International Journal of Molecular and Clinical Microbiology 5(2) (2015) 575-584

International Journal of
Molecular and Clinical Microbiology

Effect of 900 MHz microwave radiation on alpha-INT1 gene expression, proliferation and adherence of *Candida Albicans*

Shahin-Jafari Ariyo[1], Bayat Mansour[1,*], Shahhosseini Mohammad Hassan[2], Tajik Parviz [1], Roudbar-Mohammadi Shahla[3]

1Department of Pathobiology, Faculty of Veterinary Specialized Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
2Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran
3Faculty of Medical Mycology, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Article history:
Received 18 October 2015
Accepted 5 November 2015
Available online 1 December 2015

Keywords:
Candida albicans;
Alpha-INT1 protein;
Virulence factors,
Microwaves.

ABSTRACT

To date, registered users of mobile phone communication network exceeded from total numbers of the world population, while a little knowledge of the biological effects of 900-1800 MHz microwave radiation, originating from the handsets or the base transceiver stations, have been released. The current study was designed for evaluation of 900-MHz radiation effects on *Candida albicans* proliferation, adherence and alpha-Int1 gene expression. *Candida albicans* (ATCC:10231) grown in Yeast Peptone Dextrose (YPD) broth was distributed into five tubes (5 ml, 10^6 cells/ml) and exposed to 900 MHz GSM radiation for 6, 12, 18 and 24 hours, while the fifth tube was kept far from the radiation. Cell densities at 0, 6, 12, 18 and 24 hours were assayed (using turbidimetry in 600 nm). Equal cell densities (2.5 x 10^6 cells/ml, 200 ul) from exposed and unexposed yeasts were transferred into 96 well plates and incubated for 4 hours, in order to biofilm formation by the yeast. Yeast densities in biofilm network were assayed using the MTT method. Abundance of alpha-int1 mRNA was also estimated in the five yeast samples using q-RT-PCR method. Microwave exposure led to increased proliferation rate and increased biofilm formation by the yeast and the effect was prominent in 18 hours exposed samples. Quantitative RT-PCR results showed significantly increased levels of the alpha-int1 mRNA in microwave exposed yeasts. *Candida albicans* cells exposed to 900 MHz radiation are more virulent than unexposed cells, demonstrated by The significant increases in the yeast proliferation and biofilm formation after exposure to 900 MHz GSM radiation are partly mediated by changes in alpha-int1 protein expression.

1. Introduction

Wireless communication and mobile phone handsets are frequently used devices worldwide, and global registered users of the mobile phone communication network are exceeded from the total numbers of the world population. Microwave radiations produced by different devices and applications are continuously transpired into the terrestrial space, while a little knowledge of the probable biological effects of the radiation has been published.

Microwaves, frequencies between 300 MHz to 300 GHz, are radiated from radar equipments, telecommunication and data transferring tools, radio stations, satellites, mobile cellphones and so on. Microwaves belong to a broader spectrum of electromagnetic waves, ranging from 1 Hz to

*Corresponding author: Dr.Mansour Bayat
Tel: +989125874738
E-mail address: dr_mansour_bayat@yahoo.com

more than 10^{20} Hz. Mobile handsets radiate only in a very narrow band of microwave radiation, vibrating between 900 to 1800 MHz. Biological systems, including live cells, are usually considered transparent for lower portion of the microwaves, called radio waves, while they are relatively opaque to upper portion of the microwave radiation, called infrared waves. That means, a major portion of the microwave energy is steadily absorbed by opaque materials, including living cells (Stefanics et al., 2007).

Microwave absorption results in snap temperature rise or other temperature independent events in the cells or body (Mortazavi et al., 2015). Regulatory bodies usually rely on the thermal effects of the microwaves in order to issue permissible levels for the radiation, however, non-thermal biological effects of the microwave radiation are also very important and need to be exclusively investigated.

Candida albicans is a polymorphic fungus exists in yeast-like, pseudohyphal or hyphal forms (Gauthier, 2015). Phenotypic changes from yeast-like to hyphal form is a virulence factor, and germ tube formation, that is usually associated with increased pathogenicity, is a valuable tool for differential diagnosis of the *C. albicans* among other *Candida* species. *C. albicans* is the main habitant of the human skin and mucous membranes and exists in high abundance especially in the elderly and immunocompromised individuals. In healthy individuals, there is a dynamic balance state between yeast and its host, however, the balance is fragile due to the diversity of the environmental conditions. Recently, *C. albicans* has emerged as a major cause of nosocomial infections and frequently isolated from implanted medical devices. The exact reason of conversion from commensalism to an opportunistic infectious agent is not understood yet. We analysed microwave radiation effects on the three major virulence factors of the yeast, including growth rate, adherence to the surfaces and the expression level of an integrin-like protein contributes in to the yeast adherence to human epithelial and endothelial cells.

