sumy region, which occupies 3.9% (2383.2 ths. ha) of territory of Ukraine, where according to data of 01.01.2014 inhabit 2.5% (1130.8 ths. people) of Ukraine's population, is not an exception. The average population density is 47 persons per 1 square km.

Materials and methods

Deterioration in the health of the population, the growth of the primary disease and the prevalence of different nosologies aroused the search for causes, which might cause such an increase. Most scientists are agreeing that this factor may be the quality of the environment. Therefore, finding and assessment of the linkages between different types of diseases and parameters of the environment is an important component of medico-ecological research. A direct correlation between the pollution of environment and level of morbidity prevalence of the population we established by the mathematical methods. All calculations and computations, as well as graphical representations were obtained by using the capabilities of the computer package SPSS Statistic 17.0 from the SPSS inc., Microsoft Excel 2010 of Microsoft corporation and Statistica 10 from the StatSoft Inc.

Based on the correlation analysis the array data about quality of the environment, and the prevalence of specific nosology or their classes, we established dependence of the latter from the indicators of the environment. In the analysis, we obtained 5940 Pearson's pair correlation coefficients between the 44 parameters characterizing the quality of the environment and the prevalence of 135 diseases of population of Sumy region, belonging to 15 nosological classes [3].

From the resulting array we are selected the 143 pairs for which the correlation is significant at the level ≤0.05 and 50 pairs – for which the correlation is significant at the level ≤0.01, reflecting the most closely between prevalence of morbidity and the state of the environment. Thus, from all correlation array only the 193 (3.2%) coefficients of correlation are statistically significant. Of all the environmental indicators that we considered a most affect on the prevalence of diseases in population of Sumy region are chemical and radioactive contamination of soil, drinking water quality and the level of air pollution.

Results

Deterioration in the health of the population, the growth of the primary disease and the prevalence of different nosologies aroused the search for causes, which might cause such an

increase. Most scientists are agree that this factor may be the quality of the environment. Therefore, finding and assessment of the linkages between different types of diseases and parameters of the environment is an important component of medical-ecological research. A direct correlation between the pollution of environment and level of morbidity prevalence of the population we established by the mathematical methods. All calculations and computations, as well as graphical representations were obtained by using the capabilities of the computer package SPSS Statistic 17.0 from the SPSS inc., Microsoft Excel 2010 of Microsoft corporation and Statistica 10 from the StatSoft Inc.

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Soil pollution and its impact on the prevalence of diseases in populations. Ecological conditions of soil, in particular their radioactive contamination by Cs^{137}, is most strongly influences to the prevalence of diseases of the endocrine system, especially nodular goiter ($r = 0.476$, $p = 0.04$) (Fig. 1) and hypothyroidism, particularly the postoperative hypothyroidism ($r = 0.67$, $p < 0.01$).

Postoperative hypothyroidism is also reliably correlates with the radioactive contamination of crop production by Cs^{137} ($r = 0.461$, $p = 0.04$) and Sr^{90} ($r = 0.507$, $p = 0.02$) (Fig. 2). In both cases, the big prevalence of these nosologies is in the Shostka district, where this problem is most acute.

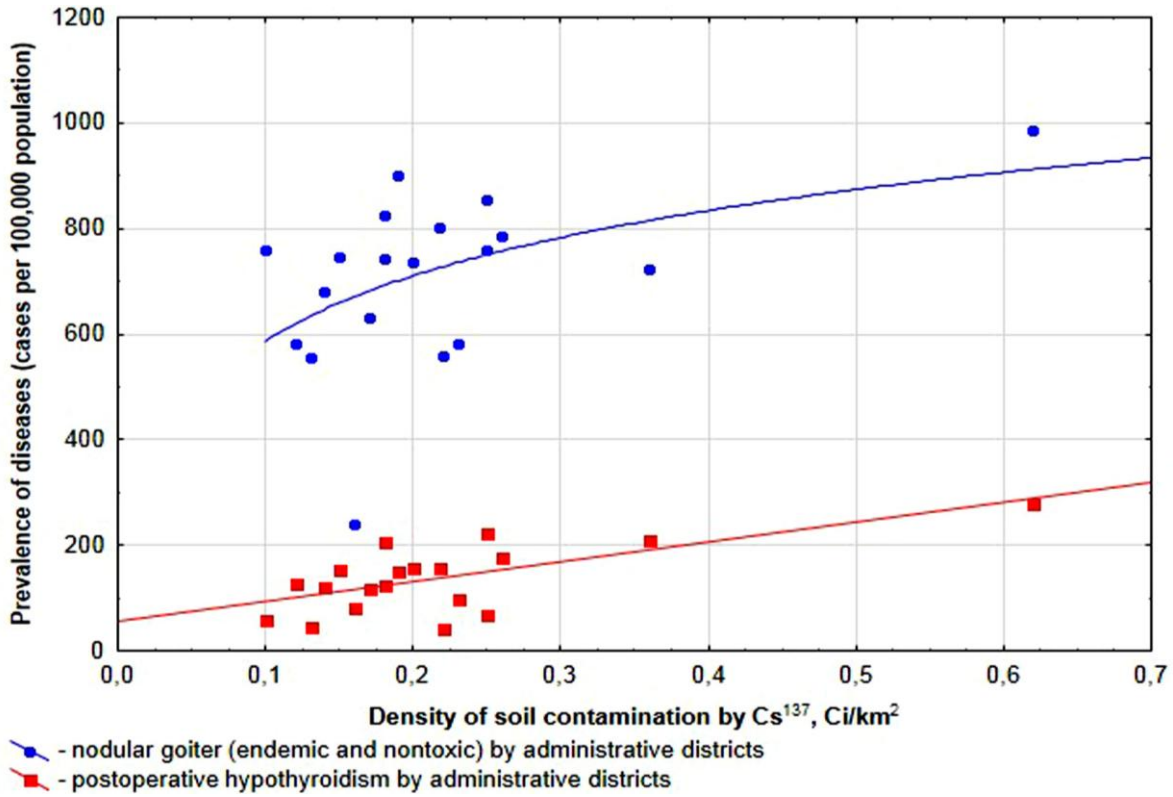

Fig. 1. Dependence the prevalence of goiter in the population of Sumy region from the density of soil contamination by Cs^{137}

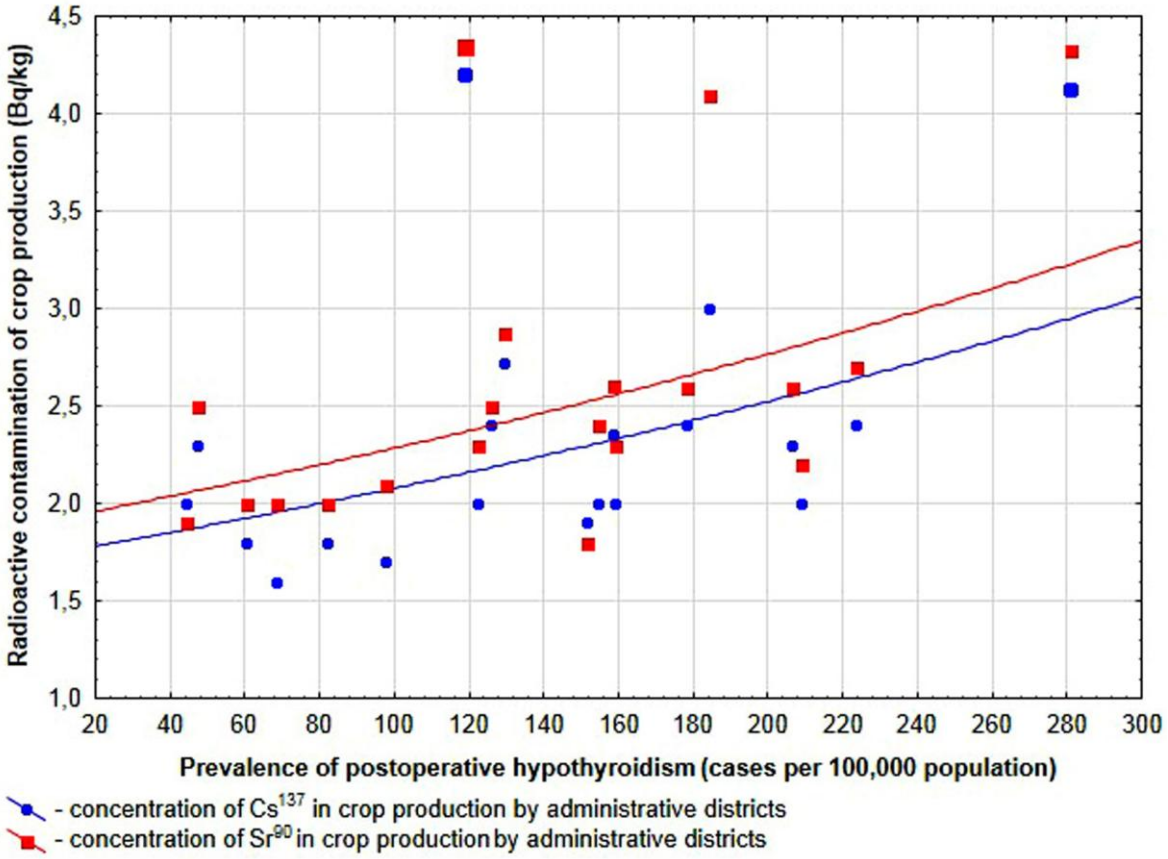

Fig. 2. Correlation between the postoperative hypothyroidism in the population of Sumy region and the radioactive contamination of crop production

No less serious is the problem of soil contamination by heavy metals. For example, the agrochemical certification [5] in 2013 were surveyed 178.8 thousand ha of agricultural land in five districts. Studies indicate that the content of Pb in soils of farms Buryn' district was 0.24-1,86 mg·kg^{-1}, Krolevets' – 0.32-1,7, Krasnopillya – 0.28-2,12, Putyvl' – 0.35-1,83, and Yampil' disrticts – 0.18-1,34 mg·kg^{-1} etc. The content of Cd in soils of administrative districts was – 0.05-0.32 mg·kg^{-1}, Cu – 0.07-0.28 mg·kg^{-1}, Zn – 0.25-2,2 mg·kg^{-1}, Co – 0.08-0.36 mg·kg^{-1}, and Mn – 6,7-38,0 mg·kg^{-1}.

