

Influence of Mobile Phone Electromagnetic Field Exposures on *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* in vitro

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Abstract

Background: Mechanism underlying the lethal effect of microwave radiation on microorganisms are yet to be discovered. Some researchers hypothesized that electromagnetic waves can increase the target temperature and destroy life.

Objectives: The aim of this study was to determine the impact of cell phone radiation on bacteria that cause otitis media.

Materials and Methods: Two bacterial isolates were selected from cases of inflammation of the middle ear (otitis media). *Pseudomonas aeruginosa* and *Staphylococcus aureus*, isolates were exposed to electromagnetic waves emitted from mobile. After the incubation period, the absorbance was measured to determine the degree of turbidity by using spectrophotometer on the wavelength 400nm.

Results: It was found that is no effect of mobile radiation up to six minutes of exposure to both bacterial Species, but influence began after the six minutes and increased influence directly proportional to the time. Effective dose 50 of *Staphylococcus aureus* was 11.5 minutes while for *Pseudomonas aeruginosa* was 14.2 minutes.

Conclusions: The radiofrequency of cell phone effects on the bacteria depend on the following factors: the exposure time to mobile radiation, water content and increasing temperature, breaking protein molecule.

Key words: Mobile phone, radiofrequency, exposure time, bacterial effects.

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Introduction

Microwaves are categorized as a non-ionizing low frequency electromagnetic wave with frequencies between 300MHz and 300GHz [1]. Many reports have been published on the lethal effects of microwave radiation on microorganisms [2, 3].

Mechanism underlying the lethal effect of microwave radiation on microorganisms is yet to be discovered. Some researchers

hypothesized that electromagnetic waves can increase the target temperature and destroy life [4]. The impact of microwaves radiation on human health is mediated via thermal (dielectric heating) or non-thermal (biological responses) effects [5, 6]. The metabolic changes in living cells under the exposure of microwaves from mobile communication systems include over expression of heat shock proteins, an increase

of reactive oxygen species level, an increase of intracellular Ca^{+2} , damage of DNA, inhibition of DNA repair, and induction of apoptosis [7].

Mobile phones generate non-ionizing radiofrequency electromagnetic radiation generally at either 900MHz or 1.8GHz [8]. The transmitted radiation is absorbed by the body and produces heat. For homogeneous matter, this absorption can be described by an exponential equation. The radiation intensity I at the depth x in the matter is

$$I = I_0 e^{-x/D}$$

Where I_0 is the radiation intensity at the surface and D is the matter thickness.

The absorption is linked to the amount of water in the matter and that heat producing. Interaction occurs between the electric field in the microwave radiation and the electric dipole moment of water molecules in the body. The amount of energy absorbed depends upon the frequency of the microwave and the energy is deposited more effectively in matter with high water content [9].

The possibility that Radio frequency (RF) radiation may cause changes in protein conformation, protein consists of a sequence or chain of amino acids connected by peptide bonds. The side chains of the amino acids are often polar. They attract or repel nearby side chains [10, 11, 12, and 13]. So the aim of this study is to determine the influence of mobile radiation on bacteria that causes inflammation of the middle ear.

Materials and Methods

Specimen collection:

Two bacterial isolates were selected from cases of inflammation of the middle ear specimens were collected with Stuart's transport medium (BBL aerobic collection and transport system; Becton Dickinson Microbiology Systems, Cockeysville, Md) from ENT department of Baquba teaching hospital in 2011 and delivered to the

laboratory within the time frame stipulated by the transport medium manufacturer were included in the study. This study conducted in Department of Microbiology in cooperation with Department of Medical Physics, College of Medicine, Diyala University in Iraq, and approved by the Scientific Committee of the College.

Specimen processing. Agar and broth media were obtained from Becton Dickinson Microbiology Systems. After initial inoculation onto appropriate agar media [14]. According to Baron and Finegold method [18].

Broths were incubated at 37°C in ambient air for up to 24 hours. Turbid broths with organisms identifiable by Gram staining and biochemical test, blood and MacConkey agar media. Organisms isolated from the primary broth cultures were identified in the same manner as those isolated from primary agar media. And diagnosed as *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Exposure to cell phone radiation. Two bacterial isolates were exposed to electromagnetic waves emitted from mobile (nokia made in Finland), the cell phone was placed on the top of culture media and allowed to ring at indicated time intervals. We prepared 20 test tubes for each broth culture with bacteria and exposed for certain time periods. Control group (which is not exposed to cell phone radiation) at the same culture protocol.

