Review Article

The effect of extremely low-frequency magnetic field (50–60 Hz) exposure on spontaneous apoptosis: The results of a meta-analysis

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Abstract

Background: This paper is a meta-analysis of the published data from *in vitro* studies to evaluate whether spontaneous apoptosis might be influenced by extremely low frequency (ELF) magnetic fields (MFs).

Materials and Methods: A comprehensive scientific literature search in electronic databases was conducted and studies covering the period 2000–2010 were selected. Then, published studies involving the desired topic were retrieved. The inclusion criteria were percentage of apoptosis in the cells exposed to 50–60 Hz ELF-MFs. The statistical analysis was performed by comprehensive meta-analysis version 2.

Results: The summary measure of association (95% confidence interval) for all 18 effect estimated from 8 studies was 1.18 (1.15, 1.20). Heterogeneity among studies was found. There was no evidence of publication bias for the association between exposure to MF and apoptosis risk.

Conclusion: Our meta-analysis provided conclusive data that ELF-MFs can increase apoptosis in cancer and normal cells. Furthermore, there is a possibly individual intensity and time range with maximum created effect according to window effect.

Key Words: Apoptosis, extremely low-frequency magnetic field exposure, meta-analysis

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INTRODUCTION

Nowadays due to the exposure to extremely low frequency (ELF) electromagnetic fields (EMF), they have been considered as potential threats to public health.[1-4] There are many studies in the literature on the effects of ELF-MFs in biological systems.[5-7] However, there is much controversy on the adverse effects of ELF on human tissue, especially in promoting cancer and carcinogenesis.[8] Epidemiological studies have shown an increase in the cancer cell growth such as childhood leukemia, lymphomas, and cancer of nervous system by exposing to ELF-MFs.[9-11] Thus, evaluating the effect of ELF-MFs on the cancer cell damage *in vitro* is important to consider.

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One of the most important procedures in controlling cancer is programmed cell death or apoptosis.[12] Apoptosis is, in fact, an active procedure playing an important role in the regulation and maintenance of the cell population in tissues.[13] In the other word, dysfunction of apoptosis can promote tumor formation.[14] Chemical or physical agents that are not intrinsically mutagen can promote tumor development by preventing the removal of tumor cells by apoptosis.[15] Various *in-vitro* studies examined this effect when a different type of cells was exposed to ELF.[8]

The ELF-MF can induce both increased[16] and decreased susceptibility[17,18] to apoptosis. It has to be noted that inappropriate apoptosis can cause to cancer with either decreased removal of cells or an over proliferation of cells.[19] Furthermore, it was shown in the literature that some tumors are developed with increasing tumor survival after oncostatic therapies.[15,20] Therefore, if ELF-MFs are able to increase apoptosis, tumor survival after treatment can decrease.

Based on the above controversies, it is difficult to come to a conclusion taking into account that different overlapped confounders in the literature.[21] In the present study, we aim to answer whether ELF 50-60 Hz MFs affect spontaneous apoptosis by performing a meta-analysis on the basis of published manuscript between years 2000 and 2010.

MATERIALS AND METHODS

In the current study, we conducted a literature search published between the years 2000 and 2010 to identify *in vitro* studies relevant to investigating effects of acute exposure to ELF-MFs. We searched publications in PubMed and Web of Science using the search words "ELF," "MFs," "*in vitro*" and "apoptosis," the manuscripts published in a peer-reviewed journal were evaluated [Figure 1]. A total of 40 manuscripts were revealed from scientific publications. After investigating all abstracts from these articles, 19 studies were taken into consideration. A selection process was done using the following inclusion criteria in the current meta-analysis:

- Exposure to an MF at the frequency of 50–60 Hz;
- Documentation of mean, standard deviation, and sample size for both control and treatment groups;
- Exact expression of apoptosis percentage in graph or table;
- The detailed description of exposure characterization.

Overall, 8 studies fulfilled our requirements. Table 1 gives an overview of the study design, cell model, flux density (intensity) and exposure duration.