Alpha-Int1 protein expression by *C.albicans* is highly associated with morphological changes and increased adherence of the fungus to the epithelial and endothelial cells (Gale et al., 1998). It has been shown alpha-Iny1 protein co-localizes with septins and functions in the axial bud site selection (Sundstrom, 2002; Gale et al., 2001). Int1 protein binds to extracellular matrix proteins and induces morphological changes in response to extracellular signals (Kinneberg et al., 1999; Calderone, 1998). *Saccharomyces cerevisiae* cells expressing alpha-Int1 protein shows adherence properties and produce a germ tube-like structures, and in a neutralization study, antibodies produced against the protein completely abolished adherence capacity of the yeast to epithelial and endothelial cells. In the same fashion, disruption of the alpha-int1 gene in *C. albicans* reduced 40% adhesion of the yeast to human epithelial cells, and as well as, mutations in alpha-int1 gene led to reduced virulence of *C.albicans* in a mouse model (Kinneberg et al., 1999; Sundstrom, 2002).

In the preliminary studies, we found increased adherence of *C. albicans* after exposure to 900 MHz GSM radiation, however, molecular assessment of alpha-Int1 gene sequence using PCR assay did not revealed any changes in the locus (Shahin-jafaria et al., 2015). Increased expression of alpha-Int1 protein may be the second possible mechanism for the increased adherence. Therefore, alpha-Int1 gene mRNA levels were determined in 900 MHz radiation exposed yeast samples. In addition, radiation induced changes in biofilm formation and proliferation of *C.albicans* were also investigated.

2. Materials and Methods

2.1. Strain and radiation source

Candida albicans, ATCC: 10231, was a kindly gift from Dr. Ghahri, the supervisor of mycology section in Resalat diagnostic laboratory of Tehran. For cell preparation, a single colony from the stock culture (slant agar) of *C. albicans* (ATCC:10231) was transferred into Yeast Peptone Dextrose, YPD, broth medium and incubated at 37°C for 48 h. Mobile phone radiation simulator capable of producing GSM microwaves at the wave frequencies of 851-960 nm with maximum output power of 900 mW was used as the source of radiation. The device was a standard mobile Jammer approved by Iranian Telecommunication organization.

2.2. Radiation effect on proliferation of Candida albicans

Yeast cells were grown to cell density of 3 x 10^8 cells/ml (equal to 1 McFarland turbidity). The cell suspension from logarithmic growth phase was diluted to the cell density of 1 x 10^6 cells/ml, and 10 ml aliquots from the suspension were transferred into 5 polystyrene tubes. Four tubes (1-4) were placed in front of the radiation source and exposed to 900 MHz GSM radiation for 6, 12, 18 and 24 hours, respectively. The fifth tube, as control, was kept far from the radiation source. The tubes were incubated at 25°C along with random agitation. ANOVA statistical test was used for statistical comparisons.

2.3. Radiation effect on biofilm formation by Candida albicans

Yeast samples from previous test were used for assessment of radiation exposure effect on biofilm formation (Coffey et al., 2014). Optical density of 4 radiation exposed and 1 unexposed yeast samples were adjusted to the cell density of 2.5 x 10^6 cells/ml by addition of fresh YPD medium and 100 ul of the adjusted suspensions was transferred into the wells of 96 well microtiter plates. Additional 100 ul from the YPD medium was added to all wells for adjusting the final volume to 200 ul. Control wells lacking yeast cells were also included. The plates were incubated at 37°C for 4 hours without agitation. Attached cells were kept and floating cells were washed off using sterile PBS and gentle agitation. Control wells preserving all of the cells, either suspended or attached, were also included and the total cell contents of these wells were precipitated to remove medium before the addition of MTT solution. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma:M2128) working solution (100 ul) was added to the wells and the plates were incubated for 2 additional hours in a dark place. Working solution (1X) was prepared by 10 fold dilution of stock solution (50 mg MTT in 10 ml PBS) using PBS. After a precipitation step, the supernatant was discarded and 100 µl acidified isopropyl alcohol (1 part HCl + 999 part alcohol) was added to each well in order to dissolve formazan crystals formed inside of the cells. Finally, the ODs of blue-brown color end product were read at 570 nm using an ELIZA-Reader.