Special attention is attracted to the Pb and Cd, given their negative impact on human health. With these contaminations with medium and large closeness of the relationship are correlate 12 diseases of such nosological classes: blood diseases and blood-forming organs (anemias), endocrine system diseases, digestive disorders, metabolic disorders (obesity), skin diseases (atopic dermatitis), and diseases of musculoskeletal systems and connective tissue (gouty arthritis and podagra).

However most closely with the content of Pb in soils are correlated the diseases of circulatory system (Fig 3): acute and repeated myocardial infarction ($r = 0.574$, $p = 0.01$), non-rheumatic involvement of cardiac valves ($r = 0.565$, $p = 0.01$), atrial fibrillation and atrial flutter ($r = 0.461$, $p = 0.05$). Also closely the contents of Pb in soils are correlated the diseases of digestive organs (Fig. 4): duodenal ulcer ($r = 0.539$, $p = 0.02$), gastritis and duodenitis ($r = 0.614$, $p = 0.01$), diseases of peritoneum and bowel ($r = 0.569$, $p = 0.011$), cholelithiasis ($r = 0.6$, $p = 0.01$).

Somewhat less noticeable is the impact on the prevalence of diseases another heavy metal – Cd. As in the case of Pb, there is a fairly reliable connection between the content of Cd in soils Sumy region and the prevalence of anemia, acute and recurrent myocardial infarction, non-rheumatic involvement of cardiac valves, atopic dermatitis. More close relationship we observe between the content of Cd and prevalence of diseases of the digestive system (Fig. 5): gastric ulcer and duodenal ulcer ($r = 0.57$, $p = 0.01$), gastritis and duodenitis ($r = 0.68$, $p < 0.01$), dyspepsia ($r = 0.509$, $p = 0.03$), peritoneum and intestinal diseases ($r = 0.59$, $p = 0.08$), cholelithiasis ($r = 0.6$, $p = 0.01$).

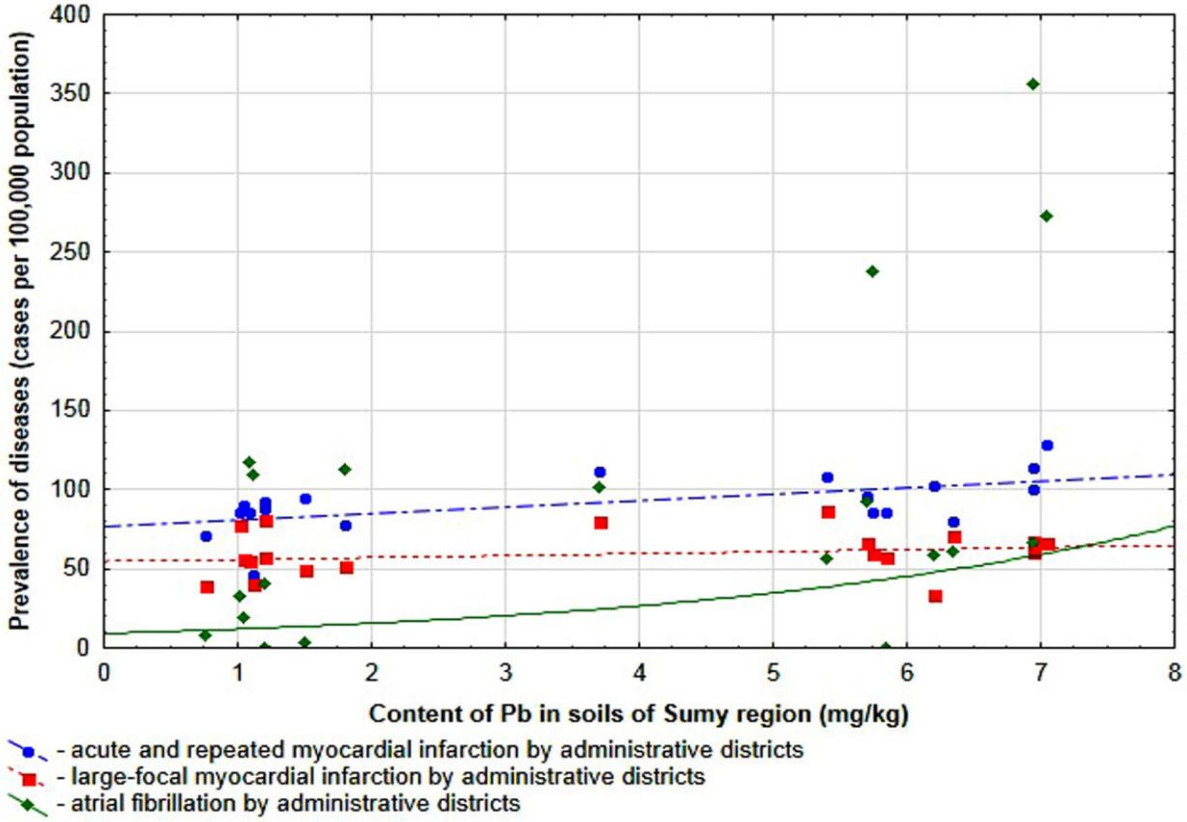

Fig. 3. The impact of soil pollution by Pb on the prevalence of some diseases of heart

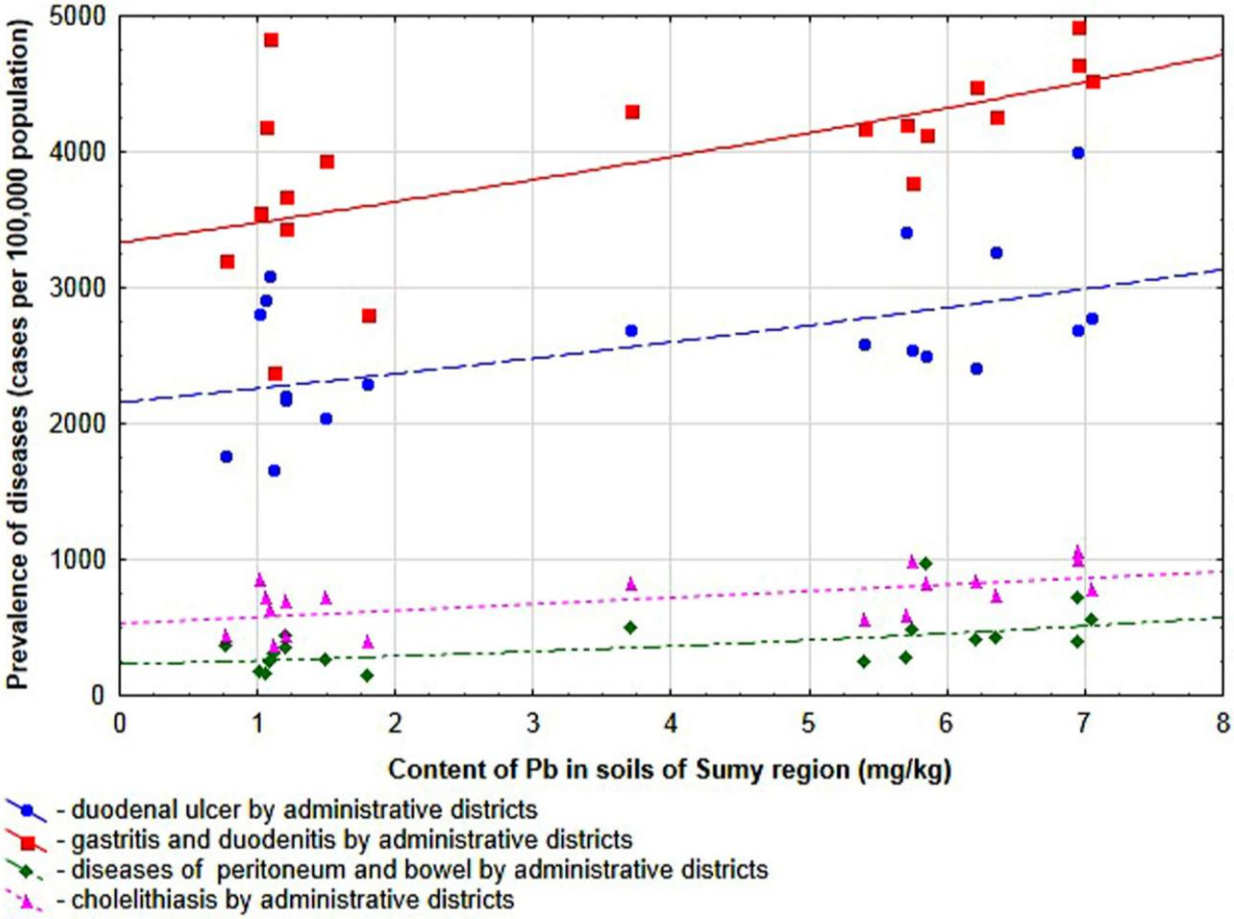

Fig. 4. The impact of soil pollution by Pb on the prevalence of diseases of the digestive system