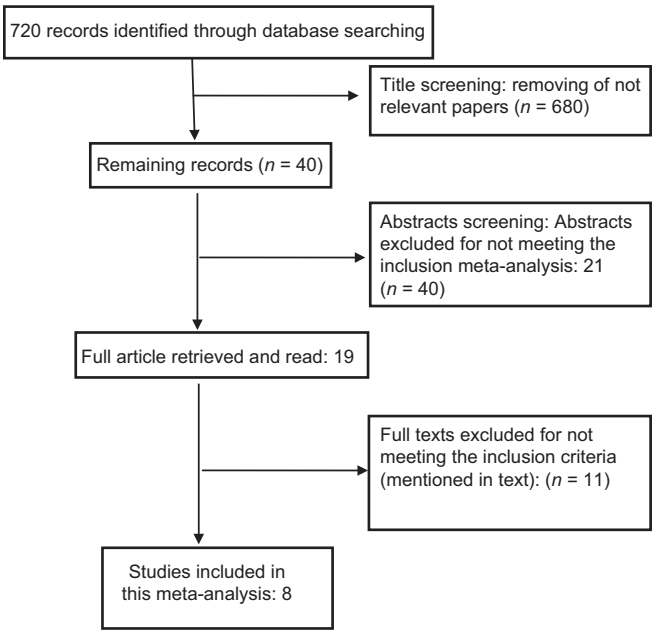

Figure 1: Article selection flow chart

We conducted the analysis as a comparison between control (nonexposed) and exposed groups. The variables related to the frequency of 50–60 Hz included flux density (intensity), exposure duration, and cell model. The flux density (intensity) was sub-classified as: (1) 0–0.5 mT, (2) 0.5–1 mT and (3) 1–5 mT.[22] The exposure duration was sub-classified as: (1) <24, (2) 24–72 h, (3) 72 h–5 days, (4) >5 days and finally model cells sub-classified as: (1) Normal cells, (2) cancer cells.

Statistical analysis

Quantitative data were entered into comprehensive meta-analysis version 2. When appropriate, results of comparable groups of studies were pooled in a meta-analysis using the random-effects model. For dichotomous outcomes, odds ratios (odds) with 95% confidence intervals (CIs) were calculated based on total sample size and number of events. P values were two-tailed, and a value of $P < 0.05$ was considered statistically significant. Heterogeneity between studies was measured by χ^2 statistics ($P < 0.1$) and quantified with I^2 statistics.[23] We determined the I^2 values of < 25% for minimal heterogeneity, <50% for moderate heterogeneity, and 50% or greater for substantial heterogeneity. In attempting to dissipate any heterogeneity, subgroup analyses were performed on studies. Since the different trials implemented various types of cells, different strategies for exposure duration, various MF intensity, and trials were divided according to the type of confounders; then the subgroup analysis was conducted. Meta-regression and subgroup analyses were conducted to explore the potential sources of

between-study heterogeneity. Potential publication bias was evaluated using funnel plots. Heterogeneity was explored through consideration of predictor variables assessed and outcomes chosen.

RESULTS

The individual study and the overall summary results for the 18 effect estimates from eight *in-vitro* studies exposure to a different dose of MF are shown in Figure 2. Four of these 8 effect estimates found a statistically significant positive association between at least exposure duration and apoptosis rate. The summary measure of association (95% CI) for all 18 effect estimates from 8 studies was 1.18 (1.15, 1.20). Exploring potential sources of between-study heterogeneity is, therefore, an essential component of the meta-analysis.

We found a sever degree of heterogeneity (I^2 = 96.2%; $P_{\text{heterogeneity}}$ = 0.001) in our pooled results. This might have been arisen from types of cells, different strategies for exposure duration, and various MF intensities. Thus, we used meta-regression to explore the causes of heterogeneity for covariates. However, no covariate having a significant impact on between-study heterogeneity was found among those mentioned above. We then performed subgroup analyses by the types of cells, different strategies for exposure duration, and various MF intensities to explore the source of heterogeneity. The result of the subgroups analysis is presented in Table 2.

In the subgroup analysis of the MF intensity, only the 0.5–1 mT level did not have statistically significant results 0.91 (0.48, 1.68).

Table 1: Publications included in the meta-analysis and their characteristics

First author	Year	MF intensity (mT)	Exposure duration	Cell model
Santi Tofani	2001	3	20 min	WiDr colon adenocarcinoma (ATCC CCL-218)
Rosamaria Mangiacasale	2001	0.06	72 h	(Human immortalized lymphoblastoid cell lines) AHH1
Gui-Rong Ding	2001	5	24, 72 h	MCF-7 human breast adenocarcinoma
Maria Teresa Santini	2003	0.5	7, 14 days	MG-63 (osteosarcoma cell lines), Saos-2 (osteosarcoma cell lines)
Pirozzoli	2003	1	2, 5, 7 days	The human neuroblastoma cell line LAN-5
Teodora Nikolova	2005	2	48 h	Mouse ES cells
Santin	2005	1, 5	2 h	K562
Ari Markkanen	2008	0.1, 0.3	24, 48 h	L929 (murine fibroblast cells)