2.4. Reverse-Transcription Quantitative PCR
2.4.1. RNA extraction

The yeast cells were harvested and the total RNA content of the test (radiation exposed) and control (unexposed) samples were isolated using heat/freeze RNA extraction method, according to the study of Schmitt, 1990 (Schmit et al., 1990). Briefly, precipitated cells from a 10 ml volume of the suspensions were added to 500 µl solution containing 50 mM Na acetate pH 5.3, 10 mM EDTA. After well mixing, 50 µl SDS (10%) was added. Equal volumes of fresh phenol solution was added and incubated at 65°C for 5 min with agitation. The mixture chilled, centrifuged and upper aqueous phase was extracted using phenol/chloroform at RT for 5 min. Na acetate (50 µl) was added, followed by addition of ETOH to precipitate RNA. One additional washing step was performed using ETOH. The final pellet was dried at RT and further purified to remove RNA molecules other than mRNA using "*Oligotex mRNA kit*" (Qiagen, according to the kit manual). RNase free DNase was used for elimination of genomic DNA contamination from the sample. All solutions were treated with DEPC, and all plasticwares were treated to degrade any contaminating RNA. RNA content was determined by reading the absorbance of the final extract at 260 nm.

2.4.2. RNA integrity analysis

Total RNA extraction step contains harsh conditions and the integrity of isolated RNAs should be checked. Isolated total RNAs from different yeast samples were resolved using electrophoresis on 1% agarose gel (including 2% formaldehyde), stained with SYBR Green II fluorescent dye and visualized using a transilluminator. The densitometry of the resolved RNA bands in gel image was analyzed using ImageJ software (Version 1.4).

2.4.3. Quantitative RT-PCR

Quantitative reverse transcription PCR, qRT-PCR, was used to estimate the abundance of *alpha-int1* gene related mRNA molecules in

the *RNA extracts*. TaqMan primer pairs specific to amplified fragment were designed using Primer Express software (V-3.0; Applied Biosystems).

Both primer sizes were 23 bp, and the Tm was 58-59°C. The amplicon had 134 bp length. The Qiagen OneStep RT-PCR kit was used for amplification of mRNA specific to alpha-INT1 gene, according to the kit manual. Primer sequences are as follows:

Forward primer: 5'-CAA GGA AAC GAG ATT TCA AAC CA-3' Reverse primer: 5'-ACT GAT GCT ACT TCA ACG GTT CC-3'. Briefly, PCR was undertaken in a 25 µl volume containing 1 U of Taq polymerase (HotStarTaq; Qiagen, Valencia, Calif.) with recommended buffer and MgCl2 added to a final concentration of 2.0 mM, 6 pmol each of the forward and reverse primers, and 2 µl of the template were added as well.

The transcript was analyzed (25 uL) using SYBR Green PCR Master Mix amplified for 40 cycles. Thermal cycling conditions were: initial enzyme activation steps of 50°C, 2 min, and 95°C, 10 min, and then 40 cycles of denaturation at 95°C, 15 s and annealing at 60°C, 1 min, extension at 72°C for 15 s followed by a final extension step of 72°C for 5 min. To quantify the transcript, a standard curve was constructed using genomic DNA isolated from *C. albicans* ATCC:10231 strain. Gene-specific primers were used to amplify the complete ORF of the gene. The PCR products were separated in the 1.0% agarose gel, stained using SYBR Green I and the acquired images were analyzed using ImageJ software. For normalization of the obtained results, serial 10-fold dilutions of the amplified ORF was used as DNA template. Three independent replicates were done.

3. Results

3.1. Radiation effect on proliferation of Candida albicans

Mean OD values and standard error's of the means obtained by turbidimetric assessment of cell suspensions at initial and at 6, 12, 18 and 24 hours later were presented in table 1. In general, radiation exposure led to enhanced proliferation of the *C.albicans*, compared to unexposed samples, and the cell growth was directly correlated with the duration of exposure. The yeast cells that continuously exposed to the radiation for 18 or 24 hours showed highest-rate of proliferation compared to the others.

