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Ripoprinting Studies on the Effect of Mobile Phone Radiations on Mice

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Abstract: ***Cell phone radiation is one form of electromagnetic radiation that called microwaves and consists of waves of electric and magnetic energy moving together through space at the speed of light. Smart phones become an important public instrument but form a health problem as there have been reports of plenty of health hazards, both mental and physical, in people of all age groups. The present study was to investigate the possible effects of mobile phone radiation on ATP2b2 gene that play an important role in calcium hemostasis and hematological parameters in mice. In this study twenty five male albino mice were used, They were divided into five groups; normal and the others exposed to the electromagnetic field (EMF) from mobile phone for different duration 1/2h, 1h, 2h, and 4h per day for three weeks. The results showed that significant decrease in Hb, HCT, MCV, and MCHC, WBCs, PLT (P < 0.05) count and ionized calcium. While the mean value of RBCs and MCH did not show significant difference when compared to the normal with exposed group. The ATP2b2 gene was slightly affected in all exposed groups.***

Keywords: Cell phone radiation, ATP2b2 gene, Calcium ions, Male Swiss albino

1. Introduction

Every second of our life, we are exposed to all forms of radiation either man-made like that emitted from cell phones, cell phone towers, wireless routers, Wi-Fi, and radio waves from radio and television broadcasts, or natural radiation such as ultraviolet light from the sun. Electromagnetic fields (EMFs) permeate our environment everywhere: in our homes, at work, in schools, and elsewhere wherever there are electric wires, electric motors and electronic equipment (Valberg et al., 2007). So we can say we live in a radiation world.

Today, Smart phones with more advanced computing facilities have come into the market. In the last 20 years, worldwide mobile phone subscriptions have grown from 12.4 million to over 5.6 billion, penetrating about 70% of the global population (WHO, 2011). Its usage has also become an important public health problem as, a variety of negative health effects have been attributed to exposure to radiofrequency electromagnetic field (RF-EMF) from mobile phones. Cell phone use indeed represents a health menace, and classified mobile phone radiation as a carcinogenic hazard, possibly carcinogenic to humans. An epidemiologic study first showed that exposure to low-frequency EMFs increased childhood leukemia (Wertheimer & Leeper, 1979).

The biological effects of exposure to EMF from mobile phones were reported to be variable, depending on many factors including duration of exposure, distance from the various sources, species and tissues as well as the conditions of exposure (Ahlbom et al., 2008). Exposure to RF from mobile phone has harmless effects on different tissues. It forms numerous spongiform vacuoles in the neuropil of the rat's brain tissues and caused congestion of the cerebral blood vessels (Usikalu et al., 2012). EMF from mobile phones cause changes in immune activity in cultured Peripheral blood mononuclear cells (Dabrowski et al., 2003) and a significantly higher response to mitogens and higher immunogenic activity (LM index) (Stankiewicz et al., 2006). It influences the distribution of cell cycle and decreases the rate of cell proliferation (Marinelli et al., 2004).

Some studies show a relation between infertility in male and Radio Frequency Radiation (RFR) emitted from cell phone as it cause reduction of weight, in sperms count and testicular size, increase apoptosis in male rat (Kesari et al., 2010). On the other hand receiving a certain period of microwave radiation from cellular phones during pregnancy has certain harm on fetal rat brains as the contents of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), noradrenaline (NE), dopamine (DA), and 5-hydroxyindole acetic acid (5-HT) in the brain changed after exposure to RF (Jing et al., 2012). It is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis (Ferreira et al., 2006), changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates (Furtado-Filho et al., 2013). But, another study suggests that the impairment of fertility was not caused by the induction of apoptosis in spermatozoa as Mobile phone radiation had no statistically significant effect on caspase 3 activity, externalization of phosphatidyl serine (PS), induction of DNA strand breaks, and generation of reactive oxygen species (Falzone et al., 2010).

In addition to the previous effects there is evidence that RF energy from mobile phones is capable of inducing physiological effects in human subjects. These effects include warmth on and behind the ear, fatigue, headache, decreased concentration, dizziness, memory loss, tingling and numbness (Sandstrom et al., 2001), Attention Deficit Hyperactivity Disorder (ADHD) in children (Yoon-Hwan Byun et al., 2013) and effect on brain function, especially tasks requiring attention and manipulation of information in working memory (Koivisto et al., 2000). Increase in rapid eye movement sleep (REM sleep) indicating a change in the ultradian rhythm of normal sleep cycles (Haitham et al., 2013). RFR changes in concentrations of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the hippocampus, hypothalamus, midbrain and medulla oblongata of adult rats (Aboul Ezz et

al., 2013),and increase in both excitatory and inhibitory amino acids in the cerebellum of adult and young rats (Noor et al., 2011).

RF-EMF exposure has no acute effects on retinal ganglion cell responses under constant temperature conditions (Ahlers et al., 2013). Exposure to electromagnetic field (EMF) emitted by an ordinary GSM mobile phone (902.4 MHz pulsed at 217 Hz) did not show significant differences in Auditory brainstem responses (ABR) suggesting that short-term exposure to mobile phone EMF did not affect the transmission of sensory stimuli from the cochlea up to the midbrain along the auditory nerve and brainstem auditory pathways (Kwon et al., 2010a).