ES: Embryonic stem, ATCC: American type culture collection

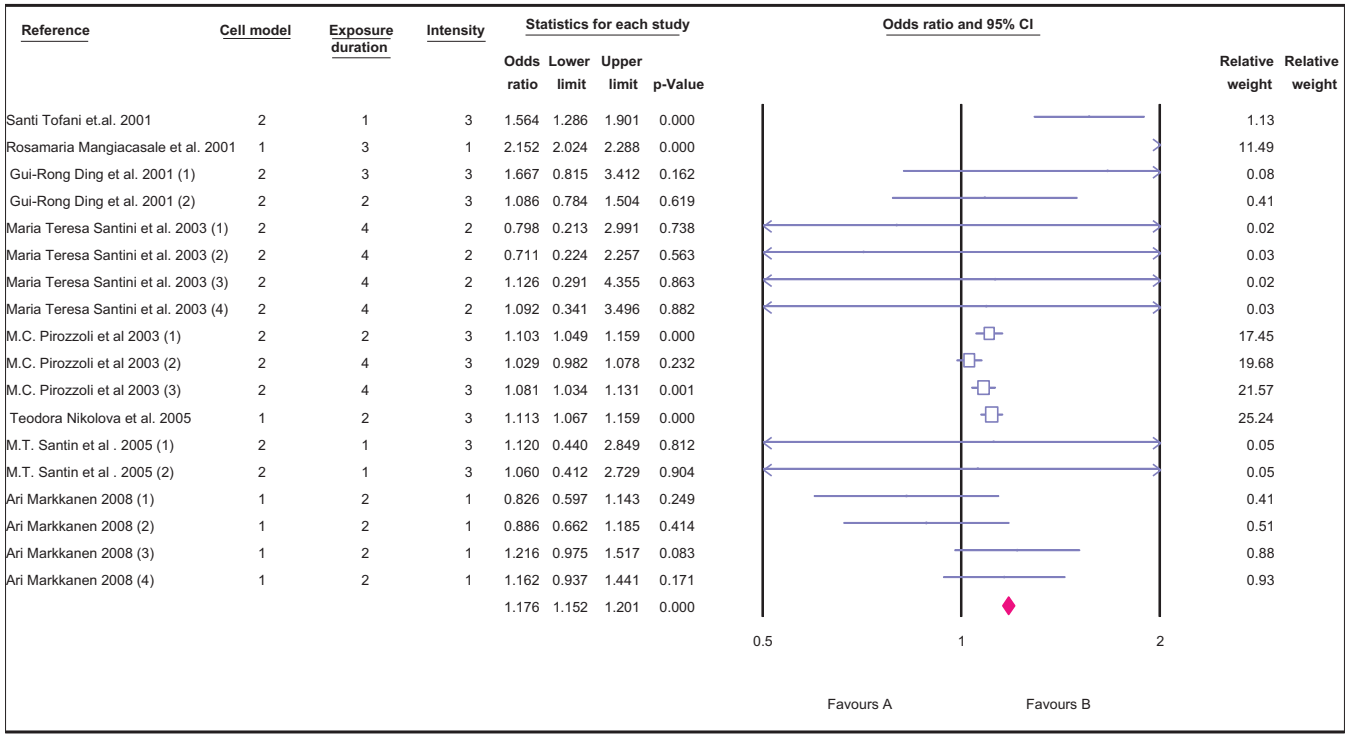

Figure 2: Forest plot of the association between exposure to magnetic field and apoptosis risk in *in-vitro* studies. Odds ratio estimate, horizontal line 95% confidence interval, diamond summary odds ratio estimate and its corresponding 95% confidence interval. All statistical tests were two-sided. For cell model: (1) Normal cell, (2) cancer cell. For exposure duration: (1) <24, (2) 24–72 h, (3) 72 h–5 days, (4) 4–>5 days. For intensity: (1) 0–0.5 mT, (2) 0.5–1 mT, (3) 1–5 mT

Table 2: Summary of OR estimates (95% [CI]) *in-vitro* studies of the association between exposure of MF and risk of apoptosis by cell model, exposure duration, and MF intensity

