

Immunophenotyping of Peripheral Blood Lymphocytes to person Exposed to electromagnetic fields

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Abstract

Background: There is considerable evidence relating electromagnetic fields (EMFs) exposure to reduce immune system competence and these changes associated with cell growth control, differentiation and proliferation of cells of immune system, trans membrane signaling cascades, gap junction communication, immune system action.

Objective: to investigate the Phenotyping of peripheral blood lymphocytes of volunteer's exposed at least 10 years to electromagnetic fields (EMFs) induced by transmission power lines in their residential area ,

Subjects and methods: forty five volunteer's aged between 25 and 65 Years, exposed for at least 10 years to electromagnetic fields (EMFs) induced by transmission power lines in their residential area and Fifteen male of similar age unexposed, away from the transmission power lines as a control group were used in this study . The electromagnetic fields (EMFs) (with range of 50 Hz) beside the homes of the volunteer's. This study carried out in three different areas as of Baghdad included (Al -Bladyat, Hay al-adel and Al-Dorra cities) , The groups of this study were divided into three sub- groups according to the distance away from the towers of transmission

power lines (1) range: from 1 to 25 meter (2) from 25 to 50 meter (3) from 50 to 75 meter . Phenotyping of peripheral blood lymphocytes had been done by direct immunofluorescent microscopy using anti -CD 3(for T-cells detection), anti-CD4 (for T- helper-cells), anti CD8 (for T-cytotoxic/suppressor cells), anti CD21 (for B-cells) and anti CD56 (for natural killer cells).

Results: A statistically significant reduction of PBL percentage bearing CD3,CD4,CD19,CD56($P<0.01$) between the exposed volunteers and control ;except CD8 which showed no significant difference between these groups . the mean percentage of CD4⁺/CD8⁺ ratio in exposed volunteers groups was significantly ($P<0.01$) lowered in comparison to control group .

Conclusion: we postulated that the chronic exposure to electromagnetic fields from power lines caused suppression in immune system.

Keywords: Electromagnetic field, CD4, CD8, CD21, CD56, Lymphocytes, phenotyping

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Introduction

Biology has preceded electronic physics because brains and cells use oscillating ion currents for controlling the release of neurotransmitters and in the cell to cell communication systems. Biological systems detect and respond to external ELF signals using their built-in receiving and decoding systems (cell-to-cell communication)⁽¹⁾.

In the last five years a large number of experiments have clearly shown various biological and medical effects of (EMFs) at the cellular level, Both human and animal studies report large immunological changes after exposure to environmental levels of electromagnetic fields (EMFs)⁽²⁾.

There is considerable evidence relating EMFs exposure to reduce immune system competence. Many of these evidence show that EMFs -caused changes in processes associated with cell growth control, differentiation and proliferation of cells of immune system, trans membrane signaling cascades, gap

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junction communication, immune system action, rates of cell transformation; which are biological processes of considerable interest to scientists who study the molecular and cellular basis of immune system⁽³⁾.

Lymphocytes phenotyping is part from the mirror image of the immunity, and can give an idea of the immunological status.

Specific reports from studies on exposures to various types of modern EMFs was found over-reaction morphological alterations of immune cells; epically mast cells, enlarged and profound increased of mast cells in the upper skin profound increases in mast cells in the upper skin layer accompany with degranulation of mast cells (electrohypersensitivity) , presence of biological markers for inflammation that are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability decreased the number of NK cells(CD56) and T lymphocytes(CD3)⁽⁴⁾

Andrew and his collages demonstrated that a 6-weeks exposure to EMFs induces a significant decrease of CD3+, CD4+, CD8+, CD19 and Natural killer cells Population Linear regression Analysis demonstrated a dose-response relationship between the changes in the immune functions and the EMFs intensities⁽⁵⁾.

Another study showed a significant decline in the absolute numbers and ratios of CD4+/CD8+ lymphocytes in favor of CD8+ cells of cows at farm A housed under a 320 kV transmission line exposed to 3.28 T magnetic fields compared to cows at a distant 198 meter Farm B considered zero exposed⁽⁶⁾.

