

OPEN ACCESS

In vitro T lymphocyte adherence capabilities under the influence of lower induction values (0.1 – 0.01 mT) of 50 Hz external magnetic fields

To cite this article: A Čoček *et al* 2011 *J. Phys.: Conf. Ser.* **329** 012031

View the [article online](#) for updates and enhancements.

You may also like

- [Topographical and mechanical characterization of living eukaryotic cells on opaque substrates: development of a general procedure and its application to the study of non-adherent lymphocytes](#)
Rafael Daza, Julia Cruces, María Arroyo-Hernández et al.
- [Interaction of nanosilver particles with human lymphocyte cells](#)
Alena Zhornik, Ludmila Baranova, Igor Volotovskii et al.
- [The influence of tube voltage and phantom size in computed tomography on the dose-response relationship of dicentric in human blood samples](#)
G Jost, P Lengsfeld, M Voth et al.



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

In vitro T lymphocyte adherence capabilities under the influence of lower induction values (0.1 – 0.01 mT) of 50 Hz external magnetic fields

**A Čoček¹, A Jandová⁴, A Hahn¹, J Mártonová¹, M Ambruš², A Dohnalová³,
M Nedbalová³, J Pokorný⁴**

¹3rd Faculty of Medicine, Charles University in Prague, Department of otorhinolaryngology, Faculty Hospital Královské Vinohrady, Prague, Czech Republic, Šrobárova 50, 100 34, Prague 10, Czech Republic

²3rd Faculty of Medicine, Charles University in Prague, Department of radiotherapy and oncology, Faculty Hospital Královské Vinohrady, Prague, Czech Republic, Šrobárova 50, 100 34, Prague 10, Czech Republic

³Physiological Institute, 1st Medical Faculty, Charles University, Albertov 5, 128 00 Prague 2, Czech Republic

⁴Institute of Photonics and Electronics, Academy of Sciences, Chaberská 57, 182 51, Prague 8, Czech Republic

Abstract. Our research thus far has concerned the impact of external magnetic fields (50 Hz) and low (0.01-10 mT) induction on adherence capabilities of T lymphocytes obtained from the blood of patients with head and neck tumors. We know that the in vitro adherence capability of T lymphocytes towards surfaces in cancer patients is less than that of control. Previously, we have found that exposure to magnetic fields (50 Hz / 0.01-10 mT) increases the capability of T lymphocytes, in larynx/pharynx cancer patients, to adhere in vitro to surfaces, achieving almost physiological values, in not only pre-treatment patients but also those receiving treatment in the course of follow-up. The capability of T lymphocytes in controls (voluntary blood donors) to adhere to surfaces was also increased (50 Hz / 0.01-0.5 mT). The present study concentrates on the significance of the level of magnetic field induction in order to determine whether low induction values can restore T lymphocytes adherence capabilities. Testing a set of 20 patients showed a statistically significant difference ($p < 0.05$) in the in vitro adherence capacity of T lymphocytes between both 0.01 and 0.05, and 0.1 mT induction levels. In the control group (patients diagnosed with chronic sensorineural hearing loss) there was even a statistically significant difference between induction values of 0.05 and 0.01 mT. Therefore, we concluded that lower induction values resulted in a more biologically significant response.

1. Introduction

Our research is concerned with the impact of external magnetic fields of power frequency (50 Hz) and low (0.01-10 mT) magnetic induction on the inhibition of T lymphocyte adherence. The research was carried out on blood obtained from patients with head and neck carcinomas. We know that the in vitro adherence capability of T lymphocytes towards surfaces in cancer patients is less than that seen in controls [1]. We have found that exposure to magnetic fields (50 Hz / 0.01 – 10 mT) increases the capability of T lymphocytes from larynx / pharynx cancer patients, to adhere, in vitro, to surfaces, achieving almost physiological values, not only pre-treatment patients but also in those receiving treatment in the course of follow-up [2]. Also, T lymphocyte adherence capabilities in a control group of voluntary blood donors were previously shown to be increased by exposure to magnetic fields (EMF).

The present paper concentrates on the significance of the level of magnetic field induction (0.1 – 0.01 mT) to determine if low induction values can significantly influence T lymphocytes adherence capabilities relative to higher inductions values.

