

# Evaluation of Levels of Macro- and Micro-Nutrients in Workers Exposed to Electromagnetic Fields and Comparison with Levels of Patients with Leukemia

Issa Nourmohammadi<sup>\*1</sup>, Hassan Ahmadvand and Mohammad Taghikhani<sup>2</sup>

<sup>1</sup> Dept. of Biochemistry, College of Medicine, Iran University of Medical Sciences, Center for Cellular and Molecular Biology Research; <sup>2</sup>School of Medical Sciences, TarbiatModarres University, Tehran, Iran

## ABSTRACT

There have been much speculation and debate concerning the effect of electromagnetic fields (EMF) on body systems. Various reports have implicated excessive exposure to EMF to certain forms of leukemia. It has also been reported that EMF may cause alteration in the levels of certain macro- and micro-nutrients such as copper, zinc, selenium, calcium and iron. We have undertaken this study to determine the status of these elements in workers exposed to EMF for more than 10 years and compare these levels with those of patients with leukemia and both groups to a set of matched controls. Statistical analysis revealed meaningful differences in the serum levels of the nutrients under study both in terms of comparison of workers and patients to controls and in comparison of workers to patients. Mean Zn levels in both patient ( $PM\ 0.0002$ ) and worker ( $PM\ 0.006$ ) groups were significantly higher than that of controls. However, statistical analysis of patients to workers revealed no meaningful variation. Selenium in both study groups also showed decreased levels when compared to controls. These results were statistically significant in comparison of patients to controls ( $PM\ 0.0001$ ) and workers to controls ( $PM\ 0.0001$ ). Evaluation of patients to workers also resulted in a significant finding ( $PM\ 0.05$ ). While we do not claim these results to be definitive, they do reflect the possibility that regular evaluation of the status of these elements in workers consistently exposed to EMF may be beneficial in terms of determining the heightened risk of these workers to development of leukemia. *Iran. Biomed. J. 5 (2 & 3): 79-85, 2001*

**Keywords:** Electromagnetic fields, Leukemia, Workers, Trace elements, Calcium

## INTRODUCTION

In recent years, there has been a scientific debate regarding the effects of electromagnetic fields (EMF) on biological systems [1]. Although the basic mechanism of interaction between EMF and cellular function still remains uncertain, there are however practical and beneficial applications of EMF in medicine [2]. Numerous experimental and epidemiological observations show that human populations in industrialized communities experience a universal exposure to radiation from power lines and the low voltage of the home and working environments [3]. Exposure to this environmental phenomenon has prompted concern that it could increase biological disorders including cancer and leukemia in children [4, 5].

Although there are considerable controversies over the validity of any link between EMF and

disturbances on a biological system [6, 7], the number of reports on this newly developed concern continues to make it a reasonable undertaking to develop a definite understanding of the mechanism and its possible consequences at the molecular or even total body level.

The key events arising from exposure to EMF may include alterations in cell membrane activity, effects on various enzyme systems [8] and protein and DNA synthesis [9, 10], interference with the regulation of protein kinase-C [11], alterations in melatonin and hormone levels [12], Ca homeostasis [13] as well as variation on other important ions such as Cu and Zn which are necessary co-factors for many biological reactions [14].

This study was designed to evaluate the status of macronutrients such as Ca and Fe and micro-nutrients such as Cu, Zn and Se in subjects exposed to electromagnetic fields and compare the levels of

<sup>\*</sup>Corresponding Author. Fax: (98-21) 805 4355; E-mail: issanour@iums.ac.ir

these same elements in leukemic patients who had not been exposed to EMF in the workplace. This study was undertaken firstly based on the observations stated above that indicate EMF may affect different aspects of the biochemistry of cells. Secondly, some studies have indicated that EMF may cause leukemia [3, 4]. Thus, it is important to determine the status of trace elements to ascertain if EMF workers are predisposed to leukemia. Thirdly, our preliminary study on several clinical parameters (unpublished data) showed interesting results in workers exposed to EMF (60 Hz, 40  $\mu$ T). Plasma vitamin C level (mean  $\pm$  S.D.) of these workers was  $0.82 \pm 0.2$  mg/dl, significantly lower than that of control group which was  $1.44 \pm 0.27$  mg/dl. Total hemoglobin for EMF-exposed workers was  $12.5 \pm 0.8$  mg/dl and that of control group  $14 \pm 0.7$  mg/dl which was also statistically significant. Total protein in workers was recorded as  $7.5 \pm 0.66$  g/dl and in controls  $9.1 \pm 0.68$  g/dl.

This study thoroughly evaluated micro- and macro-nutrients in these subjects. To the best of our knowledge, there have been rare evaluations and comparisons of patients with leukemia and workers exposed to EMF.