Another study conducted on workers of TV re-transmission and satellite communication center found

decrease in the level of serum IgG and IgA; decreased the count of peripheral blood CD8, CD4, CD56 cells and decreased the ratio of (CD4/CD8) cells⁽⁷⁾. Dabrowski *et al.* exposed samples of mononuclear cells isolated from peripheral blood of healthy donors to 1,300 MHz; the results indicate that response of lymphocytes to phytohemagglutinin (PHA) as well as the T-cell suppressive activity (SAT index) and the saturation of IL-2 receptors significantly decreased in the culture supernatants. Also, the concentration of interleukin (IL)-10, IFN γ , TNF was significant decreased in the culture⁽⁸⁾.

Material and methods

Subjects and methods:

Fourty five male volunteer's aged between 25 and 65 Years, for at least 10 years exposed to electromagnetic fields (EMFs) induced by transmission power lines in their residential area were used in this study . The EMFs was (with range 50 Hz) beside their homes this study carried out in three different cities of Baghdad included. (Al –Bladyat, Hay Al-Adel and Al-Dorra cities) during Feb. 2008, they were divided into three sub-groups according to distance from the towers of transmission power lines (1) range: from 1 to 25 meter (2) from 25 to 50 meter (3) from 50 to 75 meter.

Fifteen male of similar age. apparently healthy, with smoking habits unexposed, away from the transmission power lines about 500 meter was used as a control group. Five parameter were used for detection the effects of electromagnetic fields on the immune system included (CD 3, CD4, CD8, CD19, and CD56).

Isolation of Peripheral Blood lymphocytes:

Five ml of blood sample was drawn from each volunteer by vein puncture using disposable syringe containing 10-20 units of heparin/ml. Lymphocytes were isolated by density gradient sedimentation as described by Boyum. Blood samples were diluted 1:1 ratio in RPMI 1640 media, then layered over 2ml of Ficoll. Cooled centrifugation was carried out at 18 °C for 20 minutes at 3000 rpm. Then the interphase was collected, by Pasteur pipette, Ten µl of the cell suspension was applied to each well on immune fluorescence slides, the slides were foiled with parafilm and kept at -20°C until they used⁽⁹⁾.

Immunostaining of lymphocytes:

The determination of lymphocytes phenotyping was performed by direct immune fluorescence technique as described by⁽¹⁰⁾.

Ten µl of monoclonal antibodies includes (anti CD 3, anti CD4, antiCD8, anti CD19, and antiCD56), was dispensed over the spot in the slides. Counting of cells was performed using fluorescent microscope. A suitable countable field was located and the numbers of cells exhibiting fluorescence were counted, the calculation was made as follows;

$$\text{Percentage of labeled cells} = \frac{\text{Number of labeled cells}}{\text{Total no. of cells (200)}} \times 100$$

Measurement of ELF Frequency:

The electromagnetic fields were measured into three sub-sections according to distance (1) range: from 1 to 25 meter (2) from 25 to 50 meter (3) from 50 to 75 meter from the tower of transmission power lines. By using (Gauss Meter), in milli Gauss units, or micro Tesla (µT), 10 mG equals 1 µT.

Statistical Analysis

Experimental data were analyzed using statistical software SPSS 10.0 for Windows. Significance between control and samples was determined using Student's t-test. P value < 0.05 was considered statistically significant.

Results

Mean percentage of isolated peripheral blood lymphocytes for the exposure volunteers groups and control group was illustrates in table (1). The results demonstrate a significant reduction ($p < 0.01$) in the mean percentage of CD3 cells (45.03%), In the all exposed volunteers groups to EMFs in all cities and in all distance in comparison to the mean percentage of control group CD3 (61.35 %). as shown in figure (1).

A significant decrease ($p < 0.01$) in CD 4 cells mean percentage (26.25%) was observed in exposed groups in all cities and in all distance as compared with the mean percentage of CD 4 in control group (42.72%) figure (2).

A significant decrease of mean percentage of CD21 cells ($p < 0.01$) was observed (8.01%), In the exposed groups in all cities and in all distance in comparison with the CD21 mean percentage in control group (14.83%), figure (3).