4. Discussion

High power microwave radiation (650 W for 6 min) has a deleterious effect on any living microorganism including *C.albicans*, as Neppelenbroek reported dramatic reduction of denture related stomatitis caused by *C. albicans* after microwave treatment of the dentures (Neppelenbroek et al., 2008; Ribeiro et al., 2009; Hiti et al., 2001; Brondani et al., 2012; Rosaspina et al., 1994). Dardanoni and co-authors (Dardanonl et al., 1985; Banik et al., 2003) observed frequency, modulation and time dependent impacts of the radiation on growth rate of *C.albicans*. While 1 KHz-modulated microwave radiation reduced the yeast growth rate by 15% at 72 GHz, but 71.8 or 72.2 GHz radiations did not affect the rate. In contrast, 3 h continuous irradiation of the yeast at 72 GHz increased the growth rate by about 25% over the control. Fang et al, studied low-dose microwave radiation effect on *Aspergillus parasiticus*. Microwave exposure (2.45 GHz, 1.5 W/g) led to increased cell membrane permeability, increased Ca2+; protein and DNA leakage from the fungi (Fang et al., 2011).

Todays, candida species is accounted as a causal agents of 78.3% of the total nosocomial infections and *C. albicans*, by itself, is responsible for 59.8% of the nosocomial cases (Beck-Sagué and Jarvis, 1993). *C. albicans*, behind Aspergillus niger, is the second causative agent of serious ear infections (Jadhav et al., 2003), especially among immunocompromised patients. The higher adherence capacity to oral or gut mucosa is associated with the higher pathogenicity of the yeast (Gale et al., 1996). Alpha-Int1 is an integrin-like molecule frequently expressed on the surface membrane of *C. albicans*. Alpha-INT1 interacts with fibronectin and vitronectin molecules located on the epithelial and endothelial cells, or on extracellular matrix (ECM). Transgenic expression of alph-int1 gene in *Saccharomyces cerevisiae* gives the yeast ability of germ tube production (Gale et al., 1998). It seems, the gene also plays an important role in the germ tube formation by *C.albicans*.

Candida albicans is apparently more resistant to ionizing radiation, and by now, more than 40 articles evaluating this hypothesis have been recorded in the Pubmed library. Hogewind-De Nijs and Hogewind, 1957, for the first time, have reported in a cancerous patient, an acute esophagus candida infection induced after striking of the region by X-Ray radiation. The authors concluded that, in contrast to other microbial normal flora of the esophagus that are susceptible to killing effect of the radiation, *C. albicans* resists well against the radiation and as a consequece, rapidly colonize the region. In addition, 39 studies about the high radiation resistance of *C.albicans* were reviewed by Lalla et al., 2010. The wondrous resistance of the *C.albicans* to ionizing radiation, and probably to the other frequencies, is partly attributable to its rigid cell wall and to its highly effective DNA repairing system. When radiation induced genomic or proteomic damages are tolerable, the changed molecules are rapidly being repaired or replaced by a very effective repair system. A transient growth arrest protects mitotic cells against acute stress. However, acute exposure to the radiation usually results in cell death through apoptosis or necrosis, (Davies, 2000; Crawford et al., 1996).

Our findings showed mobile phone radiation may result in increased proliferation, increased adherence (and therefore, increased biofilm formation) and as well as increased Int1p expression in *C.albicans*, as revealed by increased levels of the gene specific mRNA. Increased proliferation of *C.albicans* due to exposure to 2.45 GHz radiation has also been reported by Dardanoni in 1994, but many other studies accented on killing effect of the radiation (Dardanonl et al., 1985). Killing or the growth promoting effects of the 900 MHz radiation are largely dependent to the wave power (intensity), frequency and exposure time. Lower wave intensity or shorter exposure time is often tolerable and the exposed cells survive, while acute exposure to the radiation is usually destructive and deadly.

Int1p is a virulence factor, contributes to hyphal morphogenesis in *C.albicans* and is required for the axial budding pattern. Int1p/septin ring complex localization in cells is also used for distinguishing yeast/pseudohyphal growth from hyphal growth (Gale et al., 2001). Int1 protein is seen during hyphal induction and germ tube formation, and the protein distally localizes to mother-daughter cell junction (Sudbery, 2001). It seems Int1p play an important role in a subset of the environmental sensing and signal transduction pathways that trigger hyphal growth in *C. albicans* (Kinneberg et al., 1999).

The radiation effects on protein expression were reviewed by McNamee (McNamee and Chauhan, 2009). Repeated exposure of cells to microwave radiation may act as a repetitive stressor, leading to continuous overexpression of HSPs in exposed cells and tissues (French et al., 2001). De Pomerai et al, was reported a significant elevation in GFP reporter gen after overnight exposure of *Caenorhabditis elegans* to 750 MHz radiation (de Pomerai et al., 2000). Although other studies did not confirm the effects (Thorlin et al., 2006). Increased proliferation, enhanced adherence, polymorphic changes and germ tube formation are virulence factors of *C.albicans*, and therefore, 900 MHz microwave exposure results in increased virulence and pathogenicity of the opportunistic fungal pathogen.