After the incubation period of 18 hours at a temperature of 37 °C absorbance was measured to determine the degree of turbidity by using spectrophotometer on the wavelength 400nm.

Preparation of standard: McFarland standards tube, which was prepared as follows, Original McFarland standards were mixing specified amounts of barium chloride and sulfuric acid together. Mixing

the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dehydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). This turbid solution is identical to estimated density cells 1.5×10^8 cells/ml.

The standard can also be compared visually to a suspension of bacteria in nutrient broth.

Statistical Analysis

Data are analyzed through the use of SPSS (Statistical Process for Social Sciences) version 10.0 application Statistical analysis system and Excel (Statistical package).

Inferential data analysis: These were used to accept or reject the statistical hypotheses, which included the following: Goodness of fit test (Kolmogorov-Smirnov Z), for testing the Normal dist. Function assumption with the studied readings of (O.D.S.) and (O.D.P.), and t-test.

Results

The results in Table (1) revealed that the test distribution is normal for all readings of the studied parameters (optical density *Staphylococcus aureus* (O.D.S) and optical density *Pseudomonas aeruginosa* (O.D.P) according to Kolmogorov-Smirnov Z.

Table (1): Normal distribution function goodness of fit test for the Optical Density *Staphylococcus aureus* and Optical Density *Pseudomonas Aeruginosa* data.

		Optical Density <i>Staphylococcus aureus</i>	Optical Density <i>Pseudomonas Aeruginosa</i>
No.		20	20
Normal Parameters	Mean	0.501	0.559
	Std. Dev.	0.248	0.198
Kolmogorov-Smirnov Z		0.690	0.634
Asymp. Sig. (2-tailed)		0.728	0.816
Test distribution is Normal			

In order to studying and analyzing the observations of the studied parameters (Strength, Wear), Stem – Leaf plotting/or Explorer method had been used. The procedure of this method depending on the order statistic of the original observations in ascending form with determining the (1st & 3rd) Quartiles.

The form (\pm) pointed the lower and upper sides or data limitation which included the ordered observations of that having less than two degree of deviation by their mean value (2 Std Dev.) and the two edges belong

to the first and third quartiles and the median value of bold line between them . In addition to that, the observations that increased in more than two deviations grades would be pointed by a circle(s) and known extreme value(s) and the observations that increased in more than three deviations grades would be pointed by a star(s) and known outlier value(s). The preceding values were known contaminated values.

For summarization to the process of creating a standardized values, we can conclude that all readings of the studied

groups in table (2) had been accepted, since non including any extreme or outlier values either at the downstairs or upstairs and we can conclude that the studied readings at the

two groups would be recommended and extremely reliable for the studied phenomenon.

Table (2): Descriptive Statistics for the two independent treated groups (O.D.S.) and (O.D.P.) with comparison significant with target value (Control).

Statistics		(O.D.S.)	(O.D.P.)
Mean		0.501	0.559
95% Confidence Interval for Mean	Lower Bound	0.385	0.467
	Upper Bound	0.617	0.652
5% Trimmed Mean		0.498	0.558
Median		0.495	0.530
Std. Error		0.055	0.044
Std. Deviation		0.248	0.198
Minimum		0.170	0.290
Maximum		0.870	0.850
Range		0.700	0.560
Interquartile Range		0.475	0.380
Control Mean value		0.87	0.85
Comparison Significant with Control value (P-value)		0.000	0.000

In addition to that, (Explorer) plot figure (1) illustrated that (O.D.S.) group registered wide grade (Range /or Interquartile Range) rather than (O.D.P.) group.

highly significant different at $P < 0.000$ was reported whether with (O.D.S.) group or with the (O.D.P.) group.

Comparison significant between control value, (i.e. the test value) showed that a

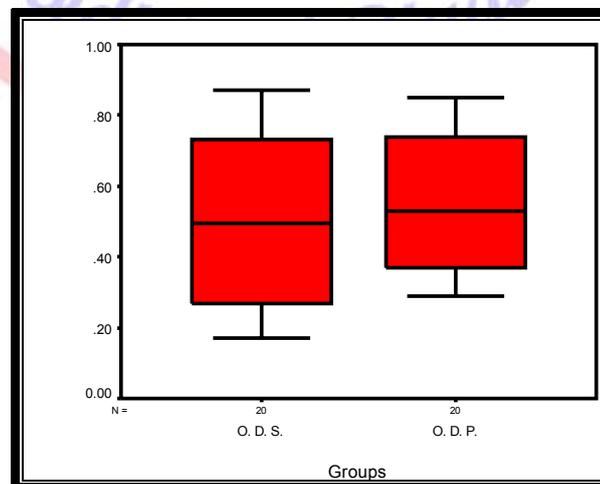


Figure (1): Explorer plot of (O.D.S.) and (O.D.P.) groups.