RF-EMF were reported to cause single-strand DNA breaks in brain (Deshmukh et al, 2013b), DNA damage in cells of human hair roots close to the phone (Cam & Seyhan, 2012), altered the structure of calf thymus DNA (Hekmat et al., 2012), induced chromosome breaks and increased risk of acoustic neuroma with the use of analogue phones (Lightfoot et al., 2005 and Hardell et al., 2006). Whenever, exposure to MWs from mobile phone did not induce DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells (Belyaev et al., 2006) and no significant change in the rate of aneuploidy of chromosomes 11 and 17 was found following exposure to GSM-900 for 24 h (Bourthoumieu et al., 2011).

Also, it was suggested that RF-EMF altered gene and protein expression in human skin (Anu Karinen et al., 2008). However, recently the gene expression did not altered in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes in the yeast cells (Chen et al., 2012). In another study, no significant changes in the expression and activation of the p53 protein by phosphorylation at serine 15 and 37 were found (Bourthoumieu et al., 2012).

Radiofrequency from mobile phones have harmless effects on different organs and tissues. In contrast another studies suggested that no effects have been observed after exposure to cell phone radiation. Thus numerous studies should be done in different fields to show more facts on the harmless effects of cell phones in order to international standards for the protection. One of the most important issues regarding RF-EMF is their effect on genetic material and the brain as the mobile phone during call is hold near the brain that directly penetrated by microwave. Thus this study aimed to investigate the effect of radiation from cell phone on the genomic stability in the brain of mice specifically on ATP2b2 gene that produced plasma membrane calcium ATPase (PMCA). PMCA play important role regulating the amount of Ca^{2+} within all eukaryotic cells. Plasma membrane Ca^{+2}ATPase (PMCA) is considered as one of the major players for Ca2+ homeostasis.

Plasma membrane Ca^{+2}ATPase gene (PMCAs) belong to the type IIB subfamily or family 2 within the large superfamily of P-type primary ion transport ATPase's (Axelsen et al., 1998). It characterized by the formation of as partyl phosphate intermediate during an ATP hydrolysis reaction cycle (Filoteo et al., 1996, 1997). PMCA cooperates with other transport systems and with soluble Ca^{+2} binding proteins to control of the cellular homeostasis of Ca^{+2}. By extruding Ca^{+2} from the cytosol against its inward gradient using energy derived from ATP hydrolysis (Carafoli, 1992). Their high affinity for Ca^{+2} makes them a highly efficient route for Ca^{+2} effluxes even at intracellular Ca^{+2} levels close to rest, but also during transients (Thayer et al., 2002).

PMCAs 2 enriched within excitable cells such as the nervous system (areas of the brain and in skeletal muscle) where their faster activation and extrusion rates thus are termed neurospecific (Brini et al., 2003). The expression, proper targeting and function of PMCA2 are essential for hearing as well as other specific physiological processes such as calcium transport into milk (Reinhardt et al., 2004), motor neuron coordination and cerebellar synaptic plasticity (Empson et al., 2010 and Huang et al., 2010).

2. Material and Methods

2.1 Experimental Animals

This study was carried out on twenty five male Swiss albino mice, weighted about 25-30 gm. Mice divided randomly in five plastic cages for one week before the beginning of the experiment for adaptation. Mice were kept under constant environmental conditions at (22°C ± 3°C) at AM and (28°C ± 3°C) at PM. The light was on a 12 hours light and 12 hours dark cycle.

Animals were divided into five groups each group with five mice. The whole body of mice exposed to the electromagnetic field (EMF) from mobile phone (Nokia 2700c-2 model), daily for three weeks. Group (1), Control group, was not exposed to the cell phone EMF, and kept under fixed conditions of housing and handling until the day of sacrifice. Group (2) was exposed to the Mobile phone EMF 1/2hr/day (1/4 hr. Am and 1/4hr Pm). Group (3) was exposed to the mobile phone EMF 1hr/day (1/2 hr. Am and 1/2hr Pm. Group (4) was exposed to the cell phone EMF 2hr/day (1hr Am and 1hr Pm). Group (5) was exposed to the mobile phone EMF 4hr/day (2 hr. AM and 2hr PM).

2.2 Exposure Technique

Tested mice groups were exposed to radiofrequency electromagnetic field (RF-EMF) of 1800 MHz frequency band mobile phone (Nokia 2700c-2 model), which, according to the GSM, operates with microwave carrier frequencies in the range 900-1800 MHZ the mobile phone was kept in the ringing position, receiving calls from another phone during hours of EMF exposure, but in silent mode, during the whole time of exposure. The exposure occurred in an open place at 8- 10 Am and at 8- 10 Pm during April 2016. Blood samples collected for detect Ca^{+2} levels in serum and complete blood count from the eye, using orbital sinus technique (Sanford, 1954). Few drops of blood were collected in a tube containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant for the hematological studies. The other portion of blood was collected in tube without any anticoagulant and

allowed to clot, then serum was separated by centrifugation at 3000 rpm for 20 min. and the clear non hemolysis serum was collected and stored for measurement of serum calcium ions level. After the collection of blood, all brains, from both control and Experimental groups, were isolated. Brain was reserved in clean Eppendorf tube and stored in freezing to be used for genetic stability detection.

2.3 Biochemical studies:

2.3.1 Determination of complete blood count:

Blood samples collected in tube containing EDTA were used to determine Complete blood count (CBC). Estimation of red blood cells count (RBCs), hemoglobin (Hb) and hematocrit (HCT), Measurements of RBC Indices are mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), platelets (PLT) count were carried out using electronic coulter counter (Mind ray cell counter device).