Subgroup	Number of studies	Summary OR (95% CI)	Between studies I^2 ($P_{heterogeneity}$)	Between subgroups Q ($P_{heterogeneity}$)
Cell model				
Normal cell	6	1.34 (1.30-1.39)	98.46 (0.001)	440.10 (0.001)
Cancer cell	12	1.08 (1.05-1.11)	48.81 (0.037)	
Intensity of magnetic files				
0-0.5 mT	5	1.88 (1.78-1.98)	96.18 (0.001)	124.99 (0.001)
0.5-1 mT	4	0.91 (0.48-1.68)	0.00 (0.940)	
1-5 mT	9	1.09 (1.06-1.11)	62.87 (0.006)	
Exposure duration				
<24 h	3	1.59 (1.26-1.83)	0.00 (0.592)	347.01 (0.001)
24-72 h	7	1.11 (1.07-1.14)	5.46 (0.685)	
72 h-5 days	2	2.15 (2.02-2.28)	0.00 (0.486)	
>5 days	6	1.05 (1.02-1.09)	0.00 (0.719)	

OR: Odds ratio, CI: Confidence intervals

Publication bias

There was no evidence of publication bias for the association between exposure to MF and apoptosis risk ($P = 0.211$ for Begg's adjusted rank correlation test and $P = 0.885$ for Egger's regression asymmetry test).

DISCUSSION

Many studies investigating the impact of ELF-MFs on spontaneous apoptosis of cells have shown contradictory results.[24] While some researchers reported a decrease of apoptosis percentage by an increase in the level of anti-apoptotic factors[18] or decrease in apoptosis indicator,[25] others observed apoptosis increase induced by ELF-MF.[26] In addition, some reports found altering of apoptosis-related genes' expressions without change apoptosis rate, suggesting the presence of compensator mechanisms.[27] However, our meta-analysis demonstrated that ELF-MFs could significantly increase the apoptosis level *in vitro*. Such a result is in good agreement with those of some other studies conducted after 2010. For example, Kim *et al*. reported that continuous exposure to a 60 Hz MF can induce duration- and dose-dependent apoptosis of testicular germ cells.[28] In addition, Akdag *et al*. showed the initiation of active-caspase-3 activity known as characteristic of apoptosis by 500 μT ELF-MF exposure.[29] Furthermore, Yang and Ye observed an induced apoptosis of MG-63 cells and, therefore, decrease in viability of the cells with exposure to 1 mT ELF-EMF.[30] Although the exact mechanism is still obscure, there are biophysical mechanisms connected to apoptosis death induction by ELF-MFs. Since effects of MF are nonthermal, some possible biophysical mechanisms were suggested for increasing apoptosis. One of the considered mechanisms is related to free radical recombination process.[31] Recombination of radical pairs is presumably activated by the direct action of the MF on electron spin of molecules and atoms with unpaired electrons.[32] This efficacy may lead to DNA damage and thus increase the apoptosis death.[33]

In the section of cell model data analysis, the results showed that ELF-MFs can induce apoptosis in cancer and normal cells. This suggested that ELF-MFs have possibly no capacity of carcinogenesis initiating, rather decreasing tumor survival. Furthermore, the results demonstrated that ELF-MFs introduce more apoptosis in normal cells as compared to cancer cells. Such a result is in contrast to the result of Radeva and Berg[34] which reported more lethality in cancer cells as compared to normal cells induced by MFs. This could be due to the limitations of the current meta-analysis despite its significant results. In fact, the reliability of the present study was reduced because of insufficient data stem from the incomplete information in the publications where only 8 studies had the required information such as sample size.

The other interesting result of this analysis is the apparent nonlinear "dose-response" with the maximum <0.5 mT. It indicated that there is an intensity "window effect" in this range of flux density. "Window effect" is a resonance-like phenomenon predicting the MF intensity windows in which the maximum biological effects occur. Thus, targets in biological systems only respond to the MF with some discrete intensity range called "intensity window." Furthermore, our study showed nonlinear time response suggested that there is similar window effect for a time in the analysis of the literature. Therefore, it could be deduced that among exposure durations used in the publications, there is possibly a time window in the range of 72 h and 5 days which can be observed the maximum ELF-MFs effect on apoptosis.

CONCLUSION

The current meta-analysis demonstrated that the ELF-MFs can increase apoptosis in normal and cancer cells. Such an increase occurs with a distinctive range of flux density and time in conformity with window effect. Nonetheless, the sample size was very small and thus makes an analysis of data difficult to accurately determine the effects of ELF-MFs on spontaneous apoptosis. However, there is an obvious need for complete studies and further investigations.

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Conflicts of interest
There are no conflicts of interest.

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