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Comparison of effects of 2.4 GHz Wi-Fi and mobile phone exposure on human placenta and cord blood

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ABSTRACT

The aim of this study was to investigate the effects of radiofrequency radiation emitted from Wi-Fi systems and mobile phones on cord blood and placenta. The study included 149 pregnant women who were divided in subgroups: unexposed (control), mobile phone exposed, Wi-Fi exposed and mobile phone plus Wi-Fi exposed groups. Immediately after birth, placenta and cord blood samples were collected and protein carbonyl (PCO), malondialdehyde (MDA), total oxidant status (TOS), total antioxidant status, 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels and DNA single strand breaks were analysed. The results of the study showed an increase in 8-OHdG, MDA, PCO and TOS in cord blood and placenta in the group exposed to mobile phones during gestation. However, the group exposed to Wi-Fi did not show alterations in the studied oxidative stress parameters. On the other hand, tail intensity and tail moment of DNA in the mobile phone exposure groups were higher than those in the control and Wi-Fi exposure groups. In conclusion, the results of this study indicated that mobile phone exposure during pregnancy could have an important potential to cause oxidative stress and DNA damage in cord blood and placenta. The results of this study also indicated that combined effects of Wi-Fi plus mobile phone exposure have a higher potential to cause synergistic harmful effects.

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Mobile phone; Wi-Fi; placenta; cord blood; oxidative stress; DNA damage

Introduction

Electromagnetic pollution has been increasing because of daily wireless communication (Mobile phones, computers, tablets, smart clocks, toys, games, films, Wi-Fi etc.). The present situation in everyday life has been causing public and scientific concern on the effects of radiofrequency radiation (RFR) on human health. Unfortunately, most scientific data on electromagnetic pollution confirm these concerns. Therefore, RFRs have been classified as 'Group 2B (Possibly carcinogenic)' by The International Agency for Research on Cancer, a branch of the World Health Organization, because of the intensively accumulating scientific evidence on the relation between brain tumours and RFRs [1]. Interestingly, children and pregnant women are the group most affected by the non-thermal effect of RF electromagnetic pollution; however, this has received less attention. Therefore, recent studies focus on the non-thermal effects of RFRs on pregnancy [2]. For instance, some studies have reported that RFR can break down the structures of biomolecules such as protein, lipid and DNA, through oxidative stress despite the fact that RFR itself does not have enough energy to break the DNA strands [2–7].

There is a correlation between radiofrequency and body penetration. When the frequency of the RFR increases, it penetrates less into the body tissues [8]. Consequently, the penetration of 2.4 GHz Wi-Fi should be lower than that of 900, 1800 and 2100 MHz, which are the mobile phone frequencies. On the other hand, there are two frequency zones (around 100 MHz and 1–4 GHz) in which children absorb more RF than adults [8]. However, the strength and distance to sources are very important for the health effects of RFR. As it is known, when RFR moves away from the source, its power density decreases in proportion to $1/d^2$ (where d is the distance to the source). People are exposed to local RFR when talking with mobile phones, whereas RFR emitted from Wi-Fi affects the entire body. It is thought that public exposure levels

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arising from Wi-Fi are lower than those from mobile phones [8]. Additionally, frequency, power density, distance and physical properties of the biological material (permeability and dielectric constant) are the determinative factors for the interaction between RFR and the biological material. It is also reported that tissues with high water content are more sensitive to RFR than others [9]. Therefore, another issue that needs attention is pregnancy because the body water content increases in pregnancy. It is also verified that RFR has potential to develop some anomalies during pregnancy [10,11]. Additionally, the specific absorption rate (SAR) levels in foetuses exposed to 900 MHz plane waves have been found 14% lower than those of their mothers [12].

The studies performed on the relation between RFR and pregnancy, including foetuses, are still limited and the results contradictive. Therefore, the aim of this study was to investigate the effects of mobile phone and Wi-Fi exposure on protein carbonyl (PCO), malondialdehyde (MDA), total oxidant status (TOS), total antioxidant status (TAS), 8-hydroxy-2-deoxyguanosine (8-OHdG), DNA damage (single strand breaks) in cord blood and placenta of human pregnancies.

Subjects and methods

Ethics statement

All participants gave informed consent forms. The study was designed according to the Helsinki Declaration. The study was also approved by the Ethics Committee of the Medical Faculty of Istanbul Medeniyet University (Report No: 2016/0037).

