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# Effect of cell phone radiation on buccal mucosa cells

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**ABSTRACT**

*Use of cell phones has increased drastically and has raised public concern on potential health effects of radiofrequency electromagnetic waves. Hence, the present study was conducted to evaluate cytotoxicity in cell phone users by performing Buccal Cytome Assay. Sixty male volunteers (20-25 years) using cell phones for 3 to 9 hours per day were recruited with prior consent as per ethical guidelines and Buccal Cytome Assay was performed. Individuals were divided into groups according to their addiction habits and call duration. Same number (n=60) of age matched individuals using cell phones for less than 1 hour and without any addiction were considered as Least exposed Individuals. Results of this study showed highly significant frequencies of various cell anomalies such as Micronucleus, nuclear buds, pyknotic cells, karyorrhectic, condensed chromatin and Karyolytic cells, in exposed Individuals as compared to least exposed Individuals. Also, the frequency of such cells was significantly higher in the maximally exposed group (7-9 hrs) as compared to the groups with lesser call duration. Amongst the exposed Individuals, the Addiction group showed significant increase in these cell anomalies as compared to the No-addiction group. The result of our study implies caution for cell phone users as they may get prone to adverse long term health effects including cancer with prolonged talk time exposure.*

**Keywords:** *Cell phones, Cytotoxicity, Electromagnetic radiation, Buccal mucosa, Exposure risks.*

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## INTRODUCTION

Cell phone has become an indispensible device in our daily lives and emits radiofrequency electromagnetic waves (RF-EMW). These phones operate at frequencies, depending on the frequency usage in different countries, and are now used not only for having conversations, but also accessing internet, data, pictures and videos even by growing children. The increased use of cell phones over the last few years has led the civilized individuals to get exposed to RF-EMW raising questions regarding health effects, especially its long term effects (WHO, 2006). Some individuals are using cell phones for long duration of talk time either due to occupational requirement or long distance communication. Studies have suggested previously that long term exposure to cell phone radiation triggers uncontrolled cell proliferation due to accumulated DNA damage and also that Radio frequency electromagnetic Waves (RF-EMW) exposure decreases the PKC (Protein Kinase C) activity which may be linked to carcinogenesis (Desai *et al*., 2009). Since last many years, there has been an increased usage of cell phones, radar installations and microwave ovens worldwide which has resulted in alarming rates of human exposure to radio frequency waves. Cell phones use microwaves as carrier waves in a frequency range between 300 Megahertz to 300 Gigahertz. Agarwal *et al*. (2008) have reported adverse effects of cell phones on semen which included reduced sperm count, motility and morphology. Further, cell phone exposure has been associated with increased oxidative stress in semen which may impair male fertility (Agarwal *et al*., 2009). Researchers have sought to link the much debated decline in human sperm quality in the last decade, with increased exposure to RF-EMW, particularly through mobile phone usage (Agarwal & Durairajanayagam, 2015).

The rate at which radiation is absorbed by human bodies is called as Specific Absorption Rate (SAR). It is a standardized unit which measures the impact of radio frequency electromagnetic waves on the human body and it is expressed as Watt/Kg. The FCC (Federal Communication

Commission) has limited the maximum legal SAR of any handheld cell device to 1.6 Watt/kg (Hamada *et al*., 2011). Toxic effects of any chemical causing harmful effects to the genetic material of the living organisms is referred to as Genotoxicity. Cell phone radiations are radiofrequency radiations which are classified under non-ionizing radiation, and hence do not have thermal effects responsible for breakage of chemical bonds (Hamada *et al*., 2011). Many *in vitro* studies have reported evidences of RF-EMW having genotoxic effects, such as Micronucleus Assay (Koyoma *et al*., 2003); Chromosomal aberrations (Garaj-Vrhovac *et al*., 1991), DNA strand breaks (Diem *et al*., 2005). In contradiction to above mentioned literature some studies have reported negative results (Bisht *et al*., 2001; Speit *et al*., 2007). WHO Research agenda for radiofrequency fields has identified genotoxic endpoints as high priority research needs (WHO, 2006). In 2011 a group of international experts from IARC (International Agency for Research on Cancer) concluded that RF/MW (Radiofrequency/Magnetic waves) radiations should be listed as a possible carcinogen (group 2B) for humans (Baan *et al*., 2011). This classification of RF as possibly carcinogenic to humans in group 2B was not supported by a genotoxicity based mechanistic evidence given by Prihoda (2012). Even though the data available so far is inconclusive, still the scientific evidences indicate some biological effects and possible adverse health effects which merit further investigations.