2. Materials and Methods

T lymphocyte adherence inhibition tests were simultaneously carried out with and without an EMF, using comparable inductions, such that T lymphocyte capabilities of individual patients could be compared, i.e. comparing the adherence capability with and without magnetic fields of 0.1 mT, 0.05 mT, and 0.01 mT ($n = 20$; 13 males, mean age 61 years, range 45-76 years, 7 females, mean age 68 years, range 44-80 years). The control group consisted of patients with chronic sensorineural hearing loss. The group was matched for age and morbidity ($n = 30$, 13 males, mean age 59 years, range 40-68 years, 17 females, mean age 69 years, range 52-71 years).

2.1. Preparation of T lymphocytes

A venous blood sample (8 ml) was transferred to a test-tube containing 500 units of heparin, and incubated for 30 minutes at a temperature of 37 °C in thermostat. The leukocyte-rich plasma was transferred to another test-tube using a dropper. The contents were diluted with redistilled water back to the original volume. The tube was then centrifuged at 2,500 rpm for 20 minutes. The supernatant was removed and leukocyte sediment diluted with 1 ml of physiological saline (NaCl 0.9 %) and bubbled with a Pasteur pipette. This achieved the immediate lysis of the remaining erythrocytes. Diluted leukocyte sediment was filtered through a nylon filter column to achieve a pool containing 92% T lymphocytes and 8% other cells [3]. The filtrate was transferred to a Bürker counting chamber and diluted to obtain 4×10^6 cells per one ml of solution [4]. This process did not affect T lymphocytes.

2.2. Preparation of organ-specific antigen (immunoactive fraction of malignant tumors)

Organ-specific antigen (an immunoactive fraction of a malignant tumor) was obtained from peroperative malignant tumor tissue. The tissue was stored frozen with dry nitrogen. After thawing, the tissue was homogenized and the product was cryolyzed (–60 and +40 °C) three times. Thereafter, the homogenized product was centrifuged at 3.5×10^3 G, at + 40 °C, for 60 min. Subsequently, the supernatant containing proteins, nucleoproteins, nucleic acids and low-molecular weight components was extracted for 15 minutes using phenol (1:1 ratio) in the presence of 1% sodium dodecyl sulfate and 0.01% 8-hydroxyquinoline to achieve a pH of 7 – 7.5.

The solution was then centrifuged at 3×10^3 G. Afterwards the water phase was removed and shaken with phenol. The water phase – a mixture of cytoplasmic ribonucleic acids – was separated in a cylinder filled with oligo-dT-cellulose (enriched with deoxythymidine), which has an affinity towards polyadenylate i-RNA residues. The residues obtained in the cylinder were rinsed with water. The eluate was subsequently purified using high-pressure gel chromatography. The cylinder was filled with Separon-Hema-300 glc® gel, containing a suspension of 2-hydroxyethyl-methacrylate with ethylene dimethacrylate with an exclusion limit of 3×10^5 kDa.

Detection was performed using an R 043 refractometer and a variable wavelength UV spectrophotometer. A saline solution was used as the mobile phase. Immunologically active and organ-specific antigens were obtained in the 25 – 50 cm³ fraction evaluated at a 340 nm wavelength.

2.3. Preparation of nonspecific antigen (LDV antigen – immunoactive fraction from inbred mice serum)

An immunologically active fraction was obtained from the serum of inbred mice from the C3H/H2K strain infected with the lactate dehydrogenase virus (LDH), which produces lactate dehydrogenase isoenzymes (NAD⁺ 1.1.1.27 oxidoreductase). The plasma, obtained from heparinized blood, was twice centrifuged for 20-30 minutes at $3 - 3.5 \times 10^3$ G. The separated supernatant was subjected to further centrifugation for one to two minutes at $9 - 11 \times 10^3$ G. The sediment was then suspended in

buffer (pH of 7 – 7.4) consisting of tris (hydroxymethyl)-aminomethane-NaCl and the dinatrium salt of ethylene-diamintetra acetate acid in a minimal volume (max = 1 ml). The sediment was divided, using a saccharose density gradient, and the area corresponding to 32 – 38% of the saccharose mass, with a maximum of 35%, was retrieved. After dilution in the above-mentioned buffer, in a 1:0.5 – 1.5 volume ratio, the sample was centrifuged for 50 – 70 minutes at $50 - 100 \times 10^3$ G. Samples were stored after obtaining the last sediment, at 2 – 6 °C. The obtained sediment was diluted in 1.5 – 3 ml of physiological saline and subjected to high-pressure gel chromatography. Fractions corresponding with a molecular mass of 3.0×10^5 to 1.5×10^4 were immunologically active. Level centrifugation was applied for 180 – 210 minutes at 150 and 180×10^3 G. The stationary phase of high-pressure chromatography utilized Sepharon-Hema-glcR, which is a suspension copolymer of 2-hydroxyethyl-methacrylate with ethylene dimethacrylate with a column exclusion limit expressed by a molecular mass of 3.0×10^5 , with a gel particle size of 32 – 40 μm . Detection was performed using a differential refractometer and an UV spectrometer with variable wavelengths. A physiological saline was employed as a mobile phase and the fraction was entrapped at a wavelength of 340 nm. This resulted in an immunologically pure fraction.