Participants

In the first step, eligible pregnant women were identified and any women with any medical treatment, chronic systemic disease and multiple pregnancies were excluded from the study. Without the use of any assisted reproductive technology, self-pregnancies were included in the study. The study included 149 pregnant volunteers aged between 18 and 40 years. Pregnant women were divided into four groups depending on the usage of mobile phone and Wi-Fi as follows:

Control Group: Pregnant women who did not use mobile phone and Wi-Fi during the gestation period.
Group 1: Pregnant women who used only mobile phone during the gestation period.
Group 2: Pregnant women who used only Wi-Fi during the gestation period.
Group 3: Pregnant women who used both mobile phone and Wi-Fi during the gestation period.

Most of the participants in the control group lived in a rural area which has no base station, mobile phone or Wi-Fi access; the others were women with a high level of education who purposefully chose not to use mobile phones and Wi Fi during pregnancy. The participants in Group 1 consisted of pregnant women who did not have Wi-Fi in their homes or workplaces. We determined the Wi-Fi exposures of the participants according to their exposure in the workplace and at home. We classified the exposure periods such as less and more than 2 h a day. The exposure periods were determined by the mobile service operators depending on the participants with their consent. A questionnaire containing all the confounding factors that could affect the results of this study was administered to the pregnant women. The newborns were clinically examined by the paediatrician just after delivery. Detailed information is given in Tables 1 and 2.

The pieces of placental tissue and samples of cord blood were collected immediately after birth. Then, PCO, MDA, TOS, TAS and 8-OHdG levels were determined by enzyme-linked immunosorbent assay (ELISA) and DNA single-strand breaks were quantified by Comet assay.

Placental tissue samples and analysis

A scalpel was used to collect 1×1 cm samples of tissue from the central section of the surface of maternal placenta. Pieces of tissue were weighed, minced into small pieces and homogenized in phosphate buffered saline (PBS; pH: 7.4; 9 mL of PBS per 1 g of tissue) with a glass homogeniser on ice. A protease inhibitor (aprotinin) was added to the PBS solution. To further disintegrate the cells, the suspension was sonicated with an ultrasonic cell disrupter (MP FastPrep-24 Tissue and Cell Homogenizer, USA). The homogenates were then centrifuged for 15 min at 15,000*g* to obtain supernatants.

The total amount of protein was measured by the Bradford method in all samples with a spectrophotometer. MDA (ELISA kit: Eastbiopharm, Cat no: CK-E10376), PCO (ELISA kit: Eastbiopharm, Cat no: CK-E11583) and 8-OHDG (ELISA kit: Eastbiopharm, Cat no: CK-E11652) in tissue homogenates were determined by ELISA in a plate reader (Thermo Scientific Multiskan FC, 2011-06, USA). TAS and TOS also were measured at 240 and

Table 1. Key anamnesis parameters of women, pregnancies and newborns according to the groups.

	Control (*n*: 20)	(Mobile phone) Group 1 (*n*: 48)	(Wi-Fi) Group 2 (*n*: 17)	(Mobile phone + Wi-Fi) Group 3 (*n*: 64)	*p*
Talking duration (minutes)	0.00000 ± 0.00000	33.75958 ± 29.50369	0.00000 ± 0.00000	44.85111 ± 35.10301	0.065
Maternal age	28.40000 ± 5.344944	29.37500 ± 5.502901	27.47059 ± 5.197567	28.31746 ± 4.788512	0.549
Paternal age	31.50000 ± 5.093856	32.62500 ± 5.483534	30.82353 ± 5.198982	32.14286 ± 5.009208	0.621
Weight gain during pregnancy (maternal) (kg)	9.50000 ± 4.135851	11.04167 ± 5.090493	12.58824 ± 6.175140	10.69048 ± 4.320272	0.269
Number of visits to gynaecologist	6.750000 ± 2.935715	9.000000 ± 5.251140	9.529412 ± 3.502100[a]	9.349206 ± 2.294163[a]	**0.047**
Birth weight (gram)	3118.250 ± 511.8112	2992.500 ± 477.3040	3182.059 ± 442.9696	3145.873 ± 438.8020	0.291
Baby head circumference (cm)	35.64000 ± 0.678543	35.35833 ± 0.862168	35.46471 ± 0.803073	35.42540 ± 0.617740	0.549
Baby height (cm)	50.86000 ± 0.707404	50.72292 ± 0.754346	50.82353 ± 0.727607	50.69048 ± 0.856985	0.821
SAR value (W/kg)	0.00000 ± 0.00000	0.569375 ± 0.209817	0.630000 ± 0.257026[a]	0.619524 ± 0.228631[a]	**0.007**

SAR: Specific Absorption Rate.
[a]Significant difference from control group.