In a recent study DNA damage was observed in buccal cells after exposure to cell phone radiation, and it was concluded that mobile phone users may get prone to malignancy and cytotoxicity. Microwaves can cause genotoxic effect to somatic cells of human system and also lead to inheritable genotoxic effects in germ cells (Verschaeve, 2005). Another study on genetic polymorphism of GSTM1 and GSTT1 in individuals exposed to radiation from mobile towers showed significant genetic damage (Gulati *et al*., 2016). The Buccal Micronucleus Cytome (BMCyt) assay is a cost effective, minimally invasive method for studying DNA damage, chromosomal instability, cell death and the regenerative potential of human buccal mucosa cells. It is now widely used in epidemiological studies for analyzing the effect of nutrition, lifestyle factors, genotoxin exposure, DNA damage, chromosomemal segregation and cell death (Thomas *et al*., 2009). While using cell phones for conversation, the placement pattern for handset is from both ears and is very close to the buccal cavity which would get maximally exposed to the RF-EMW radiations while talking. Hence, this study was undertaken to assess the cytotoxic effects in buccal cells of cell phone users and to correlate this effect on the exposed individuals according to the exposure time. Also, to further explore the effects of various confounding factors such as smoking, panmasala, tobacco chewing and alcohol consumption etc. on cell phone users.

## MATERIALS AND METHODS

The study was approved by the Institutional Ethics Committee and the samples were collected as per the ethical guidelines and with prior consent of cell phone users. A detailed questionnaire was filled up with information including, type of cell phone set, daily frequency of calls (incoming and outgoing), use period in 24 hours and in years, Specific Absorption Rate (SAR) of the model (obtained from the models website) and brand in use, age, occupation, diet, disease (if any), addiction (if any), allergy etc. Exposed individuals included 60 males who used cell phone for 3-9 hours per day, either due to occupational requirements or personal habits. The Least exposed group consisted of similar number of males (n=60). They were age matched healthy individuals with less exposure (maximum up to 1 hour) to cell phone radiation and were strictly non smoking with no other addictions.

Samples were collected using a sterile, small headed plastic toothbrush from the inner walls of cheeks, slide preparation and scoring was done as per the standard protocol (Thomas *et al*., 2009). Slides were stained with Giemsa, air dried and observed under the microscope.1000 cells were scored per subject to find the frequency of various cell types observed in buccal cytome assay. The observed cells included Normal cells, Micronucleated cells (MN), Binucleated cells (BN), Nuclear bud (NB), Pyknotic cells (PC), Karyorrhectic cells (KR) Condensed chromatin cells (CC), and Karyolytic cells (KL). All data were expressed as the mean ± standard error. The significance was considered when $p<0.05$. The differences between Least exposed and Exposed groups were analyzed using Student's t-test, while multiple comparisons amongst more than two groups (as per Addiction habits and Call duration) was done by Tukey's multiple comparison test.

## RESULTS AND DISCUSSION

The demographic details of the cell phone users and their lifestyle factors are given in Table 1.

**Table no. 1: Table showing various lifestyle factors in male cell phone users**

| Sr. No. | Details | Least exposed (<1 hour) | Exposed (3-9hours) |
|---|---|---|---|
| 1) | No of Samples | 60 | 60 |

| | | | |
|---|---|---|---|
| 2) | Age (years) | 18-25 | 18-25 |
| 3) | Daily Calls attended | 2-5 | 20-100 |
| 4) | SAR of Handsets used (Watt/Kg) | 0.3-1.6 | 0.3-1.6 |
| 5) | Exposure (Years) | 3-9 | 3-9 |
| 6) | Addiction Habits | Non addicted | Tobacco – 26.6% Panmasala – 16.6 % Smoking – 26.6 % Alchohol – 13.3 %<br>Non addicted (16.9%) |
| 7) | Diet | Veg -79.3%<br>Mixed – 20.7% | Veg -76.6%<br>Mixed – 23.33% |

The Exposed Individuals attended daily 20-100 calls because of job requirements, as many of them were in sales and marketing and were using cell phones since 3 to 9 years and the Least exposed individuals attended 2-5 calls each day. The SAR values of all the models of handsets used by the study Individuals were ranging between 0.3-1.6 Watt/Kg body weight. In present study 1000 normal buccal cells were scored and various types of cell anomalies were observed as shown in Figs. 1 to 8.