## MATERIALS AND METHODS

A group of power line station workers ( $n = 40$ ) continuously exposed to electromagnetic fields (60 Hz, 40  $\mu$ T) for at least 10 years and patients ( $n = 80$ ) with leukemia were studied. Leukemia patients were diagnosed by expert physicians and on the basis of clinical, morphological, hematological and cytogenetics evaluation, categorized as acute lymphoblastic leukemia (ALL,  $n = 30$ ), acute myeloid leukemia (AML,  $n = 40$ ) and other leukemia patients (LP,  $n = 10$ ). Controls, that did not exposed to EMF, were selected according to sex, age, weight and height to match workers and patients. Blood was collected in mineral free vacutainer tubes and stored at 5°C for subsequent analysis.

Ca, Fe, Cu and Zn were analyzed using atomic absorption spectrophotometry (AA670, Shimadzu-Japan) under the conditions detailed in Table 1.

A solution of strontium was used in the Ca determination to prevent phosphate interference. Se concentration was determined with hydride-generation atomic absorption spectrophotometry (Perkin-Elmer Norwalk, CT) with the following conditions: Lamp current: 10 mA; type of flame: air  $C_2H_2$ ; wavelength: 190 nm; and slit width: 2 nm.

All data were analyzed using the Student's *t*-test and analysis of variance (ANOVA) with software SPSS.

## RESULTS

As can be seen in Table 2, the mean age for patients and controls was not significantly different nor was that of workers to controls, indicating a matched group in relation to age. Mean height of the patients to controls and workers to controls did not differ significantly. Mean weight of the patients to controls indicated a significant difference with  $P < 0.0001$ , however workers compared to controls indicated no significant difference.

Results of the analysis of elements under study are summarized in Table 3. The mean concentration of Cu in our patients was higher than that of controls. This difference was statistically significant ( $P < 0.0001$ ). A lower serum level was found in our EMF worker group compared to controls which was also significantly different with  $P < 0.049$ . Using analysis of variance (ANOVA) to evaluate the data showed the higher level of Cu in patients to be statistically significant ( $P < 0.05$ ), however, the difference between workers and controls was not significant. In evaluation of worker to patient levels, the results indicated a significant difference with  $P < 0.05$ .

A significant difference in serum Zn levels was found ( $P < 0.0002$ ) between patients and controls with the level of Zn lower in leukemia patient group. Mean Zn levels in EMF worker group also decreased in comparison to controls with  $P < 0.006$ , also denoting a significant difference. Analysis by ANOVA showed a significant difference in comparison of both patients to controls and workers to controls with  $P < 0.05$  for both groups. However, a comparison of workers to patients indicated no significant difference.

**Table 1.** Conditions for atomic absorption analysis of elements under study.

Element	Halode cathode lamp current	Type of flame	Wavelength nm	Slit-width nm
Ca	6	Air-C <sub>2</sub> H <sub>2</sub>	422.7	0.5
Fe	8	Air-C <sub>2</sub> H <sub>2</sub>	248.3	0.2
Cu	3	Air-C <sub>2</sub> H <sub>2</sub>	324.8	0.5
Zn	4	Air-C <sub>2</sub> H <sub>2</sub>	213.9	0.5

**Table 2.** Comparison of age, height and weight of workers, patients and controls.

Group	Age (yr $\pm$ SD)	Height (cm $\pm$ SD)	Weight (kg $\pm$ SD)
Controls (n = 40)	37.79 $\pm$ 11.053	169.083 $\pm$ 5.897	73.54 $\pm$ 5.465
Patients (n = 80)	39.117 $\pm$ 16.71	167.862 $\pm$ 5.151	68.181 $\pm$ 5.151
Workers (n = 40)	38.889 $\pm$ 6.144	166.639 $\pm$ 5.846	71.667 $\pm$ 6.131
<i>P</i> value			
Patients to controls*	NS	NS	<i>P</i> < 0.0001
<i>P</i> value*			
Workers to controls	NS	NS	NS
<i>P</i> value			
Patients to controls**	NS	NS	<i>P</i> < 0.05
<i>P</i> value			
Workers to controls**	NS	NS	NS
<i>P</i> value			
Patients to workers**	NS	NS	NS