520 nm wavelengths in a plate reader (Thermo Scientific Multiskan FC, 2011-06, USA). TAS and TOS values were used in the calculation of the Oxidative Stress Index (OSI):

$$\text{Oxidative Stress Index (OSI)} = \frac{\text{TOS}}{\text{TAS}} \times 100$$

Umbilical blood samples and analysis

The umbilical cord of each newborn was clamped and a 1–2mL sample of blood was taken from the umbilical artery using syringes. Samples were transferred into test-tubes and centrifuged at 3000*g* for 10 min. The serum obtained was put into Eppendorf Tubes® and stored at –80 °C. MDA, PCO and 8-OHdG in serum were determined by ELISA in a plate reader (Thermo Scientific Multiskan FC, 2011-06, USA) using the kits described above. TAS and TOS were determined and OSI was calculated.

MDA, PCO and 8-OHDG analysis in umbilical cord blood and placental tissue samples

MDA (Eastbiopharm, Cat no: CK-E10376), PCO (Eastbiopharm, Cat no: CK-E11583) and 8-OHDG (Eastbiopharm, Cat no: CK-E11652) levels in serum and tissue homogenates were measured using commercial ELISA kits according to the manufacturer's procedures. Briefly, samples were thawed and loaded, along with standards, into appropriate wells which were precoated with Anti-Human monoclonal antibody before incubation. Biotin was added to all wells and combined with Streptavidin-horseradish peroxidase to form immune complexes. Following incubation, the wells were washed to remove the uncombined enzyme. Then, Chromogen Solution A, B was added and the reaction mixture colour changed to blue. At the effect of acid, colour change to yellow was measured (optical density) on a standard automated plate reader at 450 nm (Thermo Scientific Microplate Reader). The detection ranges of the kits were between 2 and 64 nmol/mL for MDA; 10–640 ng/mL for PCO and 10–128 ng/mL for 8-OHDG.

TAS and TOS analysis in umbilical cord blood and placental tissue samples

TAS levels were measured spectrophotometrically using a commertial kit (Rel Assay, Turkey). The assay was performed using a Perkin Elmer, 1420 Victor 3 instrument. Antioxidants in the sample reduce dark blue green coloured ABTS radical to a colourless reduced ABTS form. The change of absorbance at 660 nm is proportional to the total antioxidant level of the sample. Total antioxidant activities were expressed in mmol Trolox Equiv/L of sample. TOS levels were measured by a spectrophotometric method using a commertial kit (Rel Assay, Turkey). The assay was performed using a Perkin Elmer, 1420 Victor 3 instrument. Oxidants present in the sample oxidize the ferrous ion chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colour complex with chromogen in an acidic medium. The colour intensity, which was read spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. Results are expressed in μm H_2O_2 Equiv/L.

Comet assay analysis (single cell gel electrophoresis)

Samples of blood were taken from the umbilical cord with heparinized tubes after delivery. As soon as the samples were taken, lymphocyte separation was performed. DNA damage was detected in lymphocytes with Comet analysis.

Table 2. Additional anamnesis parameters of women, pregnancies and newborns according to the groups.