The results indicated that the mean percentage CD8 cell was the only one that had not differed significantly in the study groups as compared to controls (26.72%), (25.41%) respectively, figure (4).

Significant reduction in mean percentage of CD 56 cells ($p < 0.01$) was observed (7.22%) in exposed groups in all cities and in all distance as compared with the mean percentage of CD56 cells in control group (12.85%), figure (5).

CD4/CD8 ratio was of special importance because it presents an index that refers to the immunological balance between T-helper cells and T-cytotoxic cells in the immune system.

The result demonstrated that the mean percentage of CD4⁺/CD8⁺ ratio in the all exposure volunteers group (0.984 %)was significantly (P<0.01) lower in comparison to that in control groups(1.681%) as shown in figure (6).

Finally the result of measurement of electromagnetic field by Gauss meter for different site from the towers of

transmission power line showed significant reduction with the distance, the values of Gauss meter reading reduced when the distance increased from the towers regression, and we can get the highest reading under the towers of power line 1.01, 0.987 ,and 1.142 μ T in Al-Dora ,Hay al-adel and Al- Bldyat Respectively and the lowest reading 0.34 ,0.43, 0.61 μ T in 75 meter away from the towers of transmission power line in Al-Dora ,Hayal-Adel, and Al-Bldyat Respectively. As shown in figure (7).

Table 1: Distribution of mean percentage of CD3+, CD4+, CD8+, CD21+ and CD 56+ lymphocytes in exposed volunteers and control group in the cities under the study.

CD marker	Distance meter	AL-BLDYAT	AL-ADEL	AL-DORRA	CONTROL
CD 3	0-25 m	46.2	41.8	38.3	61.35
	25-50 m	49.2	44.5	43.2	
	50-75 m	48.5	49.4	44.2	
CD 4	0-25 m	24.9	26.3	22.8	42.72
	25-50 m	27.2	26.3	25.4	
	50-75 m	28.3	28.7	26.4	
CD 8	0-25 m	27.2	26.6	25.4	25.41
	25-50 m	26.4	27.4	28.1	
	50-75 m	29.5	26.1	23.8	
CD 21	0-25 m	7.2	7.4	6.5	14.83
	25-50 m	9.8	8.5	8.4	
	50-75 m	8.4	7.1	8.8	
CD 56	0-25 m	8.5	7.1	5.4	12.85
	25-50 m	9.6	7.2	6.4	
	50-75 m	7.8	6.3	6.7	

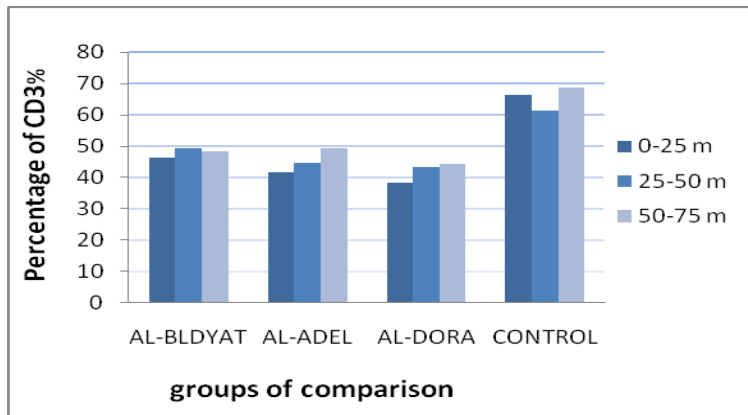


Figure1: the mean percentage of CD3 marker of PBL in all exposure and control groups with distance in meter