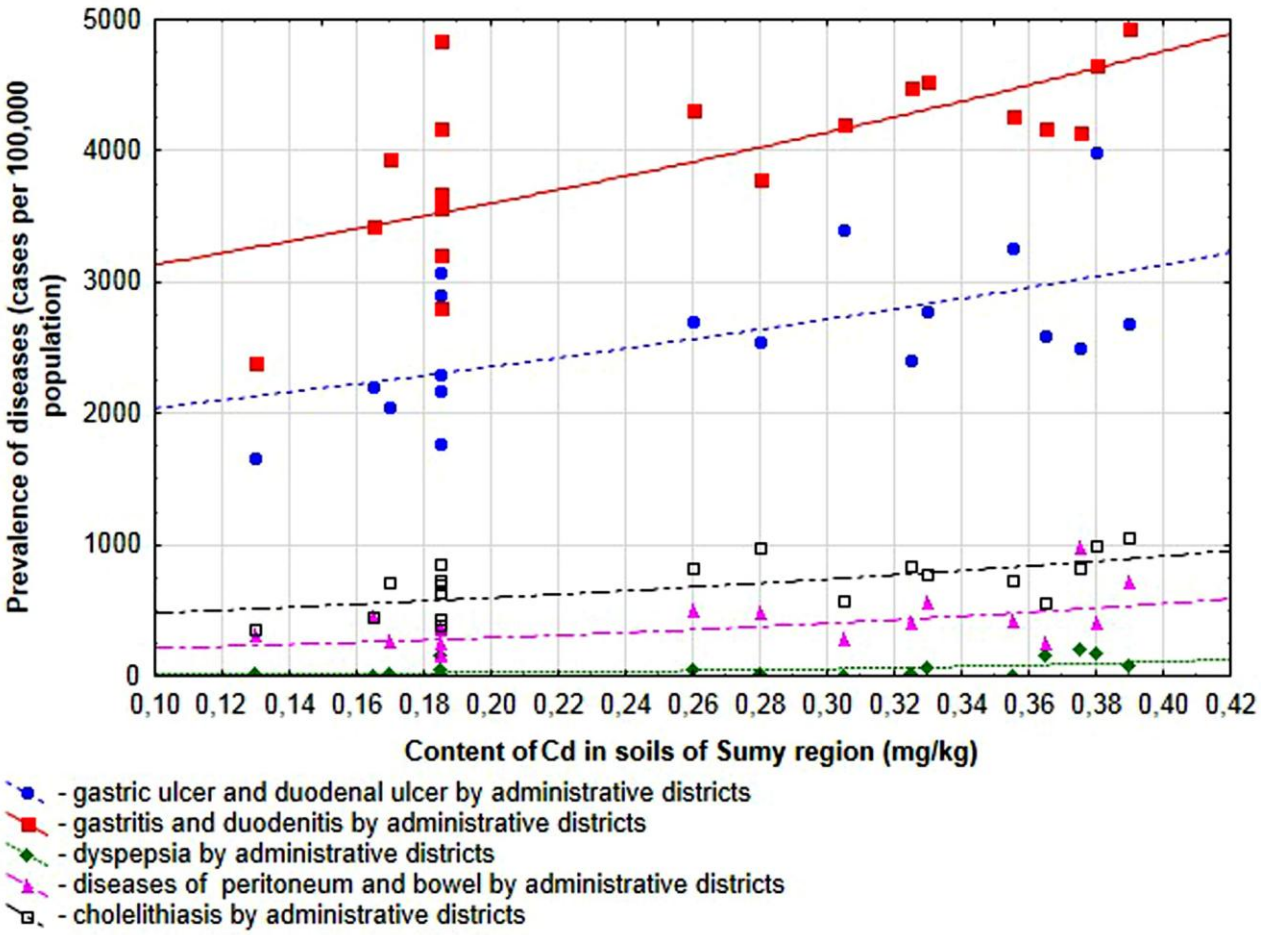

Fig. 5. The impact of soil pollution by Cd on the prevalence of diseases of the digestive system

Correlation of public health and air pollution level. Atmospheric pollution of Sumy region is due to the emission of pollutants from stationary and mobile sources, their cross-border transfer, and the conditions of the air self-cleaning. The presence and character of stationary pollution sources are determined by the development in the region the mining, chemical industry, machinery, food industry and other sectors of the economy that make emission of pollutants to the air. As of 01.01.2014, in the region there 213 industrial enterprises and objects (in 1990 there were 422), which carried out the emission of harmful substances into the atmosphere [7].

The results of many medical-ecological studies are show that there is close links between pollution of surface layer of the atmosphere and the health of the population both in time and in space. For example, in [2], which concerns to medical and ecological assessment of the Chernivtsi region was found that the most vulnerable human organs are organs of direct exposure (the respiratory organs). In the same paper was received extremely high correlation coefficients (r = 0.9) between air pollution and disease of children by bronchial asthma, as well as the dependence of mortality due to respiratory diseases from overall emissions of air pollutants. It is also alleged existence of a link between air pollution and the development of tumors and other clinical forms.

Our study did not confirm the existence of such dependencies. But most closely and with a high degree of reliability is the relationship between air pollution emissions from stationary pollution sources and prevalence of cardiovascular diseases, particularly infarctions (Fig. 5).

Since 1984, volume of emissions from mobile means was exceed the emissions from stationary sources of pollution. Since 1991, due to the rapid decline of industrial production and decrease of intensity of freight and passenger traffic, there was a sharp reduction the emissions of pollutants to the air. The emissions over the past 5 years remain at one and the same level. However, a very sparse network of monitoring stations for air pollution in the region and a small number of them do not allow objectively evaluate the air quality in the region as a whole. In addition, in the Sumy region there are no point observations for transboundary air pollution.

Emissions to the atmospheric air from the territory of Sumy region are uneven. The largest anthropogenic pressures is in atmosphere of the cities. In the city of Sumy from stationary pollution sources in 2013 was emitted – 9.86 thousand tons of hazardous substances (32.3% of emissions from stationary sources of the region), in Sumy district – 10.08 ths. tons (33%), in the town of Romny – 0.24 ths. tons and Romny district – 3.7 ths. tons (12.12%), in the town of Okhtyrka – 0.68 ths. tons and Okhtyrka district – 3.37 ths. tons, or (11.03%), in the cities of Shostka – 0.57 ths. tons, Konotop – 0.26 ths tones. The highest density of pollutant emissions from stationary sources per 1 km² in 2013 were in the cities of Sumy (67.97 tons), Okhtyrka (22.02 tons), Shostka (15.73 tons), Romny (8.23 tons).

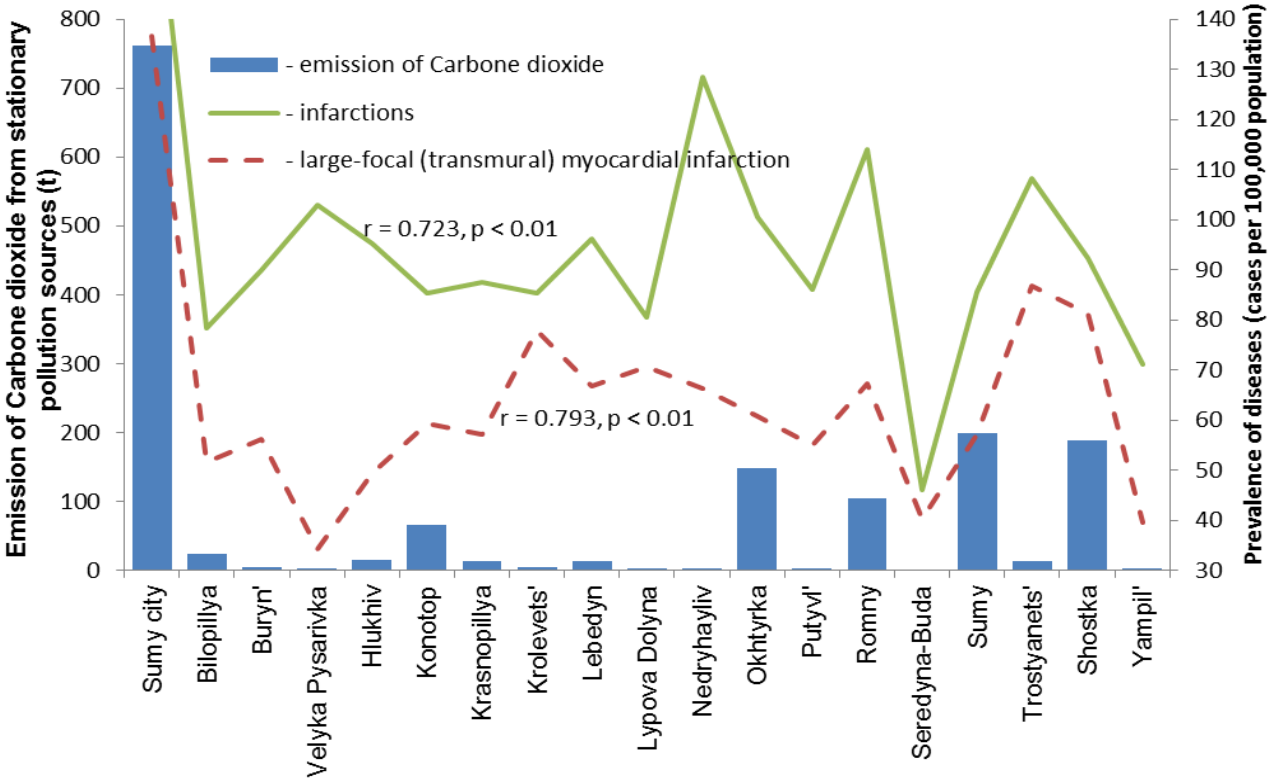

Fig. 6. The relationship between the incidence of infarction and the emission of CO_2 to the atmospheric air of Sumy region from stationary pollution sources by districts

Most closely the prevalence in the population of some administrative units of Sumy region the large-focal (transmural) myocardial infarction and infarctions at all is related with the amount of air emissions from stationary pollution sources by SO_2, C and CO_2. It is worth noting that between disease prevalence and emissions of pollutants to the atmospheric air from mobile transport means, reliable correlations can not be traced.