2.4. Sinusoidal magnetic field (EMF)

A EMF was generated in a cylinder with a diameter of 30 cm and height of 33 cm. Since the magnetic field is maximum (100%) at the center of the cylinder, test tubes (green SiAl glass) containing the T lymphocyte suspensions were positioned at the center of the cylinder. The magnetic field thus generated had induction levels of 0.1 mT, 0.05 mT and 0.01 mT. The non-homogeneity of the field within the test tube containing T lymphocytes was in the vicinity of 1%. The oscillating magnetic field had a sinusoidal form with small higher harmonics components, as verified with an oscilloscope. Field exposure lasted 45 minutes, during which samples were collected; the temperature during the experiment was 37 °C. An Immune response was triggered by mixing with a specific or nonspecific (viral) antigen. The antigen was introduced to the T-lymphocyte suspension immediately before exposure to the magnetic field.

2.5. Measurement and evaluation of results

The T lymphocyte suspension, prepared as described above, was mixed with an organ-specific or viral antigen in the following ratio: four parts T lymphocyte suspension to one part antigen. The suspension was left to sediment in a green SiAl glass test tube. Sixty minutes later the number of free non-adhering T lymphocytes (NAL), i.e. those that had not adhered to the glass test tube, were counted. The results were recorded in % (100% represents the absence of T lymphocyte adherence). Physiological NAL values in healthy people can be as high as 33%; values topping 41% are considered pathological [5].

2.6. Statistical evaluation

To assess whether statistically, the NAL values were significantly different (using low-induction field exposure (0.1, 0.05 or 0.01 mT)) compared to those without exposure, as well as between different inductions, we used variance analysis for repeated measurements and the t test for two dependent selections ($p < 0.05$) with a Bonferroni correction to test the pairs. We also evaluated the control set (patients with chronic sensorineural hearing loss) in the same way. In order to determine if statistically, the control NAL values were significantly different from the tested set for the same magnetic induction values, or in the absence of an EMF, we used a t test for two independent sets ($p < 0.001$).

3. Results

We examined a single batch of patients for their adherence capability to in vitro T lymphocytes without exposure to an EMF and with exposure to an EMF (induction levels of 0.1 mT, 0.05 mT and 0.01 mT). A statistical description of the set is available in Table 1.

Table 1. The basic statistical characteristics of values of adherence of T lymphocytes of patients with larynx/pharynx cancer without and after magnetic field exposure (0.1 mT, resp. 0.05 mT, 0.01 mT) and result of ANOVA test (n=20)

	induction (mT)	n	average (NAL)	standard deviation	standard error	min. value	max. value	ANOVA p
LDV	0	20	94.7	14.7	3.3	41.7	100	0.001
	0.1	20	61.0	19.5	4.4	33.1	83.3	
	0.05	20	37.6	16.9	3.8	16.6	68.3	
	0.01	20	34.2	15.0	3.4	6.6	68.3	
ORG	0	20	90.7	14.7	3.3	34.8	100	0.001
	0.1	20	57.6	17.2	3.9	24.4	91.4	
	0.05	20	32.3	12.4	2.8	14.6	55.6	
	0.01	20	29.7	12.1	2.7	6.6	55.6	

LDV nonspecific (LDV) antigen

ORG organ-specific (tumor) antigen

NAL non-adhering T lymphocytes

The results of mutual T tests with Bonferroni corrections, using induction pairs, is presented in Table 2. (graphically represented in Figure 1.).