2.3.2 Determination of Serum Ionized Calcium level:

Determination of ionized calcium depends on the determination of total protein and total calcium. Total protein assayed spectrophotometrically by using total protein plus ELITech Clinical Systems Kit according to (Berth & Delanghe, 2004). Total calcium determined spectrophotometrically by using CALCIUM CPC method (reagent for quantitative determination of calcium in serum and plasma or urines) according to (TIETZ, 2006). (Company: Biolabo Reagents, France). (www.biolabo.fr).
Serum Ionized Calcium level determined using the formula as follows:

$$Ca^{+2}\ \text{Ionized} = \frac{6 \times Ca.total - \frac{T.\ protien}{3}}{6 + Ca.total}$$

2.4 Molecular techniques:

2.4.1 DNA extraction:

Total DNA was isolated from the brain of mice, according to manufacture protocol of Omega Co. (USA. LMT), (Mohamed & Yacout, 2014).

2.4.2 Polymerase chain Reaction (PCR) for gene detection and amplification:

Taq DNA polymerase was purchased from sigma- Aldrich Chemi GmbH, Germany (#D9307). Taq Green master mix was used for Atp2b2 gene amplification of all groups according to manufacture protocol.

2.4.3 PCR purification:

DNA purification from the agarose gel was made using GeneJET™ PCR Purification Kit (Thermo K0701). GeneJET™ PCR Purification Kit removes unincorporated labeled nucleotides, primers, dNTPs, enzymes, and salts from PCR products and any other reaction mixture. The ATP2b2 gene primer used Forward primer (5´-GGATCCCAGCCGCCTGCGTGC-3´), and Reverse (5´-ATGAGCGTGGCCAGGCGACA-3´).

2.4.4 DNA sequencing:

Sequencing to the PCR product was made on GATC Company by using ABI 3730xl DNA sequencer by using forward and reverse primers.

Only by combining the traditional Sanger technology with the new 454 technology, can genomes now be sequenced and analyzed in half the usual project time, with a considerable reduction in the number of coatings and gaps. In addition, considerable cost advantages now make genome sequencing with the 454 technology accessible to the research community.

2.4.5 Molecular analysis (Bioinformatics tools):

T-coffee is a multiple sequence alignment online program that was used to compare all sequences in normal and exposed groups to detect mutations. (Notredame et al., 2000) (http://tcofee.org.cat/apps/tcofee/do:regular). Gene bee services is an online tool that was used for the prediction of RNA secondary structure and calculating the minimum free energy for RNA secondary structure it is available on: (http://www.genebee.msu.su/services/rna2_reduced.html)

Phylogenetic tree that shows the relationships among various biological species or other entities- their phylogeny- based upon similarities and differences in their physical or genetic characteristics was made by using gene bee. It is available on: (http://www.genebee.msu.su/services/malign_reduced.html)

3. Results

3.1 Hematological studies:

Effect of cell phone radiation was detected on blood ionized calcium level (Ca^{+2}) and Complete blood count, including red blood cells count (RBCs), hemoglobin (Hb) and hematocrit (HCT). Measurements of RBC indices are mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), platelets (PLT) count. The results showed that significant decrease in Hb, HCT, MCV, and MCHC, WBCs, PLT ($P < 0.05$) count and ionized calcium, when comparing the normal with exposed group. While the mean value of RBCs and MCH don't show significant difference as shown in the table (1) and table (2).

Table 1: Effect of cell phone radiation on hematological parameters (RBCs, Hb, PLTs, WBCs) in male mice

Parameters / Groups	RBCs 10^6/cmm	Hb g/dL	HCT Vol %	PLT 10^3/cmm	WBC 10^3/cmm
Control	4.43 ±.03	13.47 ±.03^{a}	41.40 ±.26^{a}	351.67 ±.9^{a}	6.71 ±.06^{a}
1/2 hour	3.88 ±.37	11.02 ±1.13^{c}	31.33± 2.81^{b}	363 ± 6.6^{a}	5.08 ±.45^{b}
One hour	3.78 ±.16	11.36 ±.48^{bc}	33.80 ±.47^{b}	339.75 ±5.3^{a}	4.5 ±.35^{b}
Two hour	4.28 ±.14	13.02 ±.47^{ab}	40.40 ±1.45^{a}	259.3 ± 20.5^{b}	4.5 ±20^{b}
Four hour	4.39 ±.17	13.10 ±.24^{ab}	40.87 ± 1.41^{a}	324.6 ±13.8^{a}	4.74 ±.42^{b}
p- value	.227	.044	.001	.001	.013

Values are expressed as means ± (SE) standard errors. Letters in the same column showed non- significant changes

Table 2: Effect of cell phone radiations on hematological parameters (MCV, MCH, MCHC) and ionized calcium level in male mice

Parameter / groups	MCV Fl	MCH Pg	MCHC %	Ca^{+2} mg/dL
Control	93.40 ± .45^{b}	30.17 ± .17	32.30 ±.00^{a}	1.22±.00^{a}
1/2 hour	93.43 ±.22^{b}	30.22 ±.11	32.20 ±.00^{b}	1.16 ±.00^{c}
One hour	93.20 ±.15^{b}	30.06 ±.06	32.26 ±.02^{ab}	1.19 ±.01^{b}
Two hour	94.45 ± .27^{a}	30.48± .08	32.22 ±.02^{b}	1.19 ±.01^{b}
Four hour	93.57 ± .13^{b}	30.84 ± .43	32.26 ±.02^{ab}	1.17 ±.00^{bc}
p- value	.018	.161	.029	.001

Values are expressed as means ± (SE) standard errors. Letters in the same column showed non- significant changes.