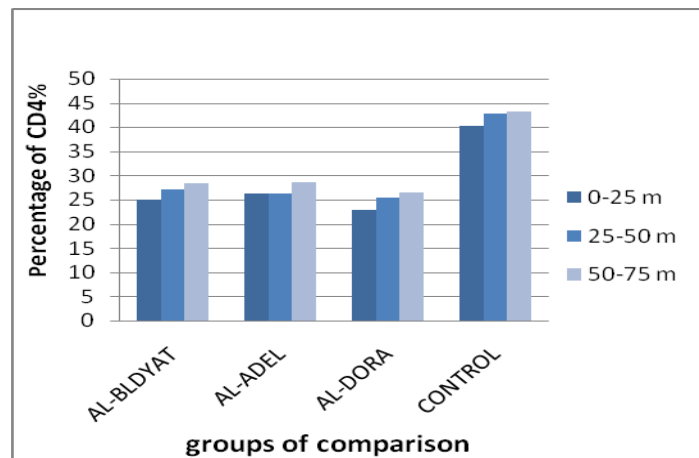


Figure 2: the mean percentage of CD4 marker of PBL in all exposure and control groups with distance in meter.

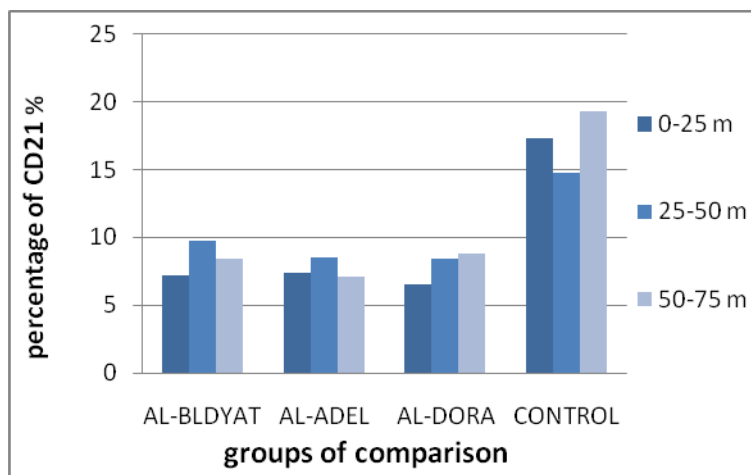


Figure 3: the mean percentage of CD21 marker of PBL in all exposure and control groups with distance in meter

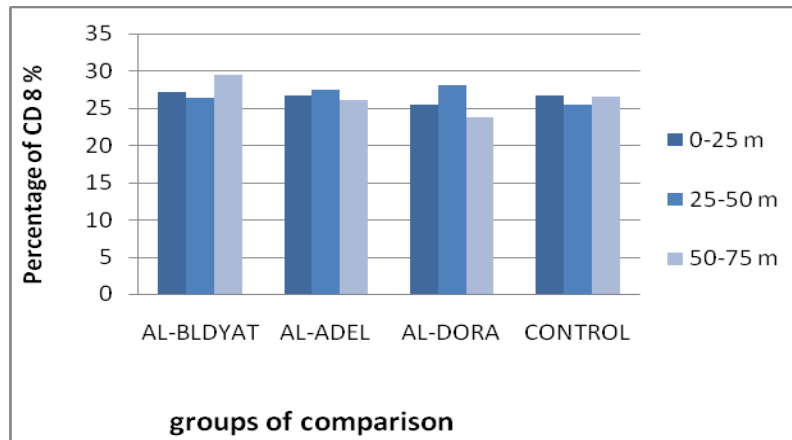


Figure 4: the mean percentage of CD8 marker of PBL in all exposure and control groups with distance in meter

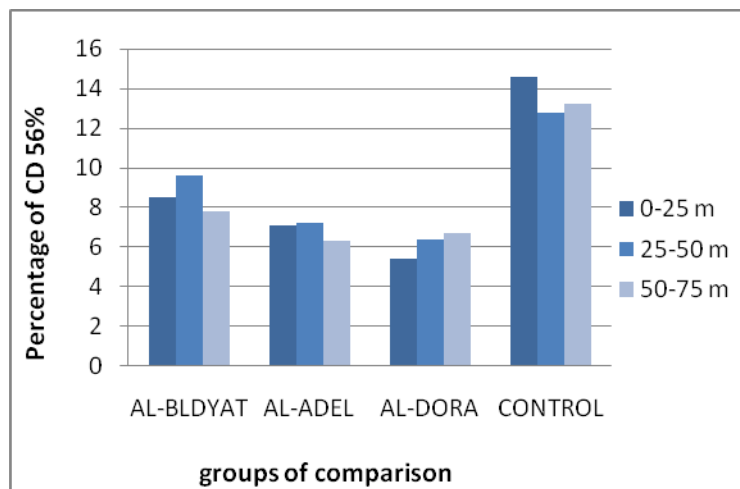


Figure 5: the mean percentage of CD56 marker of PBL in all exposure and control groups with distance in meter