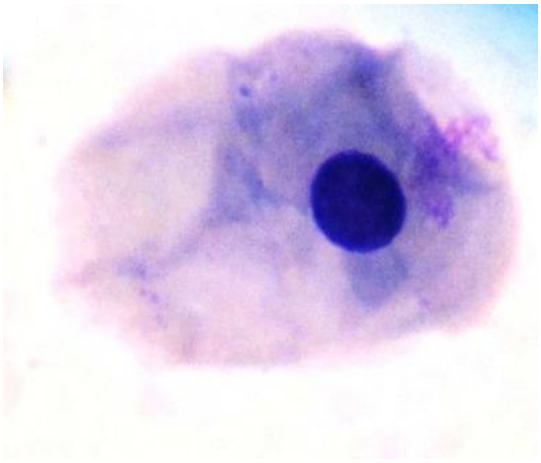

**Fig 1: Normal Buccal Cell**

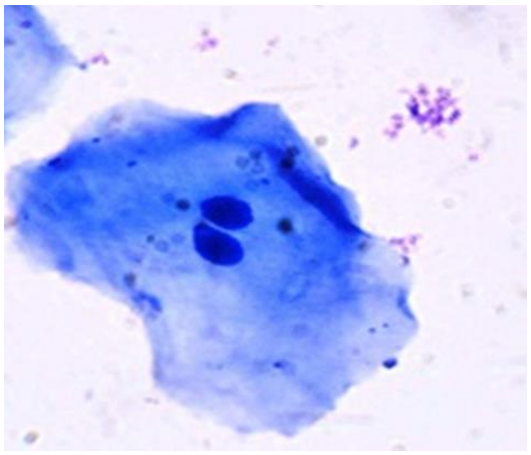

**Fig 2: Binucleated Cell**

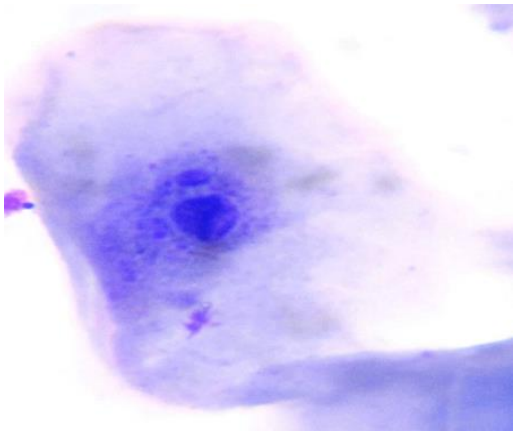

**Fig 3: Buccal cell with prominent micronuclei**

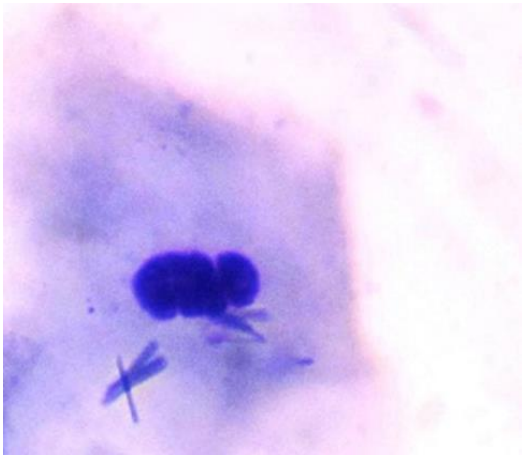

**Fig 4: Buccal cell with nuclear bud**

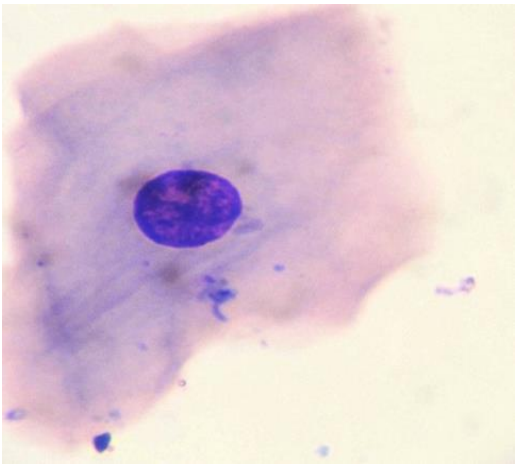

**Fig 5: Buccal cell with Condensed Chromatin**

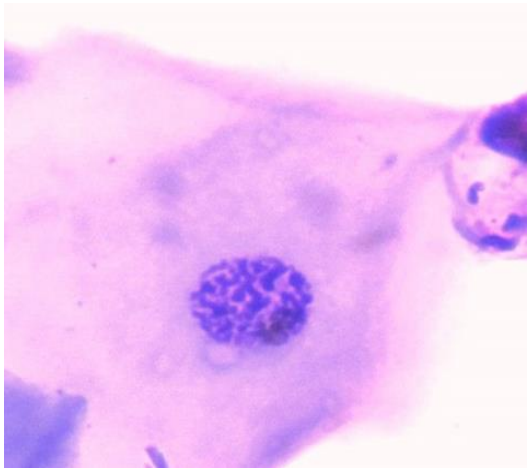

**Fig 6: Buccal cell with Karyorrhectic nuclei**

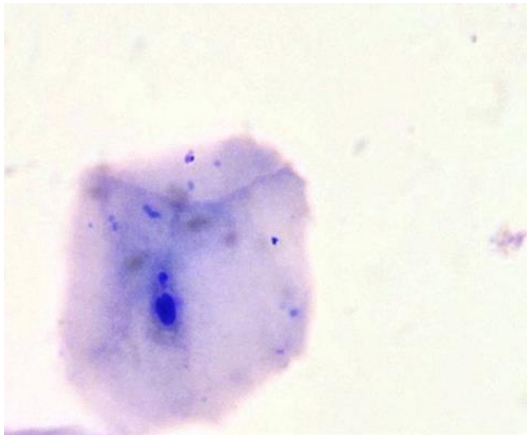

**Fig 7: Pyknotic Cell**

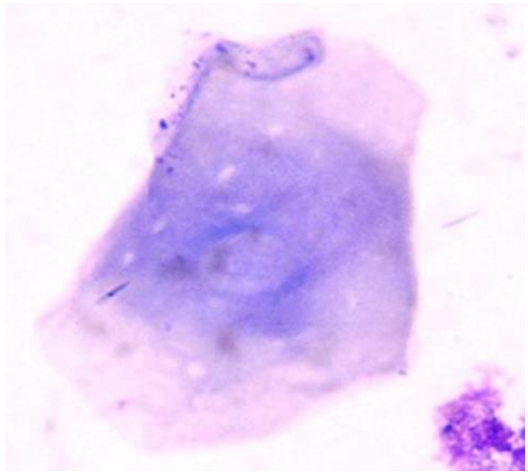

**Fig 8: Karyolytic cell**

**Table no. 2: Mean frequencies of nuclear anomalies observed in cell phone users**

| Sr.No. | Cell types | Least exposed (Mean ± S.E.) | Exposed (Mean ± S.E.) |
|---|---|---|---|
| 1) | Micronucleated cells | 0.9 ± 0.12 | 8.63 ± 0.57*** |
| 2) | Binucleated cells | 6.36 ± 0.69 | 7.07 ± 0.5 NS |
| 3) | Nuclear bud | 0.2 ± 0.08 | 2.9 ± 0.5*** |
| 4) | Pyknotic cells | 0.93 ± 0.13 | 5.57 ± 0.56*** |
| 5) | Karyorrhectic cells | 7.9 ± 0.49 | 69.9 ± 5.45*** |
| 6) | Condensed chromatin | 11.16 ± 1.05 | 70.6 ± 5.01*** |
| 7) | Karyolytic cells | 6.63 ± 0.67 | 67.8 ± 5.26*** |