3.2 Molecular studies:

After exposure to mobile phone radiation, the PCR and sequencing technique were used to detect any mutations in the partial sequence of ATP2b2 gene in all exposed groups.

The PCR products of ATP2b2 for normal and exposed groups can be seen as a single band in each lane with the same length 383 base pair as shown in figure (1). The first lane contains the DNA ladder with different lengths.

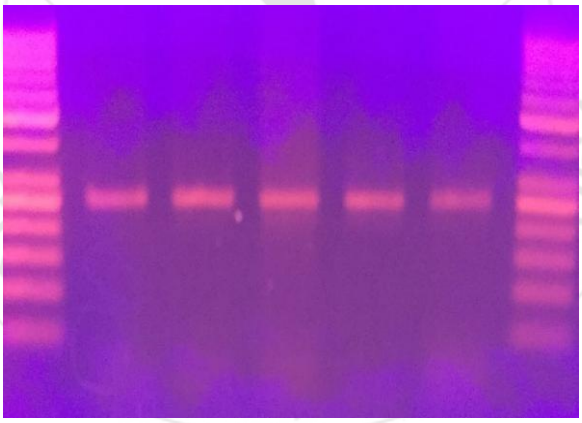

Figure 1: PCR product of ATP2b2 gene for all groups

The normal group sequence contains 383 nitrogenous bases, 122 amino acids and 5 stop codons. Figure (2) and (3) shows the alignments of DNA and protein sequences between normal and exposed group. The ATP2b2 gene showed mutated position after exposing to cell phone radiation when compared to Normal group. The group that exposed for 1/2h/day when compared to normal involves deletion of the nitrogenous base (C) at position (5) and substitution of G/C at position (370) as show in multiple sequence alignment (MSA) figure (2).

In this group occurred changes in position of amino acids and amino acids numbers become 121and 6 stop codon due to the substitution and deletion as shown in figure (3).

While the group exposed to 1h/day when compared to normal groups showed substitution of nitrogenous base G/C at position (370). This substitution caused change of amino acid Ala (A) to Pro (P) at the position 124. As the same when the normal group compared with group exposed for 2h/day and 4h/day involve the substitution of nitrogenous base G/C (370). As a result of this replacement change the amino acid Ala (A) to Pro (P) at the position 124.

```
1/2h      AGTC-CCAGAAGGGCTCAGCCCACTCCTGACCAAAGTGTTTCCCTTGCAG
1h        AGTCCCCAGAAGGGCTCAGCCCACTCCTGACCAAAGTGTTTCCCTTGCAG
2h        AGTCCCCAGAAGGGCTCAGCCCACTCCTGACCAAAGTGTTTCCCTTGCAG
4h        AGTCCCCAGAAGGGCTCAGCCCACTCCTGACCAAAGTGTTTCCCTTGCAG
Normal    AGTCCCCAGAAGGGCTCAGCCCACTCCTGACCAAAGTGTTTCCCTTGCAG
          **** **********************************************

1/2h      ATCCGCGTCGTGAAGGCGTTCCGTAGCTCTCTCTATGAAGGGTTAGAAAA
1h        ATCCGCGTCGTGAAGGCGTTCCGTAGCTCTCTCTATGAAGGGTTAGAAAA
2h        ATCCGCGTCGTGAAGGCGTTCCGTAGCTCTCTCTATGAAGGGTTAGAAAA
4h        ATCCGCGTCGTGAAGGCGTTCCGTAGCTCTCTCTATGAAGGGTTAGAAAA
Normal    ATCCGCGTCGTGAAGGCGTTCCGTAGCTCTCTCTATGAAGGGTTAGAAAA
          **************************************************

 1/2h     ACCCGAGTCTCGAACCTCCATCCATAACTTCATGGCTCATCCTGAATTCC
1h        ACCCGAGTCTCGAACCTCCATCCATAACTTCATGGCTCATCCTGAATTCC
2h        ACCCGAGTCTCGAACCTCCATCCATAACTTCATGGCTCATCCTGAATTCC
4h        ACCCGAGTCTCGAACCTCCATCCATAACTTCATGGCTCATCCTGAATTCC
Normal    ACCCGAGTCTCGAACCTCCATCCATAACTTCATGGCTCATCCTGAATTCC
          **************************************************

1/2h      GGATCGAAGATTCCCAGCCCCACATCCCCCTCATCGATGACACCGACCTG
1h        GGATCGAAGATTCCCAGCCCCACATCCCCCTCATCGATGACACCGACCTG
2h        GGATCGAAGATTCCCAGCCCCACATCCCCCTCATCGATGACACCGACCTG
4h        GGATCGAAGATTCCCAGCCCCACATCCCCCTCATCGATGACACCGACCTG
Normal    GGATCGAAGATTCCCAGCCCCACATCCCCCTCATCGATGACACCGACCTG
          **************************************************

1/2h      GAAGAAGATGCCGCGCTCAAGCAGAACTCGAGCCCGCCGTCCTCGCTCAA
1h        GAAGAAGATGCCGCGCTCAAGCAGAACTCGAGCCCGCCGTCCTCGCTCAA
2h        GAAGAAGATGCCGCGCTCAAGCAGAACTCGAGCCCGCCGTCCTCGCTCAA
4h        GAAGAAGATGCCGCGCTCAAGCAGAACTCGAGCCCGCCGTCCTCGCTCAA
Normal    GAAGAAGATGCCGCGCTCAAGCAGAACTCGAGCCCGCCGTCCTCGCTCAA
          **************************************************

1/2h      CAAGAACAATAGCGCCATCGACAGCGGGATCAACCTGACGACCGACACGA
1h        CAAGAACAATAGCGCCATCGACAGCGGGATCAACCTGACGACCGACACGA
2h        CAAGAACAATAGCGCCATCGACAGCGGGATCAACCTGACGACCGACACGA
4h        CAAGAACAATAGCGCCATCGACAGCGGGATCAACCTGACGACCGACACGA
Normal    CAAGAACAATAGCGCCATCGACAGCGGGATCAACCTGACGACCGACACGA
          **************************************************

1/2h      GCAAATCAGCTACCTCTTCAAGTCCAGGGAGCCCCATCCACAGCCTGGAG
1h        GCAAATCAGCTACCTCTTCAAGTCCAGGGAGCCCCATCCACAGCCTGGAG
2h        GCAAATCAGCTACCTCTTCAAGTCCAGGGAGCCCCATCCACAGCCTGGAG
4h        GCAAATCAGCTACCTCTTCAAGTCCAGGGAGCCCCATCCACAGCCTGGAG
Normal    GCAAATCAGCTACCTCTTCAAGTCCAGGGAGCCCCATCCACAGCCTGGAG
          **************************************************

1/2h      ACGTCGCTTTAGCTGAGGCCCCTGTCGCCTGGC
1h        ACGTCGCTTTAGCTGAGGCCCCTGTCGCCTGGC
2h        ACGTCGCTTTAGCTGAGGCCCCTGTCGCCTGGC
4h        ACGTCGCTTTAGCTGAGGCCCCTGTCGCCTGGC
Normal    ACGTCGCTTTAGCTGAGGCGCCTGTCGCCTGGC
          ********************* ************
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Figure 2: alignments of sequences of ATP2b2 gene in Normal and exposed groups