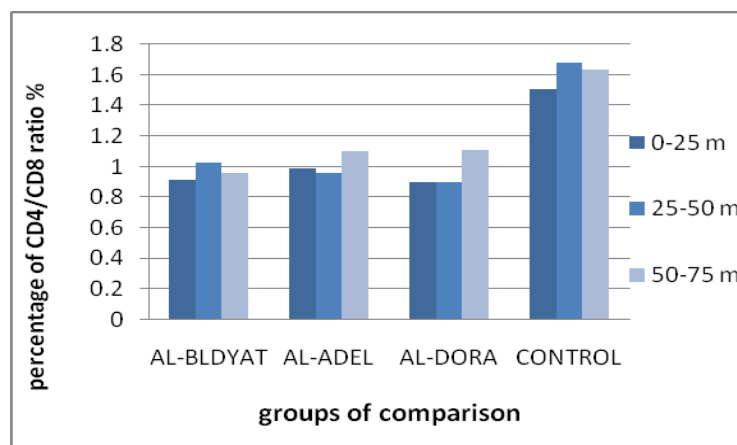


Figure 6: the mean percentage of CD4/CD8 ratio in all exposure and control groups with distance in meter

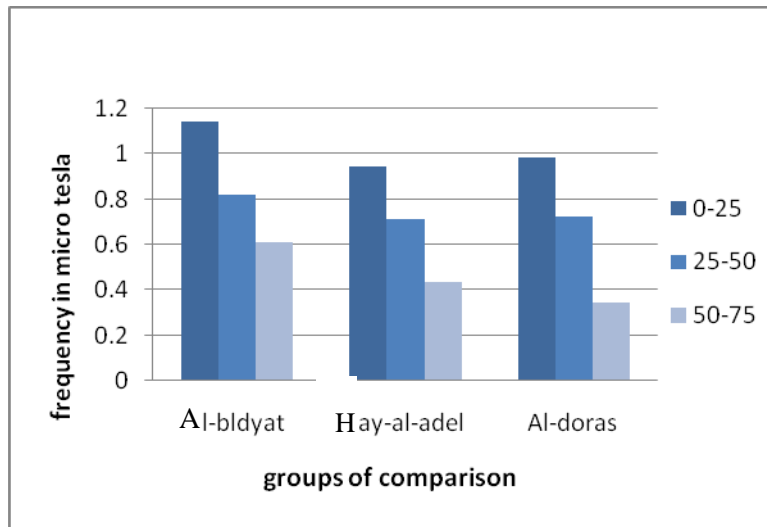
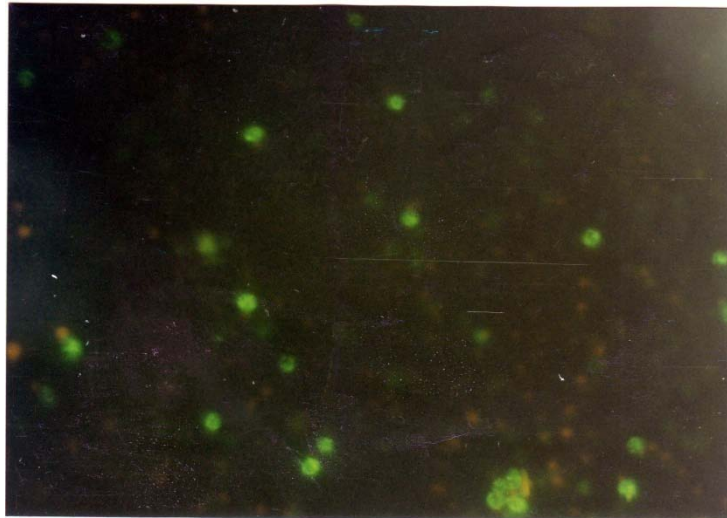


Figure 7: Measurement of EMFs in different site from the towers of power lines



A picture showed the lymphocytes subpopulation stained by direct immunofluorescence

Discussion

Surface CD markers of BPL are an important index for any research to clarify which immune defense mechanism is predominate and where are the weak points through which disease could have established⁽¹¹⁾.