*** = Highly Significant (p<0.001) NS= Non-Significant

The mean frequencies of various cell types such as MN, BN, Nuclear bud, pyknotic cells, KR, CC, and KL cells in exposed males were significantly higher (p<0.01) as compared to Least exposed Individuals (Table 2). The binucleated cells were non-significant as compared to Least exposed Individuals (Table 2) but they were significant in various groups of addiction and call duration as shown in Tables 3 and 4. The exposed Individuals were classified into groups according to their exposure time and personal habits as follows: Group-1(<1 hour), Group-2 (3-5 hours), Group-3 (5-7 hours), Group-4 (7-9 hours) and Group-A (No-Addiction), Group-B (Addiction). Group-B (Addiction) showed highly significant increase (p<0.001) in frequencies of all cell types when compared to Group-A (No- Addiction) except binucleated cells which showed significant increase (p<0.01) as shown in Table 3.

**Table no. 3: Frequency of various cell types in cell phone users as per addiction**

| Sr. No. | Cell types | Least exposed | Exposed (Mean ± S.E). | |
|---|---|---|---|---|
| | | No Addiction | No Addiction (A) | Addiction (B) |
| 1) | Micronucleated cells | 0.96± 0.12 | 3.7 ± 0.34*** | 6.61± 0.68***,### |
| 2) | Binucleated cells | 6.36 ± 0.69 | 6.9 ± 0.79** | 7.24 ± 0.66**,## |
| 3) | Nuclear bud | 0.2 ± 0.08 | 1.9 ± 0.4*** | 3.72 ± 0.76***,### |
| 4) | Pyknotic cells | 0.93 ± 0.13 | 6.7 ± 0.59*** | 5.55 ± 0.87***,### |
| 5) | Karyorrhectic cells | 7.9 ± 0.49 | 60 ± 3.76*** | 83.11 ± 7.32***,### |
| 6) | Condensed chromatin | 11.16 ± 1.05 | 78.7 ± 4.14*** | 73.88 ± 7.96***,### |
| 7) | Karyolytic cells | 6.63 ± 0.67 | 60.1 ± 3.12*** | 79.61 7.38***,### |

* = Least exposed v/s addiction /No-addiction → **: significant = p<0.01, ***: highly significant = p<0.001 #= Addiction v/s No-addiction→ ##: significant = p<0.01), ###: (highly significant = p<0.001)

Group-2 (3-5 hours), Group-3(5-7 hours) and Group-4 (7-9 hours) showed highly significant increase (p<0.001) when compared to least exposed Group (Table 4). Similarly, the frequencies of cell anomalies in Group-3(5-7 hours) and Group-4 (7-9 hours) showed highly significant (p<0.001) increase when compared with Group-2. Also, Group-4 showed highly significant (p<0.001) increase when compared with Group-3. This shows that with the increase of call duration/day, there is a gradual increase in all cell anomalies except binucleated cells and micronucleus which were significant/Non-significant as shown in Table 4.

**Table no. 4: Frequencies of various cell types in cell phone users as per call duration**

| Sr.No. | Cell types | Group-1 Least exposed (< 1 hour) | Group-2 (3-5 hours) | Group-3 (5-7 hours) | Group-4 (7-9 hours) |
|---|---|---|---|---|---|
| 1) | Micronucleated cells | 0.96 ± 0.12 | 7.2 ± 0.57** | 10 ± 1.05***, NS | 10 ± 1.81***, NS, ns |
| 2) | Binucleated cells | 6.36 ± 0.69 | 6.8 ± 0.59 * | 7.1± 0.97 **, ## | 7.8 ± 1.59***, NS, ns |
| 3) | Nuclear bud | 0.2 ± 0.08 | 1.33 ± 0.23*** | 4.2 ± 0.8***, ### | 4.8 ±2.13***,###, +++ |
| 4) | Pyknotic cells | 0.93 ± 0.13 | 4.33 ± 0.43*** | 6.7 ± 0.88***, ### | 7 ± 2.4***, ###, + |
| 5) | Karyorrhectic cells | 7.9 ± 0.49 | 60.93 ± 7.11*** | 74.3 ± 9.2***, # | 87.8 ± 14.9***, ###, ns |
| 6) | Condensed chromatin | 11.16 ± 1.05 | 63.53 ± 5.51*** | 77.3 ± 9.9***, ### | 78.2 ± 15.93***, ###, +++ |
| 7) | Karyolytic cells | 6.63 ± 0.67 | 57.66 ± 6*** | 72.3 ± 8.8***, ### | 89.2 ± 16.44***,###,+++ |