C. albicans is an opportunistic fungal pathogen that causes a wide variety of human diseases. In the environment, e.g., in the gastrointestinal tract, the organism is often associated with mucosal surfacesthat triggers several behaviors, such as biofilm formation, invasion and thigmotropism (Kumamoto and Vinces, 2005). Germ tubes of *Candida albicans* are responsible for enhanced adherence of the yeast to plastic surfaces. SDS-PAGE analysis of surface attached adhesins by Tronchin, et al., revealed contribution of four molecules with molecular weights of 68000; 60000 and over 200000 Dalton in the process (Tronchin et al., 1988). The molecular weight of Alpha-int1 candidal surface protein is about 185 kDa that seems has not been detected in Tronchin study, however, its role in blastospores and germ tube adhesion to human epithelium has been approved using the protein specific monoclonal antibodies (Gale et al, 1996).

The dual outcomes of microwave exposure have also been reported in human blood mononuclear cells, PBMCs. Capri et al, studied the nonthermal effect of direct in vitro exposure of human PBMCs on mobile phone radiation (1 hour per day for 3 days). Data obtained from exposed cells showed a slight decrease in cell

proliferation (after treatment of the cells with mitogen) and a slight increase in distribution of cell membrane phosphatidylserine (Capri, 2004). Lu et al, showed in their in vitro experiment, 900 MHz radiation exposure of human PBMCs for longer than 2 hours led to increased reactive oxygen species followed by apoptosis mediated by ROS and caspase-3 (Lu et al., 2012).

In collection, mobile phone handsets or the base transceiver stations are operating using low power microwave radiations (below 1W) and exposure time of the human to the handsets are usually short, therefore, the biological effects are expected to be negligible. However, people living near to the BTS stations are prone to radiation induced damage that the effects may be accumulative when the exposure time is increasing.

5. Conclusion

Our findings showed 900 MHz GSM radiation at the permissible intensity may potentiate *C.albicans* virulence factors and in the long period of time may prone a person to develop chronic inflammatory conditions. As well as, the radiation could alter DNA transcription and protein expression in the cells that may be used for therapeutic or preventive issues.

Acknowledgment

This work was financially supported by the Science and Research Branch of Islamic Azad University of Tehran. We are grateful to Rasoul Soleimani for his assistance in preparing figures and editing manuscript. Thanks for excellent technical assistance offered by Dr. Hassan Nekouie (An experienced member of scientific board of Pasteur Institute of Iran and the leader of Bokharest diagnostic laboratory). Thanks for all staffs of the laboratory, especially for Mrs MirSalehi due to their assistance.

Table 1. Mean and SE of turbidimetric assessments of cultured *C.albicans* cells exposed to 900 MHz radiation for 0, 6, 12, 18 and 24 hours.

		Radiation exposure time (hours)				
		0	6	12	18	24
Sampling time (hour)	0	0.5 ± 0.021	0.5 ± 0.017	0.5 ± 0.023	0.5 ± 0.022	0.5 ± 0.024
	6	0.515 ± 0.023	0.525 ± 0.020	0.525 ± 0.025	0.525 ± 0.023	0.525 ± 0.026
	12	0.665 ± 0.029	0.725 ± 0.032*	0.765 ± 0.034**	0.765 ± 0.033**	0.765 ± 0.039**
	18	0.925 ± 0.041	1.005 ± 0.044*	1.075 ± 0.047**	1.175 ± 0.052**	1.175 ± 0.053**
	24	1.25 ± 0.060	1.35 ± 0.059***	1.475 ± 0.065*	1.54 ± 0.068**	1.67 ± 0.059**

ANOVA test was applied for statistical comparison of radiation exposed yeast samples with control cells in each time points (each row in the table represents one time point). Repeated measures ANOVA is not the case for our study because of time dependent growing nature of the cells in test and control samples.

* : Significant differences ($P<0.01$) in cell density compared to control.