According to the results of analysis the variance of regression analysis of several models, Linear regression model registered the one of the best fitness compared with the others models, such as (Inverse, Quadratic, Cubic, Power, Compound, S-Shape, Logistic, Growth and Exponential).

Table(3): contents the necessary estimates of studying the nature of mentioning causes correlation ship between the two factors, (O.D.S.) readings and there contrasts of time (min.) duration.

Table (3): Simple Linear regression analysis for the effectiveness of the duration of focuses the density (per min.) and (O.D.S.) readings.

(O.D.S.) Dependent variable. Method.. Linear in the					
Correlation R	0.99196	Meaningful Linear regression Tested in two tailed alternative Statistical hypothesis			
R Square	0.98399				
Adjusted R Square	0.98310				
Standard Error	0.03222				
F =	1106.36	Sign. F =	0.0000		
Variables in the Equation					
Variable	B	SE.B	Beta	(t)	Sig. of (t)
Time (Periods)	-0.01385	0.000416	-0.99196	-33.26	0.0000
(Constant)	0.936842	0.014966	-	62.597	0.0000

Table (3) showed that a meaningful linear regression tested in two tailed alternative of the statistical hypothesis between the two factors, duration of focuses the density and (O.D.S.) readings. The slop value indicating that with increasing one unit of scale in the (duration per min.), a positive increment should be occurred in the unit of the (O.D.S.) readings and estimated with (0.936842) and that increment recorded a highly significant effect at $P < 0.000$.

In addition to that, a assignable factors given in the constant term and indicating that the initial respondents that ought to be included in each individual with highly significant of the effectiveness, as well as a meaningful causes correlation ship had been occurred between the two factors.

Figure (2) showed the Long term trend of the causes correlation ship between duration of focuses the density and (O.D.S.) readings as a dependent variable.

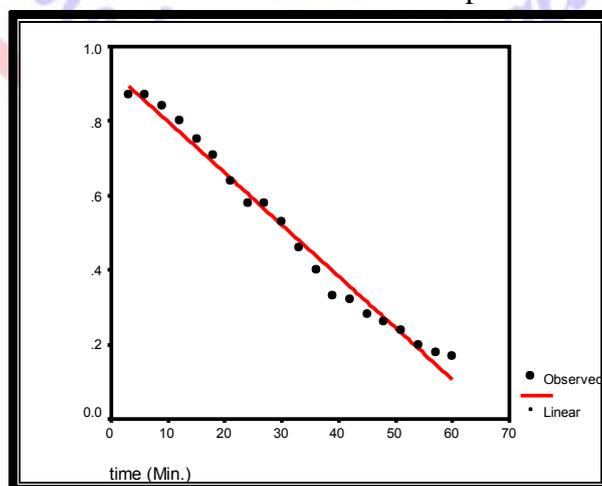


Figure (2): Long term trend plot of scatter diagram of the effectiveness of the duration of focuses the density and (O.D.S.) readings.

Once again, and according to the results of analysing of variance of regression analysis the preceding of several models , Linear regression model registered the one of the best fitness compared with the others models, such as (Inverse, Quadratic, Cubic,

Power, Compound, S-Shape, Logistic, Growth and Exponential).

Table (4) contents the necessary estimates of studying the nature of mentioning causes correlation ship between the two factors, (O.D.P.) readings and there contrasts of time (min.) duration.

Table (4): Simple Linear regression analysis for the effectiveness of the duration of focuses the density (per min.) and (O.D.P.).

(O.D.P.)Dependent variable..Method.. Linear in the					
Correlation R	0.98906	Meaningful Linear regression Tested in two tailed alternative Statistical hypothesis			
R Square	0.97824				
Adjusted R Square	0.97703				
Standard Error	0.02995				
F =	809.279	Sign. F =	0.0000		
Variables in the Equation					
Variable	B	SE.B	Beta	(t)	Sig. of (t)
Time (Periods)	-0.01101	0.000287	-0.98906	-28.45	0.0000
(Constant)	0.905895	0.013912	-	65.117	0.0000

As showed in (Table 4) a meaningful linear regression tested in two tailed alternative of the statistical hypothesis between the two factors, duration of focuses the density and (O.D.P.) readings. The slop value indicating that with increasing one unit of scale in the (duration per min), a positive increment should be occurred in the unit of the (O.D.P.) readings and estimated with (0.905895) and that increment recorded a highly significant effect at $P < 0.000$.