*= (Group -1 v/s Group -2/ Group -3/ Group - 4) →*: Significant (p<0.05), **: Significant (p<0.01), ***: Highly Significant (p<0.001)

#= (Group -2 v/s Group -3/ Group -4→#: Significant (p<0.05), ##: Significant (p<0.01), ###: Highly Significant (p<0.001), NS: Non-Significant

+= (Group -3 v/s Group - 4) → +: Significant (p<0.05), +++: Highly Significant (p<0.001), ns: Non-Significant

The primary aim of this study was to evaluate the cytotoxic effects caused by cell phone radiation using BMCyt assay since the buccal cells would be highly exposed to radiations while talking. Previously studies have been reported on RF-EMW radiations on Peripheral blood lymphocytes by Bisht *et al.* (2001) and Haematological parameters in serum sample by Jelodar *et al.* (2011). Agarwal *et al.* (2009) showed harmful effects of cell phone radiation on sperm function and Reactive Oxygen Species. Non-thermal DNA breakage in human fibroblasts as well as rat granulosa cells *in vitro* due to exposure to cell phone radiations were also reported by Diem *et al.* (2005). Studies regarding effects of cell phone radiation on Buccal cells have shown significant results with various parameters such as micronuclei (Yadav & Sharma, 2008; Hintzsche & Stopper, 2010); BN, MN, KR and KL (Rajkokila 2011). In the present study all the cell types of Buccal Cytome Assay as described by Thomas *et al.* (2009) are being reported for the first time and also the exposed individuals have been grouped according to the exposure time and addiction. This is a direct *in vivo* study on the effect of cell phone radiations on buccal cells. Our results showed significant increase in the MN, NB, PC, KR, CC and KL in exposed samples as compared to the Least exposed Individuals, and the increase was directly proportional to the duration of calls, as seen in Group-4(7-9 hrs) when compared with Group-2 (3- 5 hrs) and Group-3(5-7 hrs) (Table 4). Amongst the exposed Individuals, Group A (No-addiction) and B (addiction) showed highly significant increase in all cell anomalies (MN, BN, NB, PC, CC, KR and KL as shown in Fig. 1 - 9) when compared to least exposed Individuals. Also, the Addiction group showed highly significant difference when compared with No-addiction group. In the Exposed group, addictions reported were of Tobacco (26.6%), Pan masala (16.6 %), Smoking (26.6 %), Alcohol (13.3 %), while remaining were Non-Addicted (16.9 %). Celik *et al.* (2003) have reported that cigarette smoking significantly increased the frequencies of micronucleus and other nuclear abnormalities in both control and exposed Individuals in their study of cytome assay on petrol pump attendants.

MN test in BMCyt assay have been used to analyse the genotoxic effects and monitoring genetic damage in exposed individuals (Holland *et al.*, 2008; Bonassi *et al.*, 2011). Buccal cell MN has been identified as a useful biomarker that co-relates with oral cancer (Proia *et al.*, 2006). Hence, the frequency of MN in Buccal mucosa cells can be used as a biomarker for genotoxic and carcinogenic agents, and the highly significant MN cells in our study can have serious implications. Yadav and Sharma (2008) also reported similar results in buccal cells while Hintzsche and Stopper (2010) did not find significant results in their study of MN frequency in buccal mucosa cells of mobile phone users. In the present study, frequencies of binucleated cells were observed to be non-significant in Exposed Individuals as compared to the Least Exposed individuals, but it was found to be significant when the Exposed Individuals were further divided according to the addiction habits and duration of calls as shown in Tables 3 and4. The significance of the binucleated cells is unknown, but they are probably indicative of failed cytokinesis following the last nuclear division in the basal cell layer (Thomas *et al.*, 2009). The Nuclear bud (NBUD) is suggested to be a biomarker of genotoxic events and chromosomal instability (Fenech *et al.*, 2011). The cells with small shrunken nucleus and a high density of nuclear material with intense uniform stain were identified as pyknotic cells (Tolbert *et al.*, 1992). Karyorrhectic cells were identified with stronger appearance of nuclear chromatin aggregation (as compared to condensed chromatin cells). Cells with

condensed chromatin, karyorrhectic, pyknotic and karyolytic cells represent degenerating cells (Apoptotic) (Holland *et al*., 2008). Since the NBUD, pyknotic cells, condensed cells and karyorrhectic cells were observed significantly higher in the Exposed samples, it can be concluded that these cells were degenerating and were at early or late stages of apoptosis due to the RF-EMW effects.