** : Significant differences ($P<0.001$) in cell density compared to control.

*** : Significant differences ($P<0.05$) in cell density compared to control.

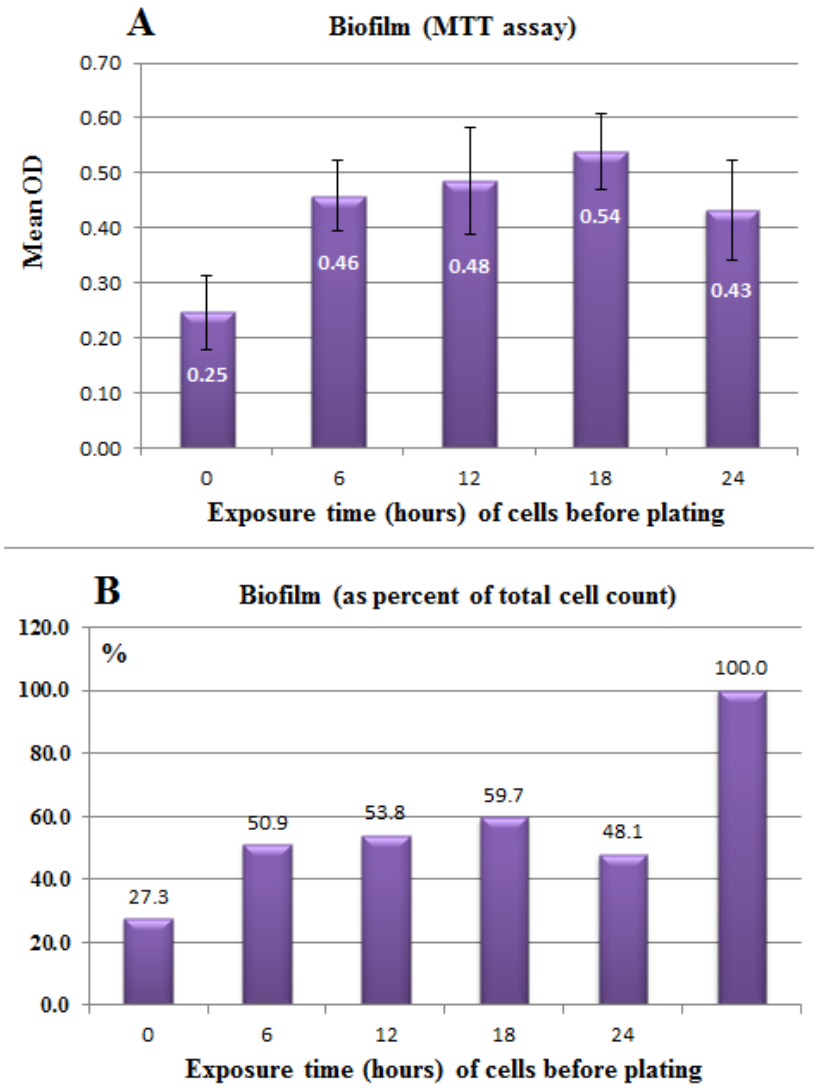

Figure 1. Density of biofilms produced by radiation exposed and unexposed yeast samples. **A**: Mean ODs resulted from the microplate MTT assay of repeated experiments of yeast samples previously exposed to 900 MHz GSM radiation for different time periods of 0, 6, 12, 18 and 24 hours. Error bars represent standard error's of the mean for each sample. Radiation exposure resulted in the increased biofilm formation by the yeast in all exposed cells, however, the differences are statistically significant only between 18 hours exposed samples and control. **B**: Percent of adherent cells (at the end of 4 hours incubation time). The right column shows 100% adherence rate of the cells [ODs from control wells retained both attached and suspended cells/itself) x 100 = 100%]. For example, 59.7% of yeast cells in 18 hour radiation exposed sample have been attached to well bottom and so on.

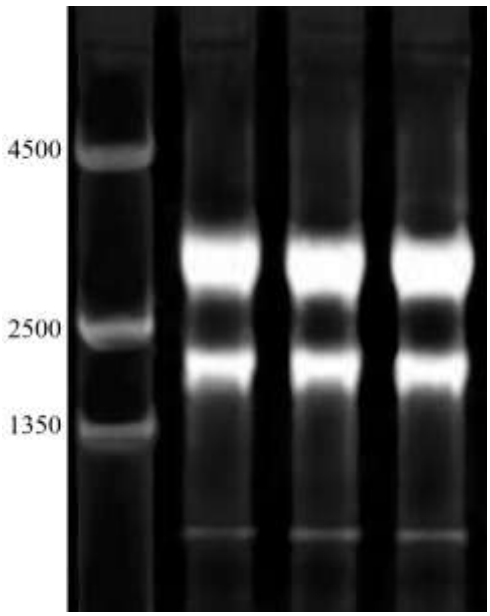

Figure 2. Gel image showing the integrity of isolated RNA molecules. Heat/freeze method was used for RNA extraction from yeast cells followed by Oligotex mRNA kit (Qiagen). Agarose gel (1%) with formaldehyde was used for electrophoresis and SYBR Green II was used for visualization of the resolved RNA bands. The presence of 3 distinct RNA bands (28s, 18s and 5s rRNA) confirms the integrity of isolated RNA samples.