```
1/2h      SPRRAQPTPDQSVSLADPRREGVP-LSL-RVRKTRVSNLHP-LHGSS-IP
1h        SPQKGSAHS-PKCFPCRSAS-RRSVALSMKG-KNPSLEPPSITSWLILNS
2h        SPQKGSAHS-PKCFPCRSAS-RRSVALSMKG-KNPSLEPPSITSWLILNS
4h        SPQKGSAHS-PKCFPCRSAS-RRSVALSMKG-KNPSLEPPSITSWLILNS
Normal    SPQKGSAHS-PKCFPCRSAS-RRSVALSMKG-KNPSLEPPSITSWLILNS

1/2h      DRRFPAPHPPHR-HRPGRRCRAQAELEPAVLAQQEQ-RHRQRDQPDDRHE
1h        GSKIPSPTSPSSMTPTWKKMPRSSRTRARRPRSTRTIAPSTAGST-RPTR
2h        GSKIPSPTSPSSMTPTWKKMPRSSRTRARRPRSTRTIAPSTAGST-RPTR
4h        GSKIPSPTSPSSMTPTWKKMPRSSRTRARRPRSTRTIAPSTAGST-RPTR
Normal    GSKIPSPTSPSSMTPTWKKMPRSSRTRARRPRSTRTIAPSTAGST-RPTR