The cells which are devoid of DNA and appear to have no nuclei were identified as karyolyticcells, which probably indicate a late stage ofcell death. In our study, karyolytic cells were found with significantly higher frequency in exposed individuals. Amongst the exposed individuals, Group-4 (7-9 hrs) and the Addiction (Group-B) showedthe maximum frequencies of karyolytic cells. The biomarkers such as pyknotic cells, nuclear bud, karyolytic cells, karyorrhectic cells etc. found in Buccal Micronucleus Cytome assay can be associated with many health hazards and it has been proved to be successful means to analyze cytotoxicity and genetic defects (Holland *et al*., 2008). A study on individuals exposed to petrol by Sellappa *et al*. (2010) have shown increased MN frequencies in Buccal mucosa cells which were linked with high risk for cancer as a long term effect and they suggested careful monitoring. Some studies have shown that RF-EMW may cause oxidative stress in human saliva (Hamzany *et al*., 2013; Abu Khadra *et al*., 2015) While another has reported no change in oxidant/antioxidant profile (Khalil *et al*., 2014). No genotoxic effects because of RF exposure were shown by many reports (Ros-Llor *et al*., 2012; Waldmann *et al*., 2013). Söderqvist *et al.* (2015) failed to show significance of short term exposure on biomarkers in volunteers exposed to cell phone radiations. Some studies have shown that even at low levels RF-EMW can cause damage to cell tissue and DNA, and it has been linked to brain tumors (Hardell *et al*., 2007), cancer, disturbed immune function, chronic allergic response, inflammatory responses (Jelodar *et al*., 2011), headache, anxiety, stress, chronic fatigue syndrome, and depression (Johannson, 2009). No risk for parotid gland tumor due to short term exposure was reported by Söderqvist *et al*. (2012). Similarly, Daroit *et al.* (2015) also concluded that, despite a significant increase in cell anomalies, the radiation emitted by cell phones among frequent users is within acceptable physiological limits. They also suggested further studies to investigate the harmful effects of cell phone radiation to draw a strong conclusion. Clearly the debate about the possible damage that RF-EMW emitted by cell phones exerts on different organs continues and there is increasing public concern regarding its health risks.

In this study, amongst the exposed individuals, Group-B (Addiction) and Group-4 (7-9 hrs) showed highest levels of DNA damage as compared to the Least Exposed Individuals (Group-1), Group-A (non- Addiction) and Group-2(3-5 hrs) and Group-3 (5-7 hrs). Hence, there is a strong co-relation between cell anomalies and exposure time, since the maximum damage is observed in the Group-4with highest call duration of (7-9 hrs). Such an increase can have long term effects including risk for development of oral carcinoma in the cell phone users after prolonged exposure (Fenech *et al*., 2011). The use of cell phones has increased only in the past few years and all long term effects might not be known as yet. This risk can further increase due to various confounding factors such as smoking, pan masala, tobacco chewing and alcohol consumption etc. as seen in the present study.

## CONCLUSION

The results of our study showed highly significant increase in buccal cell anomalies including MN, BN, NB, PC, KR, CC, and KL cells after exposure to cell phone radiations. Also, a strong co-relation was observed between the cell anomalies and cell phone exposure time. These cell anomalies were further increased due to confounding factors as seen in the cell phone users with addiction (smoking, tobacco, and alcohol) when compared to the no addiction group. The abnormal cells observed in BMCyt assay are used as an endpoint to detect cytotoxic damage in exposed individuals and this information can be helpful as an early warning of potential risk of genetic damage. Counseling and awareness of the cell phone users becomes a necessity to protect them against damaging effects of RF-EMW radiations.

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