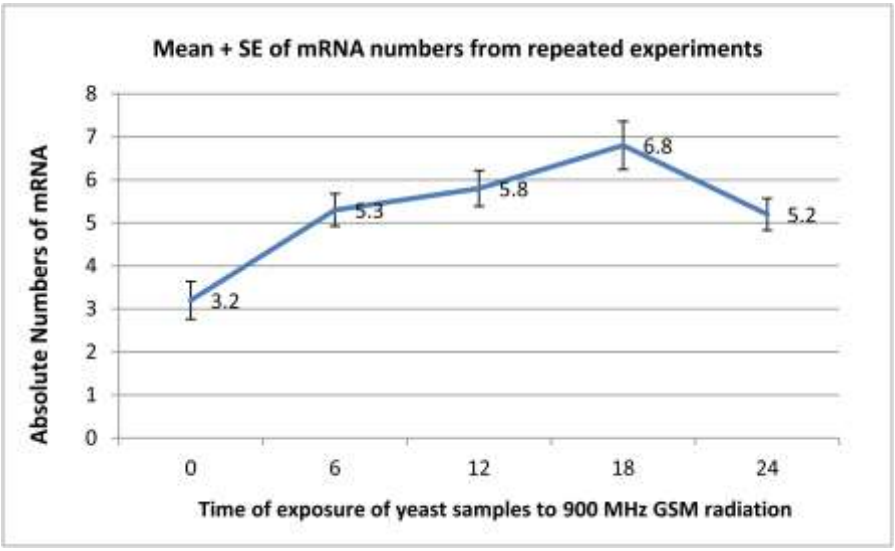

Figure 3. Absolute mRNA amount of radiation exposed and unexposed yeast samples estimated by quantitative RT-PCR results extrapolated using a calibration curve plotted based on the results of serial dilutions of the yeast genomic DNA. Radiation exposure led to increased mRNA specific to alpha-INT1 gene in *C.albicans*. The differences between exposed samples and unexposed control are statistically significant ($P<0.01$). Although mRNA levels increased after 900 MHz radiation exposure, however Int1 protein levels should also be determined.

Refereces

Banik, S., Bandyopadhyay, S., Ganguly, S. 2003. Bioeffects of microwave--a brief review. Bioresour. Technol. 87(2): 155-9.

Beck-Sagué, C., Jarvis, W.R. 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. National Nosocomial Infections Surveillance System. J. Infect. Dis. 167(5):1247-51.

Brondani, M.A., Samim, F., Feng, H. 2012. A conventional microwave oven for denture cleaning: a critical review. Gerodontology. 29(2): e6-15.

Calderone, R. 1998. The INT1 of Candida albicans. Trends Microbiol 6(8): 300-1.

Capri, M., Scarcella, E., Fumelli, C., et al., 2004. In vitro exposure of human lymphocytes to 900 MHz CW and GSM modulated radiofrequency: studies of proliferation, apoptosis and mitochondrial membrane potential. Radiat. Res. 162(2): 211-8.

Coffey, B.M, Anderson, G.G. 2014. Biofilm formation in the 96-well microtiter plate. Methods Mol. Biol. 1149:631-41.

Crawford, D.R., Schools, G.P., Salmon, S.L., Davies, K.J. 1996. Hydrogen peroxide induces the expression of adapt15, a novel RNA associated with polysomes in hamster HA-1 cells. Arch. Biochem. Biophys. 325(2): 256-64.

Dardanonl, L., Torregrossa, M.V., Zanforlin, L. 1985. millimeter-wave effects on Candida albicans cells. Journal of Bioelectricity. 4(1): 171-176.

Davies, K.J. 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life. 50(4-5): 279-89.

dePomerai, D., Daniells, C., David, H. 2000. Non-thermal heat-shock response to microwaves. Nature. 405(6785): 417-8.

Fang, Y., Hu, J., Xiong, S., Zhao, S. 2011. Effect of low-dose microwave radiation on Aspergillus parasiticus. Food Control. 22(7): 1078-1084.

French, P.W., Penny, R., Laurence, J.A., McKenzie, D.R. 2001. Mobile phones, heat shock proteins and cancer. Differentiation. 67(4-5): 93-7.

Gale, C., Gerami-Nejad, M., McClellan, M., et al. 2001. Candida albicans Int1p interacts with the septin ring in yeast and hyphal cells. Mol. Biol. Cell. 12(11): 3538-49.