With the volume of Carbon emissions from stationary pollution sources is closely related the prevalence of heart nosology (Fig. 7), such as disorder of cardial conduction ($r = 0.905$, $p < 0.001$), atrial fibrillation and flutter ($r = 0.829$, $p < 0.001$) and diseases of mammary gland ($r = 0.852$, $p < 0.001$). With emissions of methane to the atmospheric air from stationary pollution sources is reliably correlate only diseases of bowel and peritoneum ($r = 0.737$, $p < 0.001$).

In addition to CO_2 and Carbon emissions, a significant number of diseases are reliably correlated with the SO_2 air emissions from stationary pollution sources. Among them are such nosological forms as disorders of the immune mechanism ($r = 0.601$, $p = 0.006$), hypothyroidism ($r = 0.531$, $p = 0.019$), pancreatic diabetes with complications ($r = 0.606$, $p = 0.006$), contact dermatitis ($r = 0.615$, $p = 0.005$) and male sterility ($r = 0.576$, $p = 0.009$).

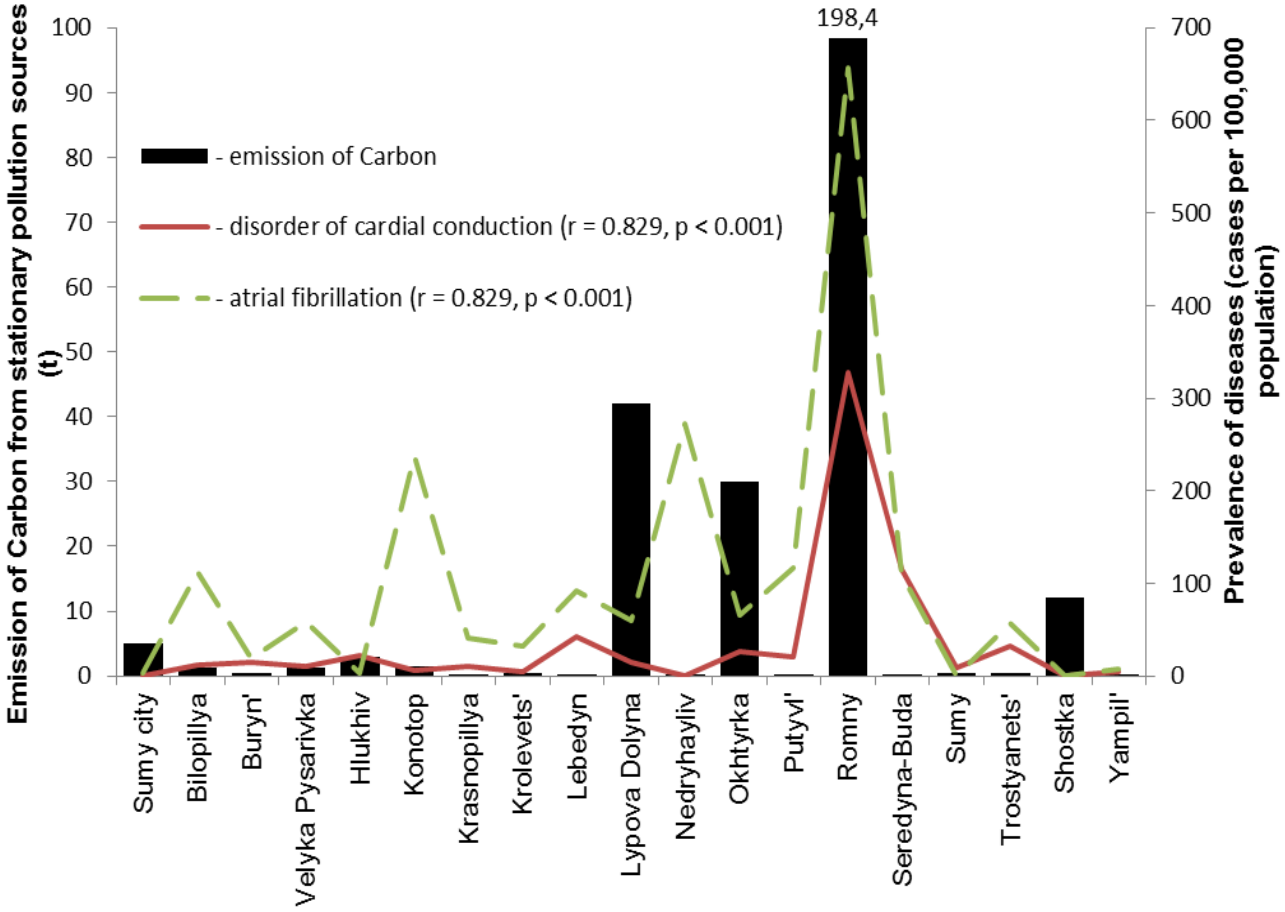

Fig. 7. The relationship between the heart nosologies and the emission of Carbon to the atmospheric air of Sumy region from stationary pollution sources by districts

Note that, such polluting component as SO_2, we have chosen not by chance, but because namely to it are normalized emissions of other pollutants, taking into account their harmfulness. Our results are differ significantly from the results obtained in [2], which can suggests the presence of significant differences of medical-ecological situation in different regions of Ukraine.

The quality of drinking water and the prevalence of diseases in the population of Sumy region. The basis of assessment of water as a food product is its compliance with sanitary standards for a number of features that are limited. Each region has its own drinking waters by chemical properties and contribution to the biochemical balance of the person. Therefore, often there is a close link between the character of the diseases, which are most often founded among the population, and sanitary parameters of sources household and drinking water. According to the WHO, 80% of all diseases are caused by consumption of contaminated water [1].

Ecological and geochemical assessment of natural waters, made by various researchers, make it possible the identify links between the different diseases and the concentrations of chemical elements and compounds in water. Among them, the occurrence of endemic goiter, which is caused by low iodine content; increased the cardiovascular diseases in connection with the use of drinking water with a low content of Ca^{2+} and Mg^{2+} cations; caries, dental fluorosis and other pathological of tissues teeth, which are caused by deficiency or conversely, an excess of fluoride with respect to its standard ratio for drinking water.

In recent years, many numbers of scientific papers are about of impact of the quality of drinking water on noncommunicable diseases. It was found that the balance of minerals in the body is important in the genesis and prevention of a number of systemic diseases, is closely related to the mineral composition of drinking water [2].

Most of the contaminants are enters into surface water with sewage, the volume of which in recent years has increased slightly. In 2013, the total discharge of wastewater into surface water bodies was amounted to 60.09 mln. m^3, of which only 7.3 mln. m^3 before being discharged into

water bodies was provided to normative cleaning at of wastewater treatment facilities, that accounting for 12.15%. The main part of polluted wastewater – 26,91 mln. m^3 (44.78%) was discharged into surface water bodies of insufficiently treated waters, and another 0.066 mln. m^3 – without treatment. Also without purification was discharged 25,82 mln. m^3 normative clean water [4]. Most of insufficiently treated and untreated sewage are fall from housing and utilities.

On the territory of Sumy region are 66 complexes of treatment facilities with a total capacity of 296 ths. m^3 per day. 48 of them are operating in a mode of artificial biological treatment with subsequent discharge of treated or inadequately treated sewage into water bodies. Total length of sewage network region is 1.03 ths. km, including dilapidated requiring replacement – 0.37 ths. km [5]. Discharge of untreated and inadequately treated wastewater is due to inefficient operation of the existing wastewater treatment facilities, and their lack of quantity. Also, do not contribute to the improvement of the ecological status of water bodies the existing technological schemes of water treatment facilities, outdated treatment technology, a significant deterioration of the existing water and sewer networks.

In connection with the moral and physical deterioration of facilities and equipment, is inefficient working of treatment plants in the towns of Konotop, Romny, Sumy, Yampil', Buryn', Lebedyn, Trostyanets', Nedryhayliv. Due to violations of the technological mode of wastewater treatment project did not come to good operation the treatment plants in town of Bilopillya and others. From these enterprises into water bodies is discharges of wastewater, that do not meet the standards for phosphates, organic substances and nutrients. With the wastewaters into surface waters in 2013 were discharged 30.3 ths. tons of pollutants. The volume of these substances in comparison with 2012 was increased by 4.37 ths. tons. The main sources of pollutants into water bodies of the region are public utilities enterprises and the chemical industry.