NS: not significant, \*Using Student's *t*-test, \*\*Using ANOVA

**Table 3.** Mean levels of elements under study in all groups and results of statistical analyses.

Group	Cu (~g/dl $\pm$ SD)	Zn (~g/dl $\pm$ SD)	Cu/Zn	Se (mg/dl $\pm$ SD)	Ca (mg/dl $\pm$ SD)	Fe (mg/dl $\pm$ SD)
Controls (n = 40)	115.69 $\pm$ 22.22	126.71 $\pm$ 35.68	0.99 $\pm$ 5.31	23.50 $\pm$ 5.53	9.65 $\pm$ 1.96	104.52 $\pm$ 47.50
Patients (n = 80)	136.16 $\pm$ 31.58	108.88 $\pm$ 20.10	1.28 $\pm$ 0.43	15.14 $\pm$ 4.76	11.07 $\pm$ 2.96	110.28 $\pm$ 34.14
Workers (n=40)	108.22 $\pm$ 25.12	104.43 $\pm$ 27.44	1.09 $\pm$ 0.25	9.58 $\pm$ 6.54	8.75 $\pm$ 1.86	86.02 $\pm$ 25.24
P value Patients to controls*	$P < 0.0001$	$P < 0.0002$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	NS
P value Workers to controls*	$P < 0.049$	$P < 0.006$	NS	$P < 0.0001$	$P < 0.016$	$P < 0.05$
P value Patients to controls**	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS
P value Workers to controls**	NS	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$	NS
P value Workers to Patients**	$P < 0.05$	NS	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$

NS: not significant, \*Using Student's *t*-test, \*\*Using ANOVA

The Cu to Zn ratio in patients compared to controls was higher with  $P < 0.0001$  indicating a significant difference. This same ratio in comparison of workers to controls was not statistically significant. ANOVA evaluation of results showed the higher Cu/Zn of patients to be significant ( $P < 0.05$ ). In comparison of workers to controls and workers to patients, the difference was not significant in either group.

The serum level of Se in leukemia patient group was significantly lower than the controls. Analysis of these data indicated a significant difference with  $P < 0.0001$ . Mean Se level in workers was also significantly lower than controls with  $P < 0.0001$ . Using ANOVA to appraise the data verified that there was a significant difference in comparison of both patient and worker groups to controls and of workers to controls with  $P < 0.05$  for all comparisons.

Analysis of mean serum Ca levels denoted a higher level in patient group over controls, and a drop in the Ca concentration in the worker group. Both results were statistically significant with  $p < 0.0001$  and  $p < 0.016$ , respectively. ANOVA analysis revealed a significant difference in comparison of patients to controls, workers to controls and workers to patients with  $P < 0.05$  for all.

There was no significant difference found in serum Fe levels between patients and controls, even though the level of patients was higher. The mean serum Fe level in workers was lower than controls with  $P < 0.05$ , indicating a significant difference. Using ANOVA to evaluate Fe data demonstrated that there was no significant difference between controls and patient or workers, but the higher level of workers compared to patients was significant with a  $P < 0.05$ .

The significant results of ANOVA evaluation of our leukemia subgroups to both workers and controls are subgroups shown in Table 4.

## DISCUSSION

Despite the discrepancy between various and actual electromagnetic fields and their expected effect on body functions, a number of claims on the bases of epidemiological studies seem to support the hypothesis that prolonged exposure to EMF may be associated with increased risk of cancers, especially childhood cancer and particularly lymphatic leukemia, lymphomas and brain tumors. [4, 15].

**Table 4.** Results of ANOVA comparison of leukemia subgroups to workers and controls.

Element		P Value
<b>Cu</b>	ALL, AML to workers	$P < 0.05$
	AML to controls	
<b>Zn</b>	ALL to controls	$P < 0.05$
<b>Cu/Zn</b>	AML to controls	$P < 0.05$
<b>Se</b>	AML to workers	$P < 0.05$
	AML, ALL, LP to controls	
<b>Ca</b>	ALL, AML, LP to workers and	$P < 0.05$
	ALL, LP to controls	

Note: Only significant results are shown. For Fe, all comparisons were non-significant, ALL: acute lymphoblastic leukemia, AML: acute

Metals such as Fe, Cu, and Zn in mammalian lymphocytes are essential for cell growth and synthesis of DNA [16]. They also help to regulate the intracellular concentrations of transferrin, ferritin, ATP, proteins and cell surface interleukin-2 receptors [17]. Research has shown that leukemic cells have elevated concentrations of Fe and Cu and decreased levels of Zn [18]. We set out to detect variations of the trace and non-trace elements Cu, Zn, Se, Ca and Fe in serum in workers exposed to EMF with the objective to assess whether EMF can cause any variation in mineral concentrations in workers and compare these results to the same levels of these elements in patients with leukemia.

In answer to the two reasons outlined in the introduction, our higher mean level for serum Cu concentration is consistent with other reports for leukemic patients and confirms that Cu levels are elevated in persons with leukemia especially those with AML. Beguinet *al.* [19] also reported this increase with chronic lymphocytic leukemia and also for chronic myelogenous leukemia. In contrast, comparing to controls, workers exposed to EMF showed a slightly decreased level of Cu. Perhaps exposure time to EMF has not been sufficient to adversely effect Cu-containing proteins such as ceruloplasmin to release copper as seen in leukemic patients. We have found no human studies in this respect but Burchard's study with dairy cows in a controlled environment also showed lower Cu plasma levels during and after exposure to EMF [20].