1/2h      QISYLFKSREPHPQPGDVALAEAPVAW
1h        ANQLPLQVQGAPSTAWRRRFS-GPCRL
2h        ANQLPLQVQGAPSTAWRRRFS-GPCRL
4h        ANQLPLQVQGAPSTAWRRRFS-GPCRL
Normal    ANQLPLQVQGAPSTAWRRRFS-GACRL
```

Figure 3: encoded amino acids of ATP2b2 gene for all groups

RNA is single strand that able to form hydrogen bond between complementary base pairs forming complicated secondary structure. RNA secondary structure contains regions with base pairs called stems and regions with single strand called loops. RNA secondary structure showed differences in their free energies when the treated groups compared with normal as shown in figures (4, 5, and 6). The free energy of normal is -75.6 Kkal/ mol and had 15 stems. While the free energy of the secondary structure of 1/2 h exposed group is -74.0 Kkal / mol and has 15 stems. The RNA secondary structures of 1h, 2h, and 4h exposed groups are the same with free energy -73.9 Kkal / mol and have 15 stems.

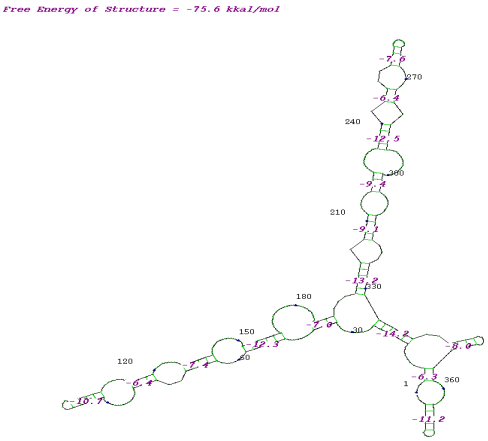

Figure 4: The predicted RNA secondary structure for normal group. Free energy = - 75.6 kkal/mol

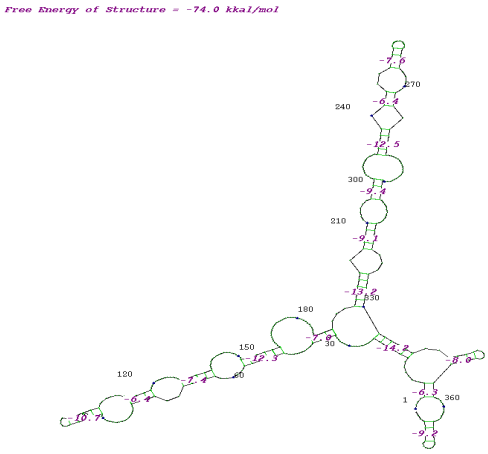

Figure 5: The predicted RNA secondary structure for 1/2h group. Free energy = - 74.0 kkal/mol

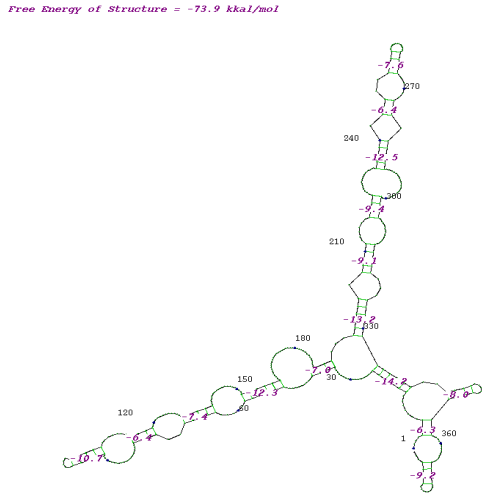

Figure 6: The predicted RNA secondary structure for 1 h, 2h, and 4h group. Free energy = -73.9 kkal/mol

4. Discussion

Assessments of blood parameters are most important means to determine the health status of experimental animals (Soud, 2004). Thus studying effects of radiofrequency radiations from cell phone on the hematological parameters serve as a useful indicator of the potential effects of RF radiations on exposed animals. This is because the blood reflects pathophysiology of the whole body (Adhikari et al., 2004).

Radiofrequency radiations from cell phone cause significant declined in RBC (Alghamdi et al., 2012, Singh et al., 2013, El-Bediwi et al., 2013, & Kumari et al., 2016), HCT, MCV, MCH and MCHC (Alghamdi, et al., 2012 & Mohammad et al., 2016), in hemoglobin, in addition to the platelet count after short and long exposure to two types of mobile phon. (Alghamdi, et al., 2012). Also RFR caused significant decrease in white blood cell count and platelets after one hour radiation exposure to electromagnetic waves generated by mobile phone (Kumari, et al., 2016). Another studies revealed that radiofrequency radiations from cell phone cause significantly elevation of white blood cell (WBC) counts (Adebayo, et al., 2010& Alghamdi, et al., 2012), Packed Cell Volume (PCV), White Cell Count (WBC), Platelet count (PLT), Red Cell Count (RBC (Adebayo, et al., 2010), white cells and lymphocytes in exposed mice to RF radiations when compared with control values (Alghamdi, et al., 2012).

The changes of the hematological parameters may be due to the electromagnetic radiation from cell phones cause stimulation of Haemopoetic activity in the bone marrows and oxidative stress that leads to uncontrolled haemopoesis might eventually lead to anemia and bone marrow atrophy or fibrosis. These disease conditions indicators for other abnormalities observed in deformation of red blood cell morphology (Adebayo, et al., 2010). Abnormalities deformities in red blood cells include change in shapes and size of red blood cells (Singh, et al., 2013 & Yong, et al., 2013), increase in number of macrocytes (enlarged cells), poikilocytes (cells of different shapes), polychromatic cells and target cells. (Adebayo, et al., 2010& Hoffman et. al., 2004) These abnormal shapes can change the superficial energy and mechanical characteristics of red blood cells. The decrease in superficial charge density of red blood cells may increase their aggregation and density leading to the formation of rouleaux and cause cell lysis. (Singh, et al., 2013 & Yong, et al., 2013) and deformities in RBCs morphology show a possible relation between exposure to cell phone radiation and depression of the bone marrow that cause multiple myeloma, clinical globulinaemia (reduced number of blood cells), myelofibrosis and thalassaemia (Hoffman et. al., 2004).

Exposure to cell phone radiation increased concentration of peroxynitrite that can interfere with proper function of cells and damage it. Also increase superoxide activity. Erythrocytes are vulnerable to oxidative damage due to continuous exposure to high oxygen tension and presence of large amount of iron, a potent catalyst for oxygen free polyunsaturated fatty acids which are major targets for peroxidation (Hasan, et al., 2014). RF fields effect on leucocytescan change the leucocyte behavior, including more rapid changes in shape (cell shrinking, expanding and rolling) and lost its ability to move (Aly,et al., 2011) causing cell lysis and reduction in white blood cells (El-Bediwi, et al., 2013).

The electromagnetic radiations from cell phones lead to damage and a clear influence on the cell walls and cause an imbalance in blood enzymes (Alghamdi, 2012 & Hasan et al., 2014). Increases cell apoptosis and functional disorders in many cell types (Atasoy et al., 2009).

These changes also may be due to cell phone cause aggregation of immature of hematopoietic tissues; some appear as a megakaryocyte and existence of caneculous gland. There are bursted primordial blood cells in bone marrow. (Kadhum, 2012), effect on erythropoietin hormone which play an important role in blood formation , Also EMR cause damage in bone marrow by affected on the precursor cells like monocyte, macrophage, promocyte by apoptosis. These cells synthesize several cytokines that share in hematopoiesis, in addition to phagocytosis (Unanue, 1993). These cytokines affects erythrocytopoiesis, Altered secretory functions and phagocytic of alveolar and peritoneal macrophages (Trosic, 2004).