Gale, C.A., Bendel, C.M., McClellan, M. 1998. Linkage of adhesion, filamentous growth, and virulence in Candida albicans to a single gene, INT1. Science. 279(5355): 1355-8.

Gale, C., Finkel, D., Tao, N., et al. 1996. Cloning and expression of a gene encoding an integrin-like protein in Candida albicans. Proc. Natl. Acad. Sci. USA 93(1): 357-61.

Gauthier, G.M. 2015. Dimorphism in fungal pathogens of mammals, plants, and insects. PLoS. Pathog. 11(2): e1004608.

Hiti, K., Walochnik, J., Faschinger, C., et al. 2001. Microwave treatment of contact lens cases contaminated with acanthamoeba. Cornea 20(5): 467-70.

Hogewind-De Nijs, J.J., Hogewind, F. 1957. [X-ray changes of the esophagus caused by Candida albicans]. Ned. Tijdschr. Geneeskd. 101(28): 1325-6.

Jadhav, V.J., Pal, M., Mishra, G.S. 2003. Etiological significance of Candida albicans in otitis externa. Mycopathologia. 156(4):313-5.

Kinneberg, K.M., Bendel, C.M., Jechorek, R.P., et al. 1999. Effect of INT1 gene on Candida albicans murine intestinal colonization. J. Surg. Res. 87(2): 245-51.

Kumamoto, C.A., Vinces, M.D. 2005. Alternative Candida albicans lifestyles: growth on surfaces. Annu. Rev. Microbiol. 59: 113-33.

Lalla, R.V., Latortue, M.C., Hong, C.H., et al. 2010. A systematic review of oral fungal infections in patients receiving cancer therapy. Support. Care. Cancer. 18(8): 985-92.

Lu, Y.S., Huang, B.T., Huang, Y.X. 2012 Reactive oxygen species formation and apoptosis in human peripheral blood mononuclear cell induced by 900 MHz mobile phone radiation. Oxid. Med. Cell. Longev. 2012: 740280.

McNamee, J.P., Chauhan, V. 2009. Radiofrequency radiation and gene/protein expression: a review. Radiat. Res. 172(3): 265-87.

Mortazavi, S.M., Rahimi, S., Talebi, A., et al. 2015. Survey of the Effects of Exposure to 900 MHz Radiofrequency Radiation Emitted by a GSM Mobile Phone on the Pattern of Muscle Contractions in an Animal Model. J. Biomed. Phys. Eng. 5(3):121-32.

Neppelenbroek, K.H., Pavarina, A.C., Palomari Spolidorio, D.M. et al. 2008 Effectiveness of microwave disinfection of complete dentures on

the treatment of Candida-related denture stomatitis. J. Oral. Rehabil. 35(11): 836-46.

Ribeiro, D.G., Pavarina, A.C., Dovigo, L.N. et al., 2009. Denture disinfection by microwave irradiation: a randomized clinical study. J. Dent. 37(9): 666-72.

Rosaspina, S., Salvatorelli, G., Anzanel, D., Bovolenta, R. 1994. Effect of microwave radiation on Candida albicans. Microbios. 78(314): 55-9.

Schmitt, M.E., Brown, T.A., Trumpower, B.L. 1990. A rapid and simple method for preparation of RNA from Saccharomyces cerevisiae. Nucleic Acids Res. 18(10): 3091-2

Shahin-jafaria, A., Bayat, M., Shahhosseiny, M.H., et al. 2016. Effect of long-term exposure to mobile phone radiation on alpha-Int1 gene sequence of Candida albicans. Saudi Journal of Biological Sciences. 23(3): 426-433.

Stefanics, G., Kellényi, L., Molnár, F., et al. 2007. Short GSM mobile phone exposure does not alter human auditory brainstem response. BMC Public Health. 7: 325.

Sudbery, P.E. 2001. The germ tubes of Candida albicans hyphae and pseudohyphae show different patterns of septin ring localization. Mol. Microbiol. 41(1): 19-31.

Sundstrom, P. 2002. Adhesion in Candida spp. Cell Microbiol. 4(8): 461-9.

Thorlin, T., Rouquette, J.M., Hamnerius, Y., et al. 2006. Exposure of cultured astroglial and microglial brain cells to 900 MHz microwave radiation. Radiat. Res. 166(2): 409-21.

Tronchin, G., Bouchara, J.P., Robert, R., Senet, J.M. 1988. Adherence of Candida albicans germ tubes to plastic: ultrastructural and molecular studies of fibrillar adhesins. Infect. Immun. 56(8): 1987-93.