By tracking the quality of drinking water in the Sumy region in 2010-2011, we have calculated the coefficient of water quality by bacteriological indicators by administrative-territorial units. Values of coefficient were calculated by formula:

$$Rib = Rib_c \cdot ke_c + Rib_d \cdot ke_d + Rib_{wm} \cdot ke_{wm},$$

where Rib – coefficient inconsistencies of water quality (bacteriological); Rib_c – the proportion of water samples from sources of centralized water supply that does not satisfy of sanitary requirements (bacteriological); Rib_d – the proportion of water samples from decentralized water sources that does not satisfy of sanitary requirements (bacteriological); Rib_{wm} – the proportion of water samples from the water mains that do not satisfy of sanitary requirements (bacteriological); ke_c – the coefficient of ensure of population by centralized water supply; ke_d – the coefficient of ensure of population by decentralized water supply; ke_{wm} – the share of housing that is connected to mains water.

With the quality of drinking water may be associated various diseases, that are related to various nosological classes. The most closely and reliably are correlated with it the mammary neoplasms ($r = 0.588$, $p = 0.01$), acuta and chronica otitis ($r = 0.667$, $p < 0.01$ and $r = 0.676$, $p < 0.01$ respectively), rheumatic fever without involvement of heart ($r = 0.669$, $p < 0.01$), disorder of cardial conduction ($r = 0.584$, $p = 0.01$), liver cirrhosis ($r = 0.643$, $p < 0.01$) (fig. 8), disasters of prostate gland ($r = 0.577$, $p = 0.01$), including the hyperplasia ($r = 0.567$, $p = 0.01$).

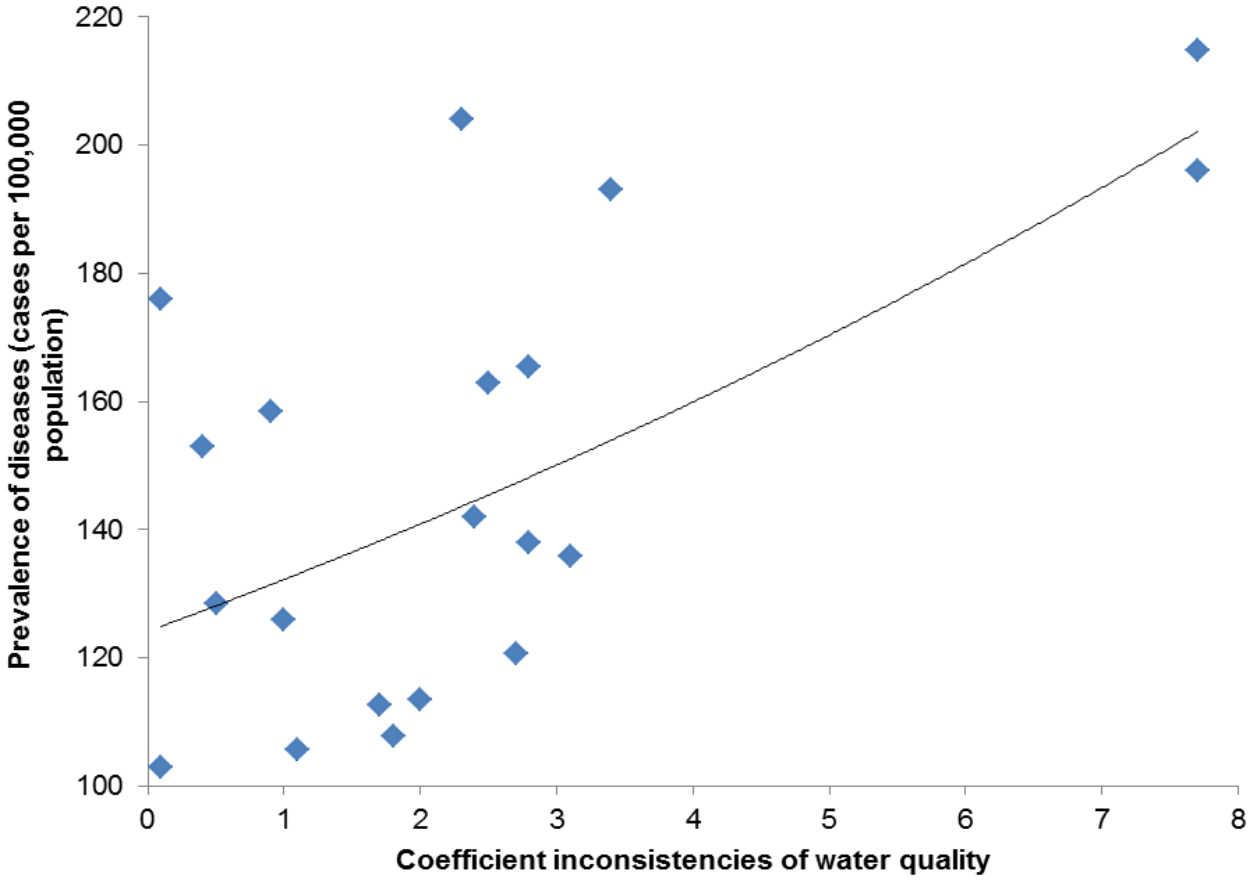

Fig. 8. Influence of drinking water quality for the prevalence of morbidity by liver cirrhosis by administrative district

Analysis of water quality in 2010-2011 shows that every tenth samples of water from the public water supply, every sixth water samples from the departmental water supply and every third samples from rural water supply did not satisfy of sanitary requirements. These standards are not consistent with every tenth sample from wells intakes. Half of water samples from wells of public water consumption are not satisfy of sanitary standards by chemical and bacteriological parameters. However, the study of influence of the drinking water quality, namely its degree of mineralization, microelements and other chemicals on human health and sanitary conditions of life in Sumy region not carried out.

The remaining 17 nosologies (anemias, immunodeficiencies, goiter of 1st degree, obesity, ischemic fit, cataract, acute rheumatic fever, atrial fibrillation and atrial flutter, atherosclerosis, pneumoconiosis, gastroesophageal reflux, infection of external integuments and subcutaneous fat, disorder of intervertebral disks of the cervical and another parts, disasters of mammary gland and neck of uterus, emmeniopathy and another female disorders have somewhat lower, albeit significant association with the quality of drinking water. The values of correlation coefficients for these nosologies are varies from 0.449 to 0.539 at a level of reliability $r \leq 0.02 \leq 0.05$.

Discussion

As you can see, the dependence of prevalence of the various diseases from various ecological parameters of the environment is quite complicated. On the one hand, due to the peculiarities and disadvantages of medical statistics, and on the other – due to the complexity of assessing the quality of the environment. The latter is also caused by disadvantages of environmental statistics, imperfect measurement of environmental parameters, an insufficient number of posts and points, where is the monitoring of environmental quality, disregard of transboundary transport of pollutants. Of course, on the prevalence of diseases is not affect environmental condition only, but other factors: socio-economic conditions, the level of medical care, etc., which act together with the

factor of environmental quality. Consequently, can be different compensations, when the influence of the environment can hide behind a greater or lesser influence of other factors, that are also affects to health. In addition, environmental factors are also doing not act alone but together, which further complicates our task.

In order to establish a universal relationship between the state of the environment and the prevalence of diseases of the population, it is advisable to move from individual parameters, that characterizing a particular component of the environment, to a more generalized indicators. For this purpose, we calculated the coefficients of correlation between the quality of the three main components of the environment: 1) air pollution, including the level of gamma-background, 2) chemical and radioactive contamination of soils and crop production, and 3) the quality of drinking water and the prevalence of diseases.

Of the many known coefficients to calculate the aforementioned dependences, we have selected the τ-b Kendall coefficient, which is a measure of rank correlation. The dependence of the prevalence of disease in the population of Sumy region from emission of pollutants to the atmospheric air from stationary pollution sources is 0.583, soil pollution is correlates with prevalence of diseases slightly better (τ-b = 0.639). And the greatest closeness of the relationship have the prevalence of diseases and the quality of drinking water (τ-b = 0.777). All obtained coefficients were checked for authenticity by using χ^2 test and are significant at level <0.05.

Conclusions

Anthropogenic factors are playing a significant role in shaping the medical-ecological situation in the administrative districts of Sumy region. Although this region has a moderate level of environmental pollution, and the decline in industrial production is continues, the diseases of population by majority of nosologies are growing, like and the prevalence of most diseases. On the one hand, this is due to an aging population, but influence of environment quality, as shown by our study, is also enough perceptible, especially for some nosologies, that are common among residents of Sumy region.

In terms of specific diseases, the greatest dependence from the state of the environment has the nosological classes of circulatory system diseases, digestive diseases and diseases of the genitourinary system. They are characterized by the maximum number of significant correlation coefficients (21, 12 and 12, respectively) with the individual parameters of the environment. Among the individual indicators is best correlated the Carbon emissions to the atmospheric air from stationary pollution sources and disorder of cardial conduction ($r = 0.905$, $p < 0.001$), fibrillation and atrial flutter ($r = 0.829$, $p < 0.001$) and diseases of mammary gland ($r = 0.852$, $p < 0.001$).

The comparative evaluation of the impact of environmental factors made possible to identify the leading of them. Found that the most influential are levels are chemical contamination of soils by Pd and Cd (12 and 9 reliable correlation coefficients, respectively), and the contamination of drinking water, especially from the centralized water supply sources (11 dependencies). In the integrated form, the quality of drinking water is the most important factor, which closely correlated with the prevalence of many diseases among the population of Sumy region. The influence of the ecological state of the air, including the gamma-background level, chemical and radioactive contamination of soils and crop production are also important, although less markedly.