The elevation of white blood cell count is also said to indicate tissue destruction, disorders of white blood cells (leukemia) and bone marrow failure. The significant elevation of the white blood cells was therefore a clear indication of a stress related effect in the exposed animals and should prompt further investigations on the immunological effect of RF radiations on exposed population. Red cell indices showed marginal variations. There was a marginal increase in the mean cell volume (MCV) of the exposed mice and a corresponding decrease in the mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in both stations. These might correspond to an increase utilization of folate and iron required for the increased hematopoietic process in the mice. (Adebayo et al., 2010).

In the present study the results showed that electromagnetic radiation from cell phone cause statically significant change in Hb, HCT, MCV, MCHC, PLT, and WBCs levels as (Alghamdi,et al., 2012; Singh et al., 2013; Mohammad et al., 2016 and Kumari et al., 2016).

Calcium has a great role in the regulation of cellular physiology. Intracellular calcium homeostasis is essential for normal cellular function. Calcium ions bound to the cell membranes surfaces are important for maintaining their stability. They help hold together the phospholipids molecules that are an essential part of their make-up (Ha, 2001). Without these ions, cell membranes become weak and are more likely to tear under the stresses and strains imposed by the moving cell contents. Although, the resulting holes are normally self-healing they still increase leakage while they are open and this can explain the bulk of the known biological effects of weak electromagnetic fields (Goldsworthy, 2007). Leaks in the membranes surrounding lysosomes can release digestive enzymes, including DNAase (Goldsworthy, 2007). This explains the serious damage done to the DNA in cells by cell phone signals**.**
Our study relieved that cell phone radiation causes statically significant decreased in ionized calcium level in plasma. The result was in complete harmony with **(**Mohamed, et al., 2011 & Maskey, et al., 2010**)**.These due to EMF increased the entry of calcium ions into the cells of bone tissue and altered the expansion of ossification (Adey, 1981). Because of these effects, EMF has been used in the treatment of bone fractures of adult humans (Andrew et al., 1984). RF radiation leads to alteration of intracellular signaling pathways that change in Ca^{+2}permeability across cell membranes (Maskey, et al., 2010). Electromagnetic radiation generated free radicals cause oxidative stress and impaired antioxidant system and genotoxic effect (Mashevich, et al., 2003). Also exposure to RF effects on the brain function due to significant increase in calcium ion influx and decrease in PKC activity (Paulraj, et al., 2012).

Genome studies play an important and excellent role in the prediction of common risks on any organism (Pennisi, 2010). Also, these studies are important to know the mutations in different genes and understanding the relation between these genes and diseases (Cooper et al., 2008).

The ATP2b2 gene encoded PMCA2 that belong P-type primary ion transport ATPase's (Axelsen et al., 1998). It characterized by the formation of aspartyl phosphate intermediate during an ATP hydrolysis reaction cycle (Filoteo et al., 1996, 1997). PMCA2 cooperates with other transport systems and with soluble Ca^{+2}binding proteins to control of the cellular homeostasis of Ca^{+2}. By extruding $Ca^{+2}$from the cytosol against its inward gradient using energy derived from ATP hydrolysis (Carafoli, 1992). PMCA2 is widely expressed in the mammalian central nervous system (Stauffer et al., 1995) and widely distributed in all areas of the brain (Furuta et al., 1998). The PMCA2 isoform is highly expressed in the cerebellum, particularly (Filoteo et al., 1996, 1997) in the plasma membrane and dendritic spines of Purkinje cells for controlling the local dendritic calcium equilibrium (Pradeep & Josef, 2013). PMCA2 also highly expressed in the inner ear: the outer hair cells of the Corti organ and the spiral ganglion neurons contain large amounts of it (Furuta et al., 1998). Several studies confirmed the dependence of auditory transduction on PMCA2 (Bortolozzi et al., 2010). And PMCA2 dysfunction leads to hearing loss in humans (Ficarella et al., 2007).

Specific missense mutations in the ATP2B2 gene have also been identified in at least two separate human pedigrees with congenital sensorineural hearing loss, providing unequivocal evidence for the involvement of this PMCA isoform in specific disease pathology (Giacomello et al., 2012). An increase in levels of PMCA2 is seen during lactation, which decreases again upon weaning. An increase in levels of PMCA2 is also observed in breast cancer cell lines (Van Houten et al., 2010).

PMCAs have been found at presynaptic sites (Jensen et al., 2007) where they provide one of the routes for pre-synaptic Ca^{+2}removal along with the Na^{+}/ Ca^{+2}exchanger (Empson et al., 2007). Ca^{+2}ions are pumped out against a concentration gradient of four order of magnitude by a plasma membrane Ca^{+2}ATPase (PMCA). The PMCAs represent the major transport system at the plasma membrane responsible for the long term regulation of resting free Ca^{+2} and counteracting transient increases that occur during Ca^{+2}signaling (Strehler & Zacharias, 2001).

In this study we investigated the effects of mobile phone radiation on ATP2b2 gene. ATP2b2 Gene assessed by DNA sequencing and bioinformatics tools. DNA sequencing is a process for determining the nucleotides within a DNA molecule. Then the multiple sequence alignments (MSA) is an initial step for driving the most common biological models, including, structural homology modeling, functional inference through domain profile comparisons, and phylogenetic reconstruction. It is one of the most challenge tasks in computational complexity. One can detect the most reliable portions from the sequenced data to be studied (Chang et al., 2014 and Pramanik et al., 2014). So we targeted ATP2b2 gene from the sequenced data. Sequence similarity searches against biological sequence databases using the algorithm computational tools have become one of the most used bioinformatics approaches (Lee et al., 2012 and Neuman et al., 2013).