Table 2. The results of mutual t test with Bonferroni corrections between pairs with different magnetic inductions (n=20)

test with	Without EMF			0.1 mT		0.05 mT
	0.1 mT	0.05 mT	0.01 mT	0.05 mT	0.01 mT	0.01 mT
LDV	x	x	x	x	x	NS
ORG	x	x	x	x	x	NS

EMF magnetic field

LDV nonspecific (LDV) antigen

ORG organ-specific (tumor) antigen

X statistically significant difference at a 0.05 level ($p < 0.05$)
 NS statistically nonsignificant difference

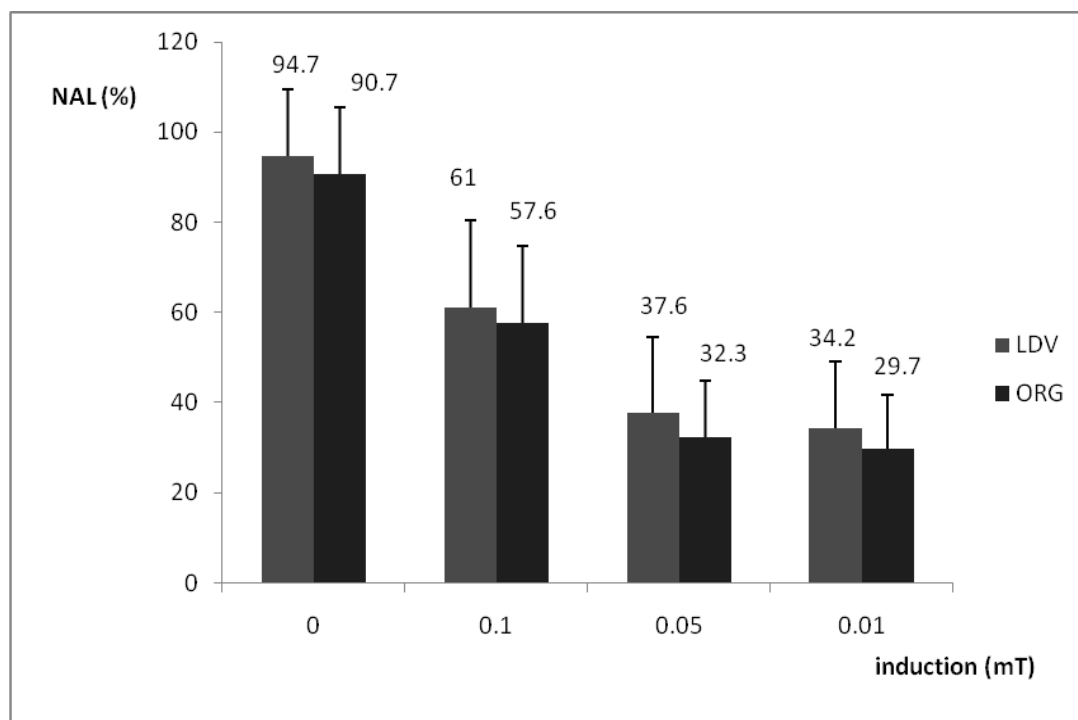


Figure 1. The average values of non-adhering of T lymphocytes of patients with larynx/pharynx cancer before and after magnetic field exposure (0.1 mT, resp. 0.05 mT, 0.01 mT; $n=20$)

NAL non-adhering T lymphocytes
 LDV nonspecific (LDV) antigen
 ORG organ-specific (tumor) antigen

Control group consists of patients with chronic sensorineural hearing loss ($n = 30$). We also ascertained, within this control group, whether the monitored magnetic induction values of 0.1 mT, 0.05 mT or 0.01 mT had a statistically significant impact on increasing T lymphocytes surface adherence capabilities (compared to adherence without an EMF, of course only with respect to a nonspecific antigen). Statistical characteristics are shown in Table 3. and the results of mutual tests with the Bonferroni correction between pairs are shown in Table 4.

Table 3. The statistical characteristics of adherence of T lymphocytes in presence of nonspecific (LDV) antigen without and with magnetic field exposure (0.1 mT, 0.05 mT, 0.01 mT). Result of ANOVA (control group – patients with sensorineural hearing loss), (n=30).

	induction (mT)	n	average (NAL)	standard deviation	standard error	min. value	max. value	ANOVA p
LDV	0	30	27.2	9.8	1.8	15.9	55.9	0.001
	0.1	30	14.5	5.5	1.0	6.6	30.3	
	0.05	30	6.9	2.8	0.5	3.2	13.9	
	0.01	30	6.1	2.6	0.5	2.7	12.9	