Groups / Variables		Control (*n*: 20)	(Mobile phone) Group 1 (*n*: 48)	(Wi-Fi) Group 2 (*n*: 17)	(Mobile phone + Wi-Fi) Group 3 (*n*: 64)	*p*
Baby gender	Male	10 (50.0%)	18 (37.5%)	9 (52.9%)	28 (43.9%)	0.659
	Female	10 (50.0%)	30 (62.5%)	8 (47.1%)	36 (56.3%)	
Birth	Normal spontaneous vaginal delivery	11 (55.0%)	25 (52.1%)	8 (47.1%)	28 (43.8%)	0.413
	Emergency caesarean spinal anaesthesia	0 (0.0%)	3 (6.3%)	1 (5.9%)	2 (3.1%)	
	Emergency caesarean general anaesthesia	0 (0.0%)	6 (12.5%)	1 (5.9%)	8 (12.5%)	
	Elective caesarean spinal anaesthesia	6 (30.0%)	5 (10.4%)	6 (35.3%)	13 (20.3%)	
	Elective caesarean general anaesthesia	3 (15.0%)	9 (18.8%)	1 (5.9%)	13 (20.3%)	
Foetal distress	Present	1 (5.0%)	13 (27.1%)	2 (11.8%)	13 (20.3%)	0.071
	Absent	17 (85.0%)	34 (70.8%)	15 (88.2%)	51 (79.7%)	
	Not evaluated	2 (10.0%)	1 (2.1%)	0 (0.0%)	0 (0.0%)	
Meconium	Present	1 (5.0%)	11 (22.9%)	2 (11.8%)	5 (7.8%)	0.068
	Absent	19 (95.0%)	37 (77.1%)	15 (88.2%)	59 (92.2%)	
Consanguineous marriages	Absent	5 (25.0%)	18 (37.5%)	12 (70.6%)	40 (62.5%)	0.016
	First degree consanguinity	12 (60.0%)	21 (43.8%)	5 (29.4%)	19 (29.7%)	
	Second degree consanguinity	2 (10.0%)	7 (14.6%)	0 (0.0%)	2 (3.1%)	
	Distant relatives	1 (5.0%)	2 (4.2%)	0 (0.0%)	3 (4.7%)	
Birth week	Term (37%–42%)	14 (70.0%)	34 (70.8%)	12 (70.6%)	45 (70.3%)	0.868
	Post-term(after 42 weeks%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	2 (3.1%)	
	Premature (29%–32%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Borderline premature (33%–37%)	5 (25.0%)	13 (27.1%)	5 (29.4%)	17 (26.6%)	
Infant weight	AGA (Appropriate for gestational age%)	16 (80.0%)	40 (83.3%)	15 (88.2%)	858 (90.6%)	0.556
	LGA (Large for gestational age%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	2 (3.1%)	
	SGA (Small for gestational age%)	4 (20.0%)	7 (14.6%)	2 (11.8%)	4 (6.3%)	
Hypertension	Preeclampsia	8 (44.4%)	10 (22.2%)	4 (30.8%)	11 (19.0%)	0.021
	Eclampsia	2 (11.1%)	11 (24.4%)	0 (0.0%)	4 (6.9%)	
	Not evaluated	2 (11.1%)	3 (6.7%)	0 (0.0%)	2 (3.4%)	
	Absent	6 (33.3%)	21 (46.7%)	9 (69.2%)	41 (70.7%)	
Systemic disease	Diabetes mellitus	1 (5.0%)	0 (0.0%)	0 (0.0%)	1 (1.6%)	0.345
	Hyperthyroidism	1 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Vasculitis	0 (0.0%)	2 (4.2%)	0 (0.0%)	1 (1.6%)	
	Absent	18 (90.0%)	46 (95.8%)	17 (100.0%)	62 (96.9%)	
Toxoplasma, rubella, cytomegalovirus, herpes simplex, HIV	Absent	18 (90.0%)	48 (100.0%)	17 (100.0%)	64 (100.0%)	NC
	Not evaluated	2 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Upper respiratory infections	Present	9 (45.0%)	20 (41.7%)	6 (35.3%)	24 (37.5%)	0.900
	Absent	11 (55.0%)	28 (58.3%)	11 (64.7%)	40 (62.5%)	
Urinary tract infections	Present	11 (55.0%)	24 (50.0%)	8 (47.1%)	34 (53.1%)	0.957
	Absent	9 (45.0%)	24 (50.0%)	9 (52.9%)	30 (46.9%)	
Vaginitis	Present	9 (45.0%)	22 (45.8%)	6 (35.3%)	29 (45.3%)	0.904
	Absent	11 (55.0%)	26 (54.2%)	11 (64.7%)	35 (54.7%)	
Placental disease	Absent	19 (95.0%)	44 (91.7%)	16 (94.1%)	59 (92.2%)	0.412
	Placenta previa	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	
	Placental insufficiency	1 (5.0%)	4 (8.3%)	0 (0.0%)	5 (7.8%)	
Amniotic fluid	Polyhydramnios	1 (5.0%)	3 (6.3%)	0 (0.0%)	0 (0.0%)	0.051
	Oligohydramnios	0 (0.0%)	4 (8.3%)	2 (11.8%)	1 (1.6%)	
	Normal	19 (95.0%)	41 (85.4%)	15 (88.2%)	63 (98.4%)	
Multiple pregnancy	Absent	20 (100.0%)	48 (100.0%)	17 (100.0%)	64 (100.0%)	NC
Smoking habit	Not smoking	13 (70.0%)	32 (66.7%)	12 (70.6%)	43 (67.2%)	1.000
	Smoking	7 (30.0%)	16 (33.3%)	5 (29.4%)	21 (32.8%)	
Number of cigarettes	0	13 (65%)	33 (68.8%)	12 (70.6%)	43 (67.2%)	0.988
	1–3 cigarettes	3 (15%)	7 (14.6%)	2 (11.8%)	11 (17.2%)	
	4–10 cigarettes	4 (20%)	7 (14.6%)	3 (17.6%)	10 (15.6%)	
	More than 10	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)	
Alcohol consumption during pregnancy	Drinking	1 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.248
	Not drinking	19 (95.0%)	48 (100.0%)	17 (100.0%)	64 (100.0%)	
Wi-Fi at home	Available	0 (0.0%)	0 (0.0%)	12 (70.6%)	40 (62.5%)	<0.001
	Not available	20 (100.0%)	48 (100.0%)	1 (5.9%)	2 (3.1%)	
	Available in the building	0 (0.0%)	0 (0.0%)	4 (23.5%)	22 (34.4%)	
Wi-Fi exposure at home	Open less than 2 h a day	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)	<0.001
	Open more than 2 h a day	0 (0%)	0 (0%)	15 (88.2%)	57 (89.1%)	
	Absent	20 (100%)	48 (100%)	1 (5.9%)	7 (10.9%)	

(*continued*)

Table 2. Continued.