The central dogma of biology defined three categories and an information flow in which DNA stores the information, and

RNA carries the information to proteins that then carry out all biological functions (Herschlag et al., 2015). So, it was appropriate to use the RNA fold web server to predict the RNA and translated protein sequences for another analysis.

Alignments are processed for single Nucleotide polymorphisms (SNPs) and ideals detection. SNPs area single base in the DNA differs from the usual and normal base at that position. SNPs are a marker of choice in genetic analysis and also useful in locating genes associated with mutations. Another type of DNA mutations, are deleted or inserted pieces of DNA. (Seal et al., 2014 and Narzisi & Schatz, 2015).These mutations caused genetic variations: however SNPs constitute the large compartment of these variations (Ma & Lu, 2011). Our data identified that there were missing in 1/2 hour exposed group and substitution of nucleotides in ATP2b2 gene of all groups these mutations effects on the predicted amino acids sequence and numbers in protein of ATP2b2 of groups.

The single- stranded nature of RNA provides plasticity needed for it to fold into diverse secondary structures and tertiary structures that govern its functional roles (Kwon et al., 2015). RNA hairpins consist of a double – stranded RNA stem, often containing a terminal loop. They can guide RNA folding, protect messenger RNA from degradation, determine interaction in ribosome, serve as a recognition motif for RNA binding proteins or act as substrate for enzymatic reactions. RNA hairpins formed in different positions within different type of RNAs; they differ in the number and length of the stems, the size of loops, the size and number of the bulges, and differ in the actual sequence of nucleotide (svoboda et al., 2006).

The classical RNA secondary structures derived from RNA sequence consider the Watson- crick G/C and A/U base pairs as well as the G/U Wobble pair (Zu siederdissen et al., 2011). Many computational algorithms for predicting RNA secondary structure are based on empirical free energy determined from thermodynamic data of small RNA model compounds (Schuster, 2006).

Thermodynamic is a major determinant of secondary structure prediction and thus evolution of structured RNAs, as the free energy minimization alone typically predicts correctly about 70% of secondary structure. However for more accuracy experiments should be carried out to mapping RNA structure. (Mathews, 2014) the models resulted from the free energy minimization could be improved by applying the statistical mechanics of RNA folding, predicting pseudoknots, and using homologous sequences (Mathews & turner, 2006).

Many factors were found to effect on the regulation of protein expression. These factors are chromatin structure, processing and modification of mRNA transcripts, transcriptional initiation, transport of mRNA into cytoplasm, stability and decay of mRNA, initiation and elongation of mRNA, post translational modification, and intracellular transport and degradation of the expressed protein. (Adeli, 2011)

In this study the RNA secondary structure was built by online gene bee services. The results showed that the numbers of the stems are the same but free energy of RNA secondary structure predicted for RNA sequence transcribed from ATP2b2 gene for 1/2h, 1h, 2h, and 4h exposed groups were slightly different than the normal sequences.

The RNA secondary structure stability in groups will be different. The rate of protein translation of 1/2h, 1h, 2h, and 4h groups will be greater than that of the normal but it could be nonfunctional due to the free energies. The free energies of secondary structures for all studied groups were less than that of normal group thus the stability of RNA secondary structure less than that of the normal.

RNA secondary structure affected protein translation rate with many ways. The unwinding of every structure in ribosome decreases the elongation rate of protein (Mao et al., 2014). Also in vivo translation of thymidylate synthase mRNA the expression was greatly influenced by GC content of 5' coding region (Pedersen et al., 1997). Moreover, the translational efficiency of ATP2b2 was inversely correlated with the stability of the mRNA secondary structure, the presence of base- pairing in the consensus Kozak sequence, the number of the start codons in the 5' – UTRs, and the length of the 5' – UTRs (McClelland et al., 2009).

In the present study, the molecular observation and bioinformatics results revealed that, there were different mutations in ATP2b2 gene, this mutation don't related to the high period of exposure to mobile phone or less period of exposure because all studied groups were slightly affected and this may be due to the period of exposure isn't sufficient to cause excess affections. The most effected group is 1/2 hour exposed group and 1hour, 2 hour, and 4 hours exposed groups are the same affected by mobile phone. These mutations lead to change in the mRNA secondary structure, free energy, and number of stems. Thus the RNA translation rate by ribosome is changed and the energy needed for translation of RNA to protein could be changed. So the structure and function of resulting hormones or enzymes may be affected. And also cause significant decrease in Hb, HCT, MCV, and MCHC, WBCs, PLT ($P < 0.05$) count and ionized calcium.

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