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Re-Education Movements of the Paretic Upper Extremity in Children age by Using Non-robotic Equipment

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Abstract

Background: Pilot study tested re-education movements of upper extremity in children with hemiparesis syndrome and this study determined the effect of therapy on Armeo® equipment on movement and the ability to grip of upper extremity**.**

Methods: This Investigation consisted of twenty-three children with impaired upper extremity. They had twenty therapies in Armeo® equipment.

Results: After rehabilitation by equipment Armeo® the children achieved greater range of motions in the upper extremity which resulted in a higher average output score than the input score. Significant better results demonstrate the improvement in hand grip which resulted in higher average output score compared with the input score.

Conclusion: By the therapy in Armeo® equipment were achieved statistically significant results in improving manual activities of upper extremity, improvement the range of motions and also improvement of grip of paretic hand.

Keywords: equipment, cerebral palsy, non-robotic, therapy, children, patient**,** neurological disorders.

Introduction

Pilot studies have tested improvements of upper extremity movements in children with hemiparesis syndrome. Functional limitations in mobility of hemiparesis have severely limit the patient's activity in all areas of life. Reasons for the development of hemiparesis may be various. Hemiparesis is most frequently caused by the development of cerebral palsy, stroke, brain injury, spinal cord injury, multiple sclerosis or brain and spinal tumors[1].

Cerebral palsy (CP) is defined as a group of permanent disorders of movement and posture, causing activity limitations attributed to a static lesion in the developing brain, often accompanied by secondary impairments. Predominant clinical manifestations found in - CP includes: weakness, loss of selective motor control, spasticity, and antagonist contraction. Significant impairments caused by this disorder may compromise motor function, and as a result, individuals with CP experience functional limitations that affect activities of daily life ranging from mild incoordination to total body involvement[2]. Children and adolescents with CP have decreased levels of physical activity compared with their peers without CP. The ability to sustain physical activity at the intensity and duration necessary for participation is an important outcome of intervention. Young children with CP may be at risk for reduced physical activity and/or ability to sustain physical activity secondary to impairments in muscle performance, limitations in mobility, high calorie demands for growth, and decreased aerobic capacity[3]. Spasticity as a major part of the damage to the central nervous system reduces the patient's mobility, self-sufficiency and ultimately the quality of life. Spasticity significantly reduces motor skills in patients with minimal palsy and a predisposition for the origin of contractures[1].

During the first year of life, infants develop rapidly and acquire the ability to actively explore and act on their environments. Researchers, who have studied the effects of self-produced locomotion (eg, crawling or walking) in children's development. They typically, view it as standard psychological changes in infants and developmental changes in social understanding, spatial cognition and communication[4]. Focused development of kinesiology for the first 12 to 18 months after the birth, it is a considerable support to the study of treatment and movement disorders. Motor development obviously has been in progress during intrauterine life and it continues also after the 18-th month. This can also continue throughout the whole childhood and in some cases entire life. Tonic and phasic muscles respond within the motor program as functional units and are linked reflexively. The weakening later muscle automatically causes changes to the join position and also occurs to the reflection feedback of the lack of response to all motor child´s skills[5]. Hemiparesis is usually a lifelong health problem, but is not unsolvable. By the effort to stifle debilitating disorder in hemiparesis and to therefore prevent its progression, it needs to be followed by restoration of lost functions and paretic upper extremity which have created different methodological techniques and concepts. These are mostly based on the neurophysiologic basis[6]. New therapeutic options are still currently created and strive to positively influence the paretic upper extremity mobility. That is why we decided to devote new medical-technical options that affect the function of the upper extremity of a patient with cerebral palsy. The central nervous system is kept informed of the activities of the muscles and changes the length of so-called proprioceptors, which is located in the muscles and tendons[7].

The therapy was implemented by means of equipment Armeo®. The Armeo® equipment is an arm orthosis equipped with various components, including a pressure-sensitive handgrip. A spring mechanism provides adjustable weight support for the arm requiring treatment which also facilitates functional arm movement. The Armeo® is used to support functional therapy for patients who lose function in their upper extremity caused by cerebral, neurogenic, spinal, muscular or bone-related disorders. The Armeo® is based on the product T-WREX™. It is a passive (non-robotic) upper extremity orthosis, which lightens the weight of the upper extremity in 3D space. It allows natural movement in the workspace of approximately 66% of normal working area in the vertical and 72% in the horizontal plane. It allows quantifying range of motion and gripping strength in the patient's interaction with the software during therapy. This facilitates for users with moderate to severe hemiparesis to achieve greater range of motion than is possible without derating weight of the upper extremity. It also allows use of upper extremity targeted and coordinated, although it retained residual possibility of movement. Since this is non-robotic, equipment requires the initiation of patient motion, which requires the active participation of the patient during training[8] (Figure 1).

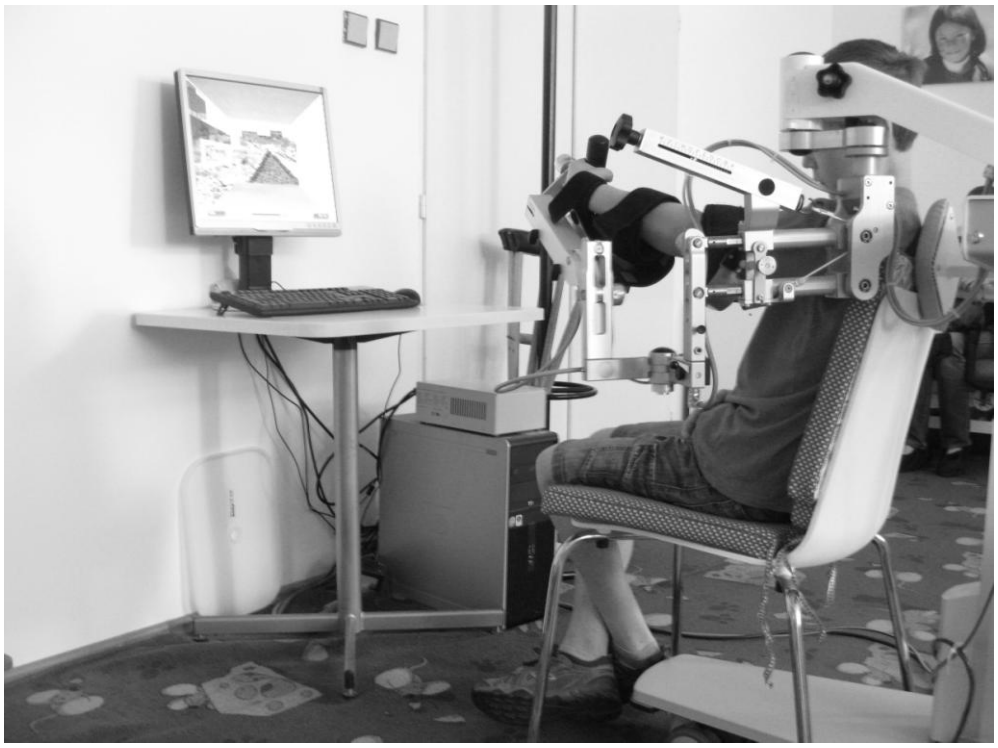


Figure 1. Training in 3D workspace

The purpose of this study was to determine the effect of therapy in the system Armeo® and on the movements and the grip´s of the ability of upper extremity in children with hemiparesis syndrome. In a pilot study, we sought to identify and verify the extent to which Armeo® equipment can effect the functionality of self-sufficiency and improve paretic of upper extremity in children with hemiparesis syndrome. Even though we know that the complete elimination of hemiparesis is impossible, we believe that hemiparesis upper extremity can effect to a large extent, so much so that children can improve their independence and quality of life.

Methods

The object of investigation consisted of twenty-three children, 10 to 16 years old with impaired upper extremity. They all have taken twenty therapies in Armeo® system. One therapy lasted 45 minutes of active exercise and frequency was minimal to twice a week. We mention the first results of tested children by using Armeo® device for the period of 2012-2014. We realized that following these studies, we required more children and further investigation that will be depended on the homogeneity of the children. Classifying criteria for the therapy by Armeo® equipment were: diagnosis of CP - hemiparesis according International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) G 80.2, child and adolescent age, the ability to self-sustaining seating with leaned back lower extremities and the opportunity to cooperate. The children with severe cerebral palsy were not integrated, uncooperative patients with severe cognitive deficits, inability to properly set up the patient to the Armeo® equipment and early discontinuation of treatment. Children were tested before and after completion of therapy using goniometric investigation[9] and by testing grip of paretic's hand (cylindrical, spherical, lateral, hook...)[9].

For processing the collected data was chosen a numerical evaluation and statistical methods.

It was used a descriptive analysis, Student's paired dependent t-test and Wilcoxon Signed Ranks Test. Student's paired dependent t-test was used to evaluate the range of motion of the upper extremity for shoulder flexion and wrist flexion only. This test investigates the differences of two quantitative variables in the same investigating population. The result of the test is the *t* value (positive or negative), and significance. If the significance of the test is on the value *a* higher than

0.05, then our observation of an intervention is not random. For other ranges of motion of the upper extremity and for evaluating the hand grip was used the Wilcoxon Signed Ranks Test - nonparametric statistical test, because in comparing to the test of the range of motion didn't work the test of normality for variances. This test does not compare the obtained values, but order of assigned values from the smallest to the largest. Data were processed by using the software Microsoft Office Word 2007, Microsoft Office Excel, 2007. For mathematical - statistical evaluation was used descriptive statistical methods SPSS 16.0. The study was conducted in accordance with ethical principles, based on the Declaration of Helsinki (1964)[10].