The pivotal idea we can get from PBL phenotype is that all the exposure groups are immunosuppressed when we compared with the control group and this immune suppression which is reflected

by low percentage of different PBL subsets could be result of the electromagnetic fields from transition power lines, as demonstrated by different recent studies⁽¹²⁾.

Modulation signals are one important component in the delivery of EMF signals to which cells, tissues, organs and individuals can respond biologically. It is likely a key factor in determining whether and when

biological reactivity might be occurring⁽¹³⁾.

We hypothesized that the frequency bands between 50 -60 Hz have been to alter immune responses and intercellular communication between lymphocytes such as altering the balance of cytokines, which regulate the growth of cells and determine whether the immune system will produce cells to proliferation and these reflected by the decrease in mean percentage of CD3, CD4, CD21, CD56, and the ratio of CD4/CD8⁽¹⁴⁾.

The cell membrane of lymphocytes contains docking ports on its surface called receptors that allow the cell to pick up distant chemical signals (cytokines, lymphokines, hormones, neurotransmitters) sent by other Cells through the blood stream and local chemical signals generated by components of immune cells, we think that many of these cell receptors also function as antennas for particular frequencies of electromagnetic fields and thus might lead to modulate the cell membrane receptor-mediated enzyme cascade in pre-B lymphocytes, with the implication and programmed-cell-death (apoptosis), cell cycle kinetics, and cytokine expression⁽¹⁵⁾.

Also exposure to EMFs may alter gene and/or protein expression in certain cell types, mRNA functions, immune responses and intercellular communication and this process may altered genes that responsible for the proliferation of different cell type of immune system such as Lymphocytes⁽¹⁶⁾.

There is epidemiologic evidence that extremely low frequency (ELF \leq 50 Hz) magnetic fields (MF) exposure associated with a decrease in melatonin production. (Melatonin is a hormone produced primarily by the pineal gland,

located in the center of the brain), and this hormone has been found to protect cells, hemopoietic system tissues and organs against oxidative damage induced by a variety of free radical generating agents and processes⁽¹⁷⁾. According to this decrease damage to hemopoietic tissue, growth factors, cytokines, and genes involved in, apoptosis, signaling pathways and DNA repair might occur, which may decrease the percentage of lymphocytes subpopulations⁽¹⁸⁾.

Several studies demonstrated that EMFs have genotoxic effects on human and animals. Significant increases in DNA damage including single and double strand breaks and cross-link chromosome conformation and micronucleus formation. Leaks in the membranes surrounding lysosomes could release digestive enzymes, including DNAase (an enzyme that destroys DNA). This explained the serious damage done to the DNA in cells by electromagnetic fields signals⁽¹⁹⁾.

(EMFS) might interfere with regulation of the onset of differentiation and proliferation of B cell and apoptotic processes of actively proliferating cells. This mechanism reflects the low percentage of CD 21 and other subpopulation of lymphocytes⁽²⁰⁾ as shown in the results.

Finally we think that the chronic exposure to electromagnetic fields from power lines caused suppression in immune system which demonstrated in the results from the significant reduction in CD4/CD8 ratio as compared with this ratio in control group because the lower CD4/CD8 ratio the more immune suppression expected⁽²¹⁾. also we think that the more important mechanisms which caused immune suppression included DNA breaks in single or double strands and /or modulation signals

between immunological cells and cytokines whom responsible for the differentiation and proliferation of immune systems such as interleukines ,interferon ,Th1,and Th2.⁽²²⁾ .

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