Serum Zn levels in our patients with ALL and workers were significantly lower than controls. Beguinet *al.* [19] also saw this result with leukemic patients. This decrease in plasma Zn concentrations was also reported in dairy cows exposed to 60 Hz and 30  $\mu$ T, during and after exposure, but cerebrospinal fluid showed increased levels of Zn [20]. However, cerebrospinal fluid does not alter appreciably, as it maintains a relatively steady state. It is difficult to speculate on the physiological implication of such variations as reported by Burchardet *al.* [20], especially in human studies. This low concentration of Zn in leukemic patients has been attributed to anorexia, tissue catabolism and other forms of metabolic stress [21], since Zn has been proven to be an essential element for metabolic functions, especially under conditions of stress. In a similar fashion, Zn as an antioxidant, may be trying to combat the detrimental effects caused by EMF with the Zn pool going to tissue in order to increase immunocompetence in both cases. In addition, as Beguin and others [19, 21, 22] have postulated, low dietary intake, intercurrent infections and loss through sweat and urinary excretion could also partially account for lower Zn levels.

In relation to trace elements, Cu/Zn ratio, because of the antagonistic actions of Cu and Zn, is an important marker in showing the status of these two elements. According to our results, Cu/Zn ratio in leukemia patients, especially those with AML, and workers increased, supports the hypothesis that a high concentration of Cu and a high Cu/Zn ratio denote the extent of leukemic disease [19]. The high Cu/Zn ratio in our patients and workers may be due to an increase in Cu absorption and a decreased Zn absorption or competition of these two elements for binding to metallothionein or elimination of Zn in combating free radicals.

Se as an antioxidant exerts a protective effect against some human cancers and its levels have been reported to be lowered in some neoplasia [23]. Our leukemia patients, especially those with AML and our worker group had significantly decreased Se values. This low level of Se may be due to its scavenging properties, its function in eliminating free radicals as a reducing agent and acting as an important property of enzymes such as glutathione peroxidase. The important implication of these results is that Se could possibly be used as an indicator for the extent of disease. This matter points toward the need for a more meticulous and comprehensive study of Se in relation to EMF exposure and leukemic disease.

Role of cellular Ca is in control of cell signals and events such as cell movement and division [13]. If EMF influences the cellular activity then this Ca homeostasis can be disturbed. Several *in vitro* and *in vivo* studies on Ca disturbance and EMF showed that low-intensity EMF can alter Ca distribution in cells probably due to an effect on receptor operated Ca and or ion channels [24]. There have been reports on the efflux of Ca-ions from the brains of chickens and cats as well as neuroblastoma cells when exposed to EMF [25, 26]. On the other hand, several others reported that Ca uptake by cells exposed to EMF increased in rat thymocytes and in embryonic chicken tibia [27, 28]. Ca-ion uptake by normal and leukemia lymphocytes increased with weak EMF of 13.6 Hz, 20  $\mu$ T, a result indicating that EMF affects the Ca-dependent functions of normal and leukemia lymphocytes as reported by Lyle *et al.* [29] and such persistent alteration of Ca-ions by magnetic fields could influence the process of cancer promotion and effect the growth control in normal and malignant cells. The lower serum Ca concentrations in our workers may be due to increased uptake or may be influenced by the conductance of the cell membrane as well as the permeability of intercellular stores. Unlike our findings, Ca concentration of plasma and cerebrospinal fluid tends to increase in dairy cows exposed to EMF [20]. Mean levels of Ca in our patient group were significantly higher. This increase in the level of Ca has been shown in other disorders such as breast cancer, lymphoma and leukemia [30, 31].

Results of our study on Fe concentration indicated a decrease in Fe levels in workers compared to controls. Even though little data are available in human studies, concentration of Fe decreased also in cerebrospinal fluid of dairy cows exposed to EMF [20]. Fe concentration of the patients did not show a significant difference compared to controls but several other studies reported Fe levels to increase in AML leukemic patients [32].

There is little available information on the effects of exposure to electromagnetic fields on the status of essential micro- and macro-nutrients in the human body. Similarly, there are few studies on the status of trace elements in Iranian leukemic patients. While the results of this study are not definitive, we believe they do show some meaningful association in relation to the status of essential elements and their alteration by exposure to EMF as well as their possible efficacy as a marker to determine whether EMF workers could be at risk to develop leukemia. EMF workers are consistently exposed to stressful

working environments. It is essential that these workers undergo routine checks for changes in biochemical indexes. Nutritional supplementation, including increased Zn and Se, could be beneficial in helping to alleviate some of the biochemical stress exposure to EMF causes and increase cellular immunity.

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