Results

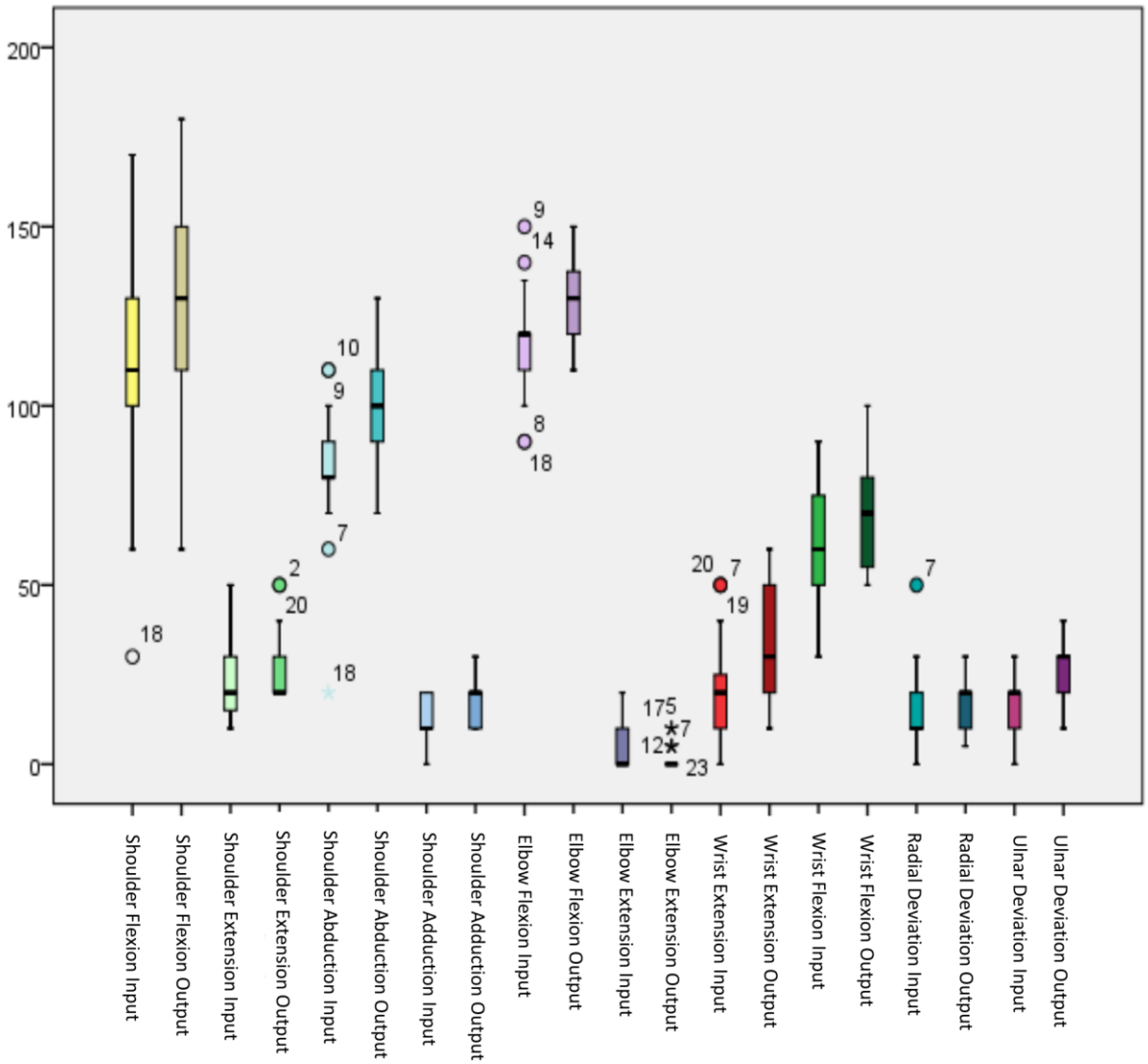
After rehabilitation by equipment Armeo®, the children achieved greater range of motions in the upper extremity. After the testing of obtained input and output data, we used tests of normality (Kolmogorov - Smirnov and Shapiro - Wilk). The tests have confirmed homogeneous and inhomogeneous distribution of the data in the study, we used parametric statistical test - Student's paired dependent t-test and nonparametric statistical test - Wilcoxon Signed Ranks Test. After the treatment has occurred in children to statistically significant improvements in range of motions of the upper extremity which resulted in a higher average output score in shoulder flexion (M = 130, SD ± 31) than the input score (M = 110, SD ± 30), t (23) = -9,045, p = 0,000, a higher average output score in shoulder abduction (M = 100, SD ± 14) than the input score (M = 80, SD ± 18), Z (23) = -4,141, p = 0,000, a higher average output score in elbow flexion (M = 130, SD ± 12) than the input score (M = 120, SD ± 15), Z (23) = - 3,669, p = 0,000, a higher average output score in elbow extension (M = 0, SD ± 3) than the input score (M = 0, SD ± 7), Z (23) = - 3,035, p = 0,002, a higher average output score in wrist extension (M = 30, SD ± 18) than the input score (M = 20, SD ± 15), Z (23) = - 3,858, p = 0,000, a higher average output score in radial deviation (M = 20, SD ± 7) than the input score (M = 10, SD ± 11), Z (23) = - 2,560, p = 0,010 (Table 1, Graph 1).

Table 1: Descriptive statistic of the measurement range of motion of the upper extremity

Descriptive

	Count	Mean	Maximum	Minimum	Median	Standard Error of Mean	Standard Deviation	Student t-test/Wilcoxon Signed Ranks Test	Sig. (2-tailed)
Shoulder Flex. Input	23	111	170	30	110	6	30	t = -9,045	,000
Shoulder Flex. Output	23	131	180	60	130	7	31		
Shoulder Ext. Input	23	22	50	10	20	2	10	Z = -3,357	,001
Shoulder Ext. Output	23	28	50	20	20	2	10		
Shoulder Abd. Input	23	83	110	20	80	4	18	Z = -4,141	,000
Shoulder Abd. Output	23	97	130	70	100	3	14		
Shoulder Add. Input	23	14	20	0	10	1	6	Z = - 2,646	,008
Shoulder Add. Output	23	17	30	10	20	1	6		
Elbow Flex. Input	23	117	150	90	120	3	15	Z = - 3,669	,000
Elbow Flex. Output	23	129	150	110	130	2	12		
Elbow Ext. Input	23	5	20	0	0	1	7	Z = - 3,035	,002
Elbow Ext. Output	23	2	10	0	0	1	3		
Wrist Ext. Input	23	21	50	0	20	3	15	Z = - 3,858	,000
Wrist Ext. Output	23	33	60	10	30	4	18		
Wrist Flex. Input	23	60	90	30	60	4	19	t = -6,521	,000
Wrist Flex. Output	23	70	100	50	70	3	17		
Radial Deviat. Input	23	14	50	0	10	2	11	Z = - 2,560	,010
Radial Deviat. Output	23	18	30	5	20	2	7		
Ulnar Deviat. Input	23	17	30	0	20	2	8	Z = 4,184	,000
Ulnar Deviat. Output	23	26	40	10	30	1	7		

Flex. - flexion, Ext. - extension, Abd. - abduction, Add. - adduction, Deviat. - deviation



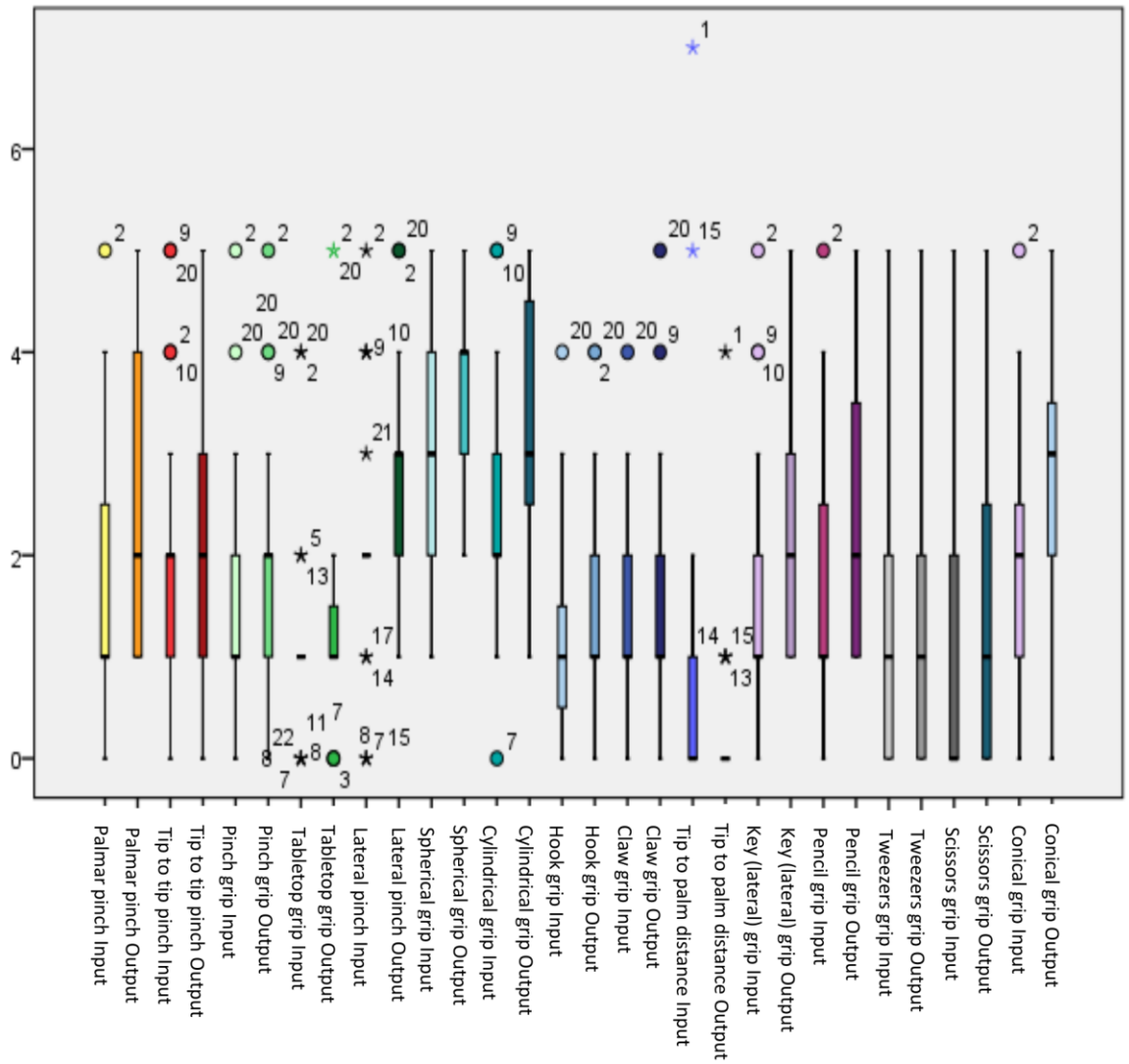
Graph 1: Graphical representation of the measurement range of motion of the upper extremity