In addition to that, an assignable factors given in the constant term and indicating that the initial respondents that ought to be included in each individual with highly significant of the effectiveness, as well as a meaningful causes correlation ship had been occurred between the two factors.

Figure (3) showed the Long term trend of the causes correlation ship between duration of focuses the density and (O.D.P.) readings as a dependent variable.

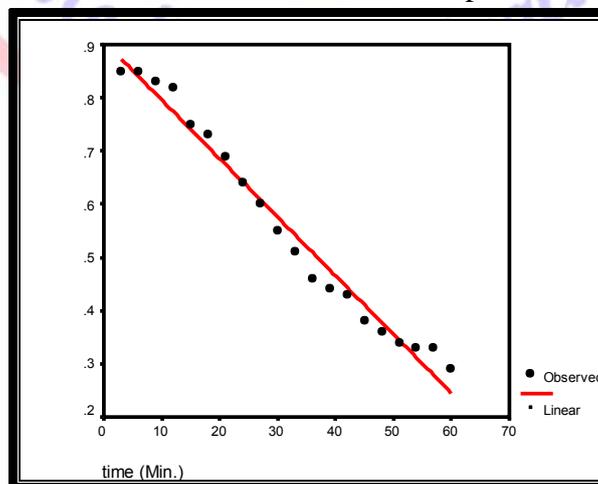


Figure (3): Long term trend plot of scatter diagram of the effectiveness of the duration of focuses the density and (O.D.P.) readings.

Table (5) showed that the mean value of differences between the two factors, (O.D.S.) and (O.D.P.) along the different periods of time (per min.) at each contrasts under the

null of the statistical hypothesis would be meaningful, at $P < 0.000$, and that indicating a real difference between the two Optical Densities indeed.

Table (5): Matched Paired Differences t-test for the differences between (O.D.S.) and (O.D.P.) readings at the studied periods of time (per min.).

Paired Samples Test	Matched Paired Differences t-test					
	Mean Diff.	Std. Dev.	Std. Error Mean	T	d.f.	Sig. (two-tailed)
<i>Staphylococcus Aurous</i> <i>Pseudomonas Aeruginosa</i>	-0.059	0.053	0.012	-4.896	19	0.000

Discussion

The results of present study observed that the viable cell count reduced relatively with an increase in time of mobile irradiation. It might be due to radio frequency energy absorption by biological materials with increasing in temperature [15]. The water molecule has a permanent electric dipole because the center of the net, positive charges in the nuclei of the three atoms that make up the molecule is not in the same place as the center of the net negative charges: The slight displacement of the centers of charge in the molecules results in a permanent electric dipole in the water molecule. The electric field from microwaves tries to align the electric dipole of the water molecule with it. In the alignment process work is done and energy is absorbed by the matter, thus producing heat [9]. The temperature was increased because substances with high dielectric constant such as water absorb radio waves and convert the energy to heat. Microbial lethality radio wave radiation may be due to the penetration of electromagnetic waves into biological wet material, heating up the intra and extra cellular fluids by the transfer of energy from the polar water molecules and dissolved ions, these results in the generation of heat within the material itself due to molecular activity [16].

Micro wave treatment has been reported to cause protein denaturation and aggregation in cytoplasm as well as to induce heat shock proteins, which cause microbial inactivation. It was very interesting to observe that gram positive bacteria were found more sensitive to micro wave radiations than gram negative. It might be due to the difference in chemical composition of cell walls [17].

Conclusions

The present work concluded that the physical effects of mobile radiation on the bacteria depend on the exposure time to mobile radiation and is directly proportional to the water content which increases the temperature and the mortality of bacteria. The whole effects of mobile radiation on bacteria follows the equation $I = I_0 e^{-kD}$ (Cameron). Under the same conditions, (*staphylococcus aureus*) was more sensitive to mobile radiation than (*pseudomonas aeruginosa*) since the water content of the former is more than later. In addition to the factors above, breaking protein molecule due to increase in temperature, contributes the process of the killing of the bacteria.

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