The effect of electromagnetic waves on the growth of Entamoeba histolytica and Entamoeba dispar

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ABSTRACT

Objectives: The aim of this study was to investigate the influence of electromagnetic radiation of a digital Global System for Mobile Communication mobile telephone (900 MHz) on *Entamoeba histolytica (E. histolytica)* and *Entamoeba dispar (E. dispar)* (cysts or trophozoites, or both) in a 24-hour period.

Methods: This study was carried out from April 2004 to May 2004 at the Department of Parasitology, Medical Faculty of Dokuz Eylul University in Izmir, Turkey. The cultivated isolate tubes, which were exposed to electromagnetic field at 37^{O}C, were evaluated as study group, whereas the tubes without exposure were assessed as control group. Finally, only living parasites in all tubes were counted using a hemacytometer. The effect of the temperature was evaluated for both control and study groups.

Results: The influence of electromagnetic field and temperature was assessed separately for the study group. The parasite number of *E. histolytica* decreased after exposure at 37^{O}C and room temperature (p=0.009) compared to the decrease in the control group (p=0.009). The parasite number of *E. dispar* also decreased after exposure at 37^{O}C and room temperature (p=0.009). In comparison to control tubes, this was a significant decrease (p=0.008). In the case of exposure of *E. histolytica* the results did not reveal any significant difference between temperature degrees to magnetic field (p=0.459) and *E. dispar* (p=0.172).

Conclusions: Our findings show that exposure to electromagnetic field for a certain period of time may cause damage that can lead to death in single-cell organisms.

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Amebiasis, which affects 10% of the world population and causes mortality and morbidity especially in developing countries, is caused by the protozoon parasite *Entamoeba histolytica* (*E. histolytica)*.[1] *Entamoeba dispar (E. dispar)* is a morphologically identical but genetically distinct species causing no clinical manifestation.[2] Cultivation of parasites using various types of medium, provides better understanding of their biological characteristics, life cycles, pathogenesis and adaptation to environmental conditions.[3] Xenic culture is used routinely for both diagnosis and in vitro studies aimed at different purposes. It is known that electromagnetic field (EMF) produced by many telecommunication systems, has short and long term biological effects on living cells.[4,5] Related to this subject, numerous in vivo and in vitro researches are carried out either on humans and animals or microorganisms.[6-8] When researches on the effects of EMF are investigated, it is seen that the number of studies on the influence of

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electromagnetic radiation on single cell protozoa is very limited.[9] From this point of view, we aimed to show the possible effects of EMF, produced by a 900 MHz frequency Global System for Mobile Communication (GSM) mobile telephone turned to speech position, on *E. histolytica* and *E. dispar* cysts or trophozoites or both. We also aimed to show the effects of EMF on single-cell organisms.

Methods. This study was carried out from April 2004 to May 2004 at the Department of Parasitology, Medical Faculty of Dokuz Eylul University in Izmir, Turkey.

Clinical isolates. Isolates were obtained from 2 of the patients who consulted the outpatient departments of Dokuz Eylul University Hospital with various gastrointestinal complaints. Amebae cysts or trophozoites or both were detected by light microscopic examination and a commercial enzyme-linked immunosorbent assay kit (Entameba Celisa-path, Cellabs, Australia) screening antigen in stool samples was used to identify and differentiate the amebae species; one of the isolates was *E. histolytica* and the other was *E. dispar*.

Stool samples diagnosed as *E. histolytica* and *E. dispar* were inoculated into Robinson medium as previously described.[10] These isolates were sub cultivated 2-3 times until they reached the logarithmic reproduction rate. The study groups consisted of medium tubes containing *E. histolytica* or *E. dispar* cysts or trophozoites exposed to EMF, whereas the medium tubes of the 2 isolates being settled under standard reproduction conditions were evaluated as the control groups. To observe the influence of the temperature on the growth of the amebae; both the study and the control groups of 2 isolates were divided into 2 sub-groups of 5 tubes each according to the temperature conditions (37°C and 22°C). The parasites were taken from the sub cultivation tubes and were inoculated with a concentration of 7.5 x 10^5 /ml into each study and control tube containing fresh medium. For the study sub-groups under the incubator temperature, a commercially available mobile phone (Panasonic G-600, GSM 900 type) was placed in the incubator just in front of the tubes. The mobile phone was positioned similarly for the other study sub-groups, which were under room temperature. The study sub-group tubes under room temperature were placed in a plastic coated box, which was heat insulated; so the temperature changes during the day were stable for the experiments. The mobile phones were turned to speech position for 60 seconds at 1-hour intervals during a total period of 24 hours. Control groups of isolates divided into 2 temperature sub-groups were placed in another laboratory environment to avoid the EMF produced by the mobile phones. The parasites both in the incubator and under room temperature were left to their standard growing.