Significantly better results demonstrated the improvement in hand grip which resulted in a higher average output score in lateral pinch (M = 3 SD ± 1) compared with the input score (M = 2 ± SD 1), Z (23) = - 3,900, p = 0,000, a higher average output score in spherical grip (M = 4 SD ± 1) compared with the input score (M = 3 ± SD 1), Z (23) = -3,827, p = 0,000, a higher average output score in cylindrical grip (M = 3 SD ± 1) compared with the input score (M = 2 ± SD 1), Z (23) = -4,001, p = 0,000, a higher average output score in key (lateral) grip (M = 2 SD ± 1) compared with the input score (M = 1 ± SD 1), Z (23) = -3,500, p = 0,000, a higher average output score in conical grip (M = 3 SD ± 1) compared with the input score (M = 2 ± SD 1), Z (23) = -3,500, p = 0,000 (Table 2, Graph 2).

Table 2: Descriptive statistics of the testing grips of paretic´s hand

Descriptive

	Count	Mean	Maximum	Minimum	Median	Standard Error of Mean	Standard Deviation	Wilcoxon Signed Ranks Test	Asymp. Sig. (2-tailed)
Palmar pinch Input	23	1,91	5	0	1	0	1	-3,051	,002
Palmar pinch Output	23	2,39	5	1	2	0	1		
Tip to tip pinch Input	23	1,91	5	0	2	0	1	-3,162	,002
Tip to tip pinch Output	23	2,35	5	0	2	0	2		
Pinch grip Input	23	1,52	5	0	1	0	1	-3,464	,001
Pinch grip Output	23	2,04	5	0	2	0	1		
Tabletop grip Input	23	1,13	4	0	1	0	1	-2,449	,014
Tabletop grip Output	23	1,39	5	0	1	0	1		
Lateral pinch Input	23	2,09	5	0	2	0	1	-3,900	,000
Lateral pinch Output	23	2,83	5	1	3	0	1		
Spherical grip Input	23	2,87	5	1	3	0	1	-3,827	,000
Spherical grip Output	23	3,78	5	2	4	0	1		
Cylindrical grip Input	23	2,48	5	0	2	0	1	-4,001	,000
Cylindrical grip Output	23	3,39	5	1	3	0	1		
Hook grip Input	23	1,22	4	0	1	0	1	-2,449	,014
Hook grip Output	23	1,48	4	0	1	0	1		
Claw grip Input	23	1,26	4	0	1	0	1	-3,162	,002
Claw grip Output	23	1,70	5	0	1	0	1		
Tip to palm distance Input	23	0,78	7	0	0	0	2	-2,032	,042
Tip to palm distance Output	23	0,30	4	0	0	0	1		
Key (lateral) grip Input	23	1,65	5	0	1	0	1	-3,500	,000
Key (lateral) grip Output	23	2,26	5	1	2	0	1		
Pencil grip Input	23	1,83	5	0	1	0	1	-2,972	,003
Pencil grip Output	23	2,35	5	1	2	0	1		
Tweezers grip Input	23	1,13	5	0	1	0	1	-2,646	,008
Tweezers grip Output	23	1,43	5	0	1	0	1		
Scissors grip Input	23	0,96	5	0	0	0	1	-3,162	,002
Scissors grip Output	23	1,39	5	0	1	0	1		
Conical grip Input	23	1,96	5	0	2	0	1	-3,500	,000
Conical grip Output	23	2,57	5	0	3	0	1		



Graph 2: Graphical representations of the testing grips of paretic´s hand

Discussion

Krebs[11] published a study, where he tested in children with cerebral palsy (CP). He tested whether or not motor habilitation resembles motor learning. Twelve children with hemiplegic CP, aged 5 to 12 years with moderate to severe motor impairments underwent a 16-session robot-mediated planar therapy program to improve their upper extremity reach, with a focus on shoulder and elbow movements. Participants were trained to execute point-to-point movements (with robot assistance) with the affected arm and were evaluated (without robot assistance) in trained (point-to-point) and untrained (circle-drawing) conditions. Outcomes were measured at baseline, midpoint, immediately after the program, and 1 month post completion. Outcomes measured were the Fugl-Meyer (FM), Quality of Upper Extremity Skills Test (QUEST), and Modified Ashworth Scale (MAS) scores; parent questionnaire; and robot-based kinematic metrics. After robotic intervention, the authors found significant gains in the FM, QUEST, and parent questionnaire. Robot-based evaluations demonstrated significant improvement in trained movements and that improvement was sustained at follow-up. Furthermore, children improved their performance in untrained movements indicating generalization. Therapy in our study was focused to determine the effect of non-robotic therapy for children with hemiparesis syndrome. We focused on improving the range of motion in the upper extremity and improving grips of paretic hand.

Armeo® Spring is an effected tool for rehabilitating the affected arm in patients with hemiparesis secondary to ictus, even in the chronic stage[12]. We agree with the authors of the study,

and we deliver treatment success by using Armeo® in children with cerebral palsy, namely primary hemiparesis.

Studies have confirmed significant improvement in mobility of the upper extremity in patients with hemiparesis. It has increased the muscle strength, increased the range of joint mobility, improved the neuromuscular coordination, improved the upper extremity function, and increased the patient's motivation and lastly the improvement of self-sufficiency. The results of the available studies have supported the current theory of motor learning by repeating the motions, which it describes the correlation between the repetition of activities and improving motor function, therefore being the key to stimulate motor plasticity[13]. We agree with the authors opinion in regards to the pursuance of acquired results, we described similar findings in a child with cerebral palsy after treatment in non-robotic device Armeo®.

Robotic and non-robotic training devices are increasingly being used in the rehabilitation of upper extremity function in subjects with neurological disorders. As well as being used for training such devices can also provide ongoing assessments during the training sessions. Therefore, it is mandatory to understand the reliability and validity of such measurements when used in a clinical setting[14]. We consent, therefore started using non-robotic Armeo® equipment in our rehabilitation centre.

We agree with the author's opinion in regards that in pursuance of acquired results we described similar findings in a patient with cerebral palsy after treatment in non-robotic equipment Armeo®. Therapeutic allowances, such as robotic therapy can be viewed as a promising development. Robotic therapy allows for patients to practice independently without a therapist, and thus help to improve their own functional level. In particular, there is strong evidence for robotically assisted therapy, because it will increase compliance with therapy by means of introduction of incentives to the patient, such as games[15]. We have to agree here with the authors of international clinical studies, because it has showed greater interest in the therapy from the patient´s side and greater motivation especially in children and adolescence age, where it is well known that it is difficult to motivate and to improve attention in therapy.

Robot assisted upper extremity therapy has been shown to be effective in adult stroke patients and in children with cerebral palsy (CP) and other acquired brain injuries (ABI). The patient's active involvement is a factor with its effectiveness. However this demands focused attention during training sessions, which can be a challenge for children[16]. We agree with the authors, however with our children, we would like to highlight the increased attention needed, because then the games would interest them and they would be completely focused on the therapy.

Conclusion

Significantly positive results were achieved due to Armeo® system. Therapy has improved the range of motion in the hemiparetic upper extremity and similarly significant results have been shown in improvements in grip ability of paretic hand. The co-operation with children during the non-robotic therapy was very good. They were coming to the therapy regularly and really looking forward to it. We can say based on the analysis results, that non-robotic therapy of Armeo® positively effects the rehabilitation of the children with cerebral palsy (hemiparesis). We would like to emphasize not only the positive effect of therapy, but also the patient's successfulness of motivation in the adolescent age. Although the therapy in system of Armeo® is more costly than conventional methods, successfulness of the treatment has a very high rate, as indicated by other authors in their articles. As we know, we can never completely get a patient with hemiparesis back to full health, but we can help them to improve the function and self-sufficiency of paretic upper extremity with interesting non-robotic therapy with Armeo® device.

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