LDV nonspecific (LDV) antigen

NAL non-adhering T lymphocytes

Table 4. The results of mutual t tests with Bonferroni corrections between pairs with different magnetic inductions (control group – patients with sensorineural hearing loss), (n=30).

test with	Without EMF			0.1 mT		0.05 mT
	0.1mT	0.05 mT	0.01 mT	0.05 mT	0.01mT	0.01mT
LDV	x	x	x	x	x	x

EMF magnetic field

LDV nonspecific (LDV) antigen

X statistically significant difference at 0.05 level ($p < 0.05$)

In conclusion, we examined whether statistically, T lymphocyte adherence values significantly differed between cancer patients and those in the presence of a nonspecific antigen with EMF exposure (inductions of 0.1 mT, 0.05 and 0.01 mT) and without EMF exposure. A basic statistical characterization of pre- and post-EMF exposure for both the control group and patients is given in Table 5 and graphically presented in Figure 2.

Table 5. The basic statistical characterization of adherence of T lymphocytes in presence of nonspecific (LDV) antigen pre- and post-magnetic field exposure (0.1 mT, 0.05 mT a 0.01 mT) for both the control and the patient group and result of t test between the control and the patient group

induction (mT)		n	average (NAL)	standard deviation	t-test	p
0 mT	control	30	27.2	9.8013	-19.5	0.001
	tumor	20	94.7	14.7138		
0.1 mT	control	30	14.5	5.4657	-10.4	0.001
	tumor	20	61.0	19.4938		
0.05 mT	control	30	6.9	2.7584	-8.05	0.001
	tumor	20	37.6	16.874		
0.01 mT	control	30	6.1	2.6363	-8.28	0.001
	tumor	20	34.2	15.0155		

Control control group (patients with sensorineural hearing loss)