Parasite counting. At the end of the experiment duration (24 hours), the living parasites in all of the tubes were counted using a hemacytometer and the results were noted. The viability of the parasites was determined with 1% eosin saline solution. Unstained amebae were considered as alive, whereas pink-red stained amebae were considered as dead was.

Statistical analysis. Significances of differences between the viable parasite numbers of the groups were analyzed by Mann–Whitney U-test, *p* value of less than 0.05 was considered significant.

Results. At the end of 24 hours, there was a statistically significant decrease in the number of *E. histolytica* exposed to EMF in 37°C incubator compared to *E. histolytica* not exposed under the same temperature conditions (p=0.009). Similarly, it was found that after the exposure of *E. histolytica* to EMF at room temperature (22°C), the parasite number decreased. In comparison to non-exposed tubes at the same temperature conditions, it was a significant decrease (p=0.009). The results obtained from *E. dispar* experiments revealed that exposure to EMF at 37°C reduced the parasite number significantly (p= 0.009) and 22°C (p=0.008) compared to control group.

In the presence of EMF, the number of *E. histolytica* at the end of 24 hours was significantly higher both at incubator temperature and room temperature (U:1.500, p=0.016) compared to *E. dispar* (U:0.000, p=0.008). In the presence of EMF, the difference between the numbers of *E. histolytica* is higher at room temperature (p=0.008) and *E. dispar* (p=0.016) in comparison to incubator. Concerning the viability of *E. histolytica* and *E. dispar* exposed to EMF, no significant difference was observed between 37°C (p=0.459) and 22°C (p=0.172).

Discussion. It is reported that electromagnetic radiation from mobile phone depending on the duration of the irradiation has distinctive biological effects such as cell damage or weakened immune system.[4] Several studies are aimed at observing these effects of EMF using different models of living organisms.[11,12]

Some of the researchers investigating the biological effects of EMF, used mobile phones[13] for their experiments since the others established the similar mechanisms themselves.[5] We preferred to produce EMF by means of a GSM mobile phone turned to speech position at definite time intervals. The EMF, depending on the duration and the intensity, alters the reproduction rates of microorganisms[8] and destroys various kinds of highly organized microorganisms.[14] In a study, the effects of the 50 Hz EMF on the swimming behavior of *Paramecium*, a ciliated protozoon, were investigated and it was found that the swimming velocity and the directional changes of the

Paramecium cells were increased by the electromagnetic field.[15] These results may indicate that even low-level EMF can cause different effects on protozoa. We believe that the ameba, which is one of the most important intestinal protozoons, should be a good model for investigating the influence of EMF. Therefore, we investigated the effects of EMF produced by a 900 MHz frequency GSM mobile telephone on 2 different entamoeba species; one of which was pathogen (*E. histolytica*) whereas the other was non-pathogen (*E. dispar*). Our data revealed that mobile phone turned to speech position for a minute at one hour intervals during 24 hours, decreased the growth of the parasites.

Berk et al[9] studied the effects of EMF, produced by an established mechanism, on 3 distinct free living amebae cultivated in the medium. After 24-72 hours (depending on the medium being xenic or axenic), they found the differences in final number of the EMF-exposed group ranging from 9-72% lower than non-exposed control group. They suggested examining the relationship between temperature and magnetic field, both of which affect the amoebae population. Considering this idea, we investigated the effects of EMF on two distinct amoebae species (pathogen and non-pathogen) at 2 different temperature levels during a total period of 24 hours. An incubator maintains 37°C, which is the optimal temperature for the parasite growth. At room temperature, 22°C was used to understand the interaction between EMF and the incubator. It was not possible for us to determine how the EMF produced by mobile phone interacted with the EMF produced by incubator itself. So, we decided to perform the experiment at room temperature in addition to incubator temperature. Data obtained from our experiments revealed that EMF independent from the temperature had a decreasing effect on the number of parasites of 2 different entamoeba species. The medium used for the growth of amebae was a xenic medium and it was suitable to observe the effects of EMF on amebae cultivated in it. Different from the results of Berk et al,[9] we found that the parasite number decreased after 24 hours of exposure.

There are several studies examining the effects of EMF, using distinct animal or microorganism models, but the number of the studies on amebae is very limited. However, we believe that amebae are good models to investigate the effects of EMF on single-cell living organisms; they can be easily produced in medium, we know relatively more on their enzymatic activities and they are similar to the human macrophages. The mechanism of how EMF reduces the growth of amoebae is not clear. Hileman[16] hypothesis: EMF, after a period, may cause the longer existence and effectiveness of free radicals, which are known to damage DNA. We did not search the metabolic activities of the parasites after exposure to EMF but determined their viability according to whether they were stained by 1% eosin or not. Our data showed that EMF from a 900 MHz frequency GSM mobile telephone decreased the number of *E. histolytica* and *E. dispar* independent of the temperature levels.

In conclusion, we believe that further researches using transectional electron microscopic technique will shed light on the mechanism of the damage to *E. histolytica* and *E. dispar* exposed to EMF.

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