Tumor group of patients with larynx/pharynx cancer

NAL non-adhering T lymphocytes

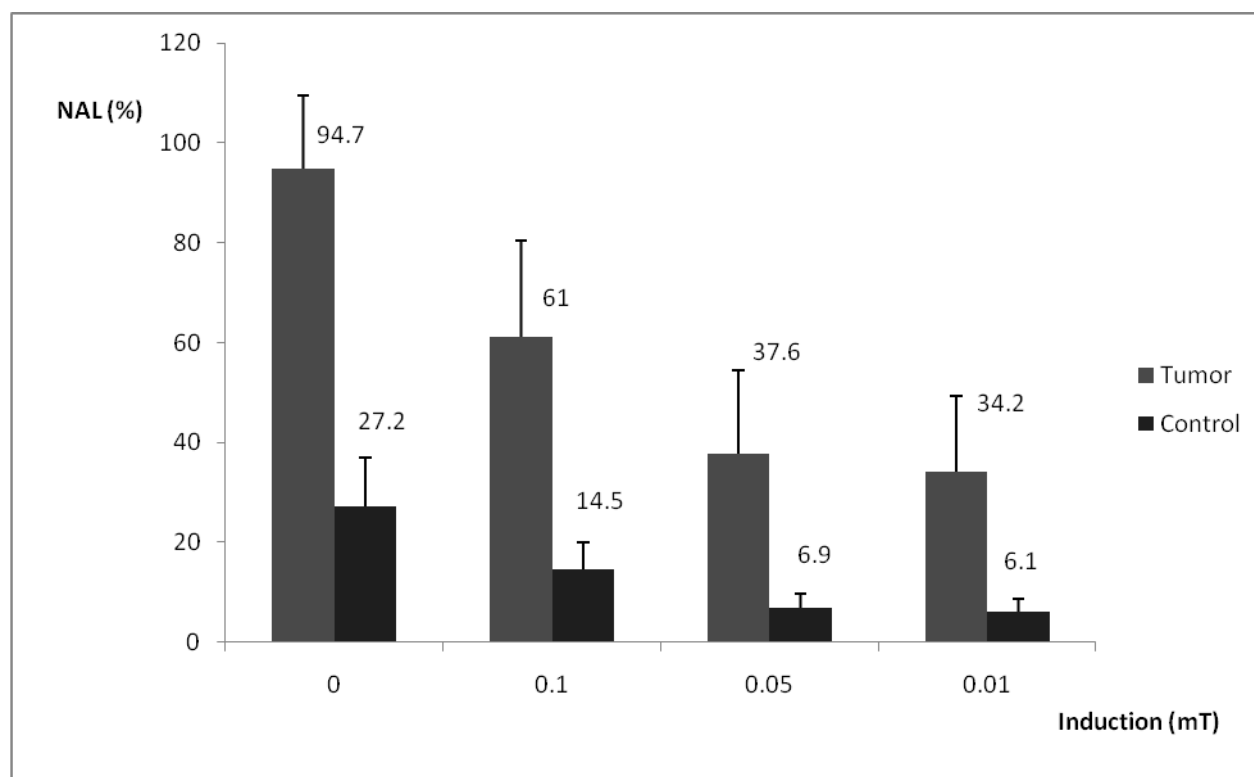


Figure 2. The average number of non-adhering T lymphocytes without and with magnetic field exposure (0.1 mT, 0.05 mT a 0.01 mT) in both tumor (n=30) and control group (n=20) in the presence of nonspecific (LDV) antigen

NAL	non-adhering T lymphocytes
Control	control group (patients with sensorineural hearing loss)
Tumor	group of patients with larynx/pharynx cancer

4. Discussion

T lymphocytes transfer information about mitochondrial dysfunction from target cells. The effects of specific and the nonspecific antigens on T lymphocytes are assumed to reflect mitochondrial dysfunction [6]. Mitochondrial dysfunction is a hallmark of cancer processes [7,8,9].

The relationship between mitochondria and cancer was the reason a nonspecific LDH antigen was used in this research. Riley and Wroblewski (1960) [10], and Rowson and Mahy (1975) [11] analyzed the effect of LDH virus infections and the origin of tumors. They found a correlation between the fast-growing Ehrlich carcinoma, in mice, and LDH activity in the serum – specifically increased levels of isoenzyme 2 and 3 in the initial phase, following tumor implantation, and isoenzyme 4 and 5 during the fast growth phase of the tumor. The lactate hydrogenase virus (LDV, LDH virus, Riley virus) is characterized as a slow virus. It is an ideal cellular parasite. At the start of infection, in mice, it can be detected as dark particles associated with mitochondria. Then the RNA replicates, and when leaving the cell, ceases to be alien to the body, and becomes indigenous. It parasitizes the energy production system of the infected cell [6].

Cellular adhesion capability is an important cell property, crucial to the organization of tissues. A loss of this cell property among cancer cells leads to their proliferation; therefore an adherence inhibition test was used.

We found statistically significant differences in the adherence capabilities in response to all induction levels vis-à-vis adherence capabilities in the absence of an magnetic field ($p < 0.001$). At a statistical significance level of $p < 0.05$, there was a difference between the adherence capabilities of T lymphocytes not exposed to an magnetic field and T lymphocytes exposed to an magnetic field, at all measured induction levels. Significant differences were also found between induction levels, except for between 0.05 mT and 0.01 mT. In other words, even micro-induction levels of 0.05 mT and 0.01 mT influence the adherence capability of T lymphocytes more than levels as low as 0.1 mT. The results were identical for nonspecific and organ-specific antigens.

An identical test was carried out with the control group (T lymphocytes of sensorineural hearing loss patients without current or an anamnestic malignity). The EMF, at all induction levels (0.1 mT or 0.05 mT and 0.01 mT), increased in vitro T lymphocytes adherence capabilities in the presence of a nonspecific antigen at a statistical significance level of $p < 0.001$ compared to cells tested in the absence of an EMF. While testing pairs (without an EMF and individual magnetic induction levels) we managed to demonstrate an even more pronounced effect regarding lower vs. higher magnetic induction values. Without residual values at the defined statistical significance level, the control group proved that the lower induction level, the greater the achieved biological efficiency (i.e. increased capability of T lymphocytes to adhere in vitro).

Finally we tested the differences of T lymphocyte adherence in 20 larynx / pharynx cancer patients against controls ($n = 30$) in the presence of a nonspecific antigen with (0.1 mT, 0.05 mT, and 0.01 mT) and without exposure to an EMF. We found that the T lymphocyte adherence capabilities of controls was, under all conditions, always higher than that of cancer patients at a statistical significance level of $p < 0.001$. It follows from the above that T lymphocyte surface adherence capability in ailing patients is lower than that of T lymphocytes in controls. Under the influence of an magnetic field with defined magnetic induction levels, the adherence capability of the both groups increased; however, control T lymphocytes always had greater in vitro surface adherence capabilities.

This work is the first research on the influence of defined EMFs on T lymphocyte in vitro surface adherence capabilities. T lymphocytes in vitro surface adherence capability is a very complicated problem that is dependent on many factors. EMFs can influence adherence in two ways: (1) they can directly improve T lymphocyte surface adherence capabilities and (2) they can indirectly

improve adherence capabilities by disrupting processes that inhibit adherence. Even though various physical mechanisms between interactions of electromagnetic and magnetic fields and biological systems have been proposed, a detailed assessment that offers a complete explanation for the observed biological effect is still missing [12,13]. Fröhlich emphasized that his theory was only a hypothesis, which needed to be clarified and verified experimentally. So far it is impossible to explain why lower EMF induction values have greater biological effects.

5. Conclusion

The results of the present study demonstrated that an magnetic field, with extremely low magnetic induction values (0.01 – 0.1 mT), improves the in vitro adherence capabilities, to solid surfaces, of T lymphocytes taken from patients with larynx / pharynx cancer. Improvement was also demonstrated with controls (patients diagnosed for chronic sensorineural hearing loss).

Testing a group of 20 patients showed a statistically significant improvement in adherence with induction levels of 0.05 and 0.01 mT compared to 0.1 mT. In the control group, a statistically significant difference occurred even between induction values of 0.05 and 0.01 mT. Therefore our paper has corroborated the literary assumption of lower induction values lead to biologically more significant responses.

Although it is not possible to assume that the exposure to an external electromagnetic and magnetic fields represents treatment for cancer transformed cells, this project demonstrates that an external magnetic field can affect a specific phenomenon, in this case, in vitro T- lymphocyte adherence.

References

- [1] Čoček A, Hahn A, Jandová A, Nedbalova M and Dohnalova A 1997 Long-term monitoring of an immune response to tumor and LDH viral antigen in patients with carcinoma of otorhinolaryngologic origin. *Med. Sci. Monit.* **3** 657-60
- [2] Čoček A, Hahn A, Ambuš M, Dohnalova A, Jandova A and Pokorny J 2008 Changes of Leukocyte Adherence Ability Under the Influence of Magnetic Field in the Course of Treatment of Patients with Laryngeal and Pharyngeal Carcinoma. *Electromagn. Biol. Med.* **27** 1-12
- [3] Tlaskalová-Hogenová H, Čoupek J, Pospíšil M, Tučková L, Kamýnková J and Mancal P 1980 Affinity chromatography of human lymphocytes on Spheron immunoadsorbent columns. *J. Polymer. Sci.* **68** 89-95
- [4] Jandová A, Hurych J, Pokorný J, Čoček A, Trojan S, Nedbalová M, Dohnalová A 2001 Effects of Sinusoidal Magnetic Field on Adherence Inhibition of Leukocytes. *Electro- and Magnetobiology* **20** 397 – 413
- [5] Čoček A, Hahn A, Nedbalová M and Jandová A 1995 Assessment of the immune reaction to the Riley virus (lactate-dehydrogenase virus, LDH virus, LDV) in head and neck oncology. *Otolaryngology (Prague)* **44** 40-43
- [6] Jandová A, Pokorný J, Kobilková J, Trojan S, Nedbalová M, Dohnalová A, Čoček A, Mašata J, Holaj R, Tvrzická E, Zvolský P, Dvořáková M and Cifra M 2009. Mitochondrial Dysfunction. *Neural Netw. World* **19** 379-91.
- [7] Warburg O 1956 On the Origin of Cancer Cells. *Science* **123** 309
- [8] Cuezva J, Krajewska M, López de Heredia M, Krajewski S, Santamaría G, Kim H, Zapata JM, Marusawa H, Chamorro M and Reed JC 2002 The bioenergetic signature of cancer: A marker of tumor progression. *Cancer Res.* **62** 6674-81
- [9] Carew J and Huang P 2002 Mitochondrial defects in cancer. *Mol. Cancer* **1** 9-20
- [10] Riley V and Wroblewski F 1960 Serial Lactic dehydrogenase activity in plasma of mice with growing or regressing tumors. *Science* **132** 151-52
- [11] Rowson and Mahy 1975 *Lactic dehydrogenase virus* vol 13, ed S Gard and C Hallauer (Wien, New York: Springer) p 121

- [12] Markov MS 2007 Magnetic Field Therapy: A Review. *Electromagn. Biol. Med.* **26** 1-23
- [13] Simkó M and Mattsson MO 2004 Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. *J. Cell. Biochem.* **93** 83-92