Groups / Variables		Control (*n*: 20)	(Mobile phone) Group 1 (*n*: 48)	(Wi-Fi) Group 2 (*n*: 17)	(Mobile phone + Wi-Fi) Group 3 (*n*: 64)	*p*
Wi-Fi in workplace	Available	0 (0.0%)	0 (0.0%)	5 (29.4%)	23 (36.5%)	<0.001
	Not available	20 (100.0%)	48 (100.0%)	12 (70.6%)	40 (63.5%)	
Wi-Fi exposure in workplace	Open less than 2 h a day	0 (0%)	0 (0%)	2 (11.8%)	1 (1.6%)	<0.001
	Open more than 2 h a day	0 (0%)	0 (0%)	3 (17.6%)	23 (36.5%)	
	Absent	20 (100%)	48 (100%)	12 (70.6%)	39 (61.9%)	
Vitamin usage	Not used	0 (0.0%)	2 (4.2%)	0 (0.0%)	0 (0.0%)	0.032
	Irregular usage	14 (70.0%)	23 (47.9%)	4 (23.5%)	27 (42.2%)	
	Regular usage	6 (30.0%)	23 (47.9%)	13 (76.5%)	37 (57.8%)	

NC: Not calculated.

The alkali version the comet assay method was used in our study. All the steps were conducted under dimmed light. First, each microscope slide was pre-coated with a layer of 0.5% normal melting agarose in distilled water and dried thoroughly at room temperature. Next, 10 μL of the cell suspension was mixed with 85 μL of low-melting agarose (0.7% in PBS solution, pH 7.4; kept in 37 °C) and dripped onto the first layer. Slides were allowed to solidify for 5 min at 4℃ in a moist box. The coverslips were gently placed on the mixture and the slides were left in the humid chamber for 7 to 10 min at 4 °C. The coverslips were delicately removed, and the slides were placed in lysis solution (146.1 g $NaCl_2$, 1.2 g Trisma Base, 37.2 g etylenediaminetetracetic acid (EDTA), %1 Triton-X, pH 10.0). The slides were removed from the lysis buffer, drained and placed in a horizontal electrophoresis unit filled with fresh alkaline electrophoresis solution (TBE), containing 54 g Tris, 27.5 g boric acid and 20 mL EDTA (pH 8.4), for 20 min to allow the DNA to unwind. Electrophoresis was performed for 18 min at room temperature at 25 V and was adjusted to 300 mA. Subsequently, the slides were washed with distilled water for 5 min in order to remove the alkali ions and detergents. Observations were made at a magnification of 400× using a fluorescent microscope (Leica DM 1000 Led, Germany). The images of 100 randomly chosen nuclei were analysed by the comet assay software IV (Perceptive Instruments, Suffolk, UK). The comet parameters tail intensity and tail moment variables were examined. TM is expressed as tail diameter/head diameter or DNA% in tail × tail length.

Statistical analysis

All statistical analyses were performed with the STATA/MP11 program. Data were expressed as numerical values and percentages (means ± standard deviation). Normal distribution data were analysed by analysis of variance and post hoc Bonferroni test, while those without normal distribution were analysed by Kruskal-Wallis and post hoc Dunn test. Differences were considered statistically significant at a value of $p < 0.05$.

Results and discussion

Summarized data about the characteristics of the participants and their RFR exposure are presented in Tables 1 and 2. In this study, the subjects in the control group were especially important because finding pregnant women who do not to use a mobile phone is very difficult in today's world. Additionally, the RF exposure, birth parameters, habits, and so forth. of the pregnant women were compared between groups.

Comet assay

The results of the Comet test (tail intensity, tail moment) of the samples of cord blood are shown in Table 3. The values of tail intensity and tail moment in Group 1 (mobile phone only) and Group 3 (both mobile phone and Wi-Fi) were considerably higher than the control ($p < 0.001$). In Group 2 (Wi-Fi only), there were significantly lower tail intensity and tail moment than in Group 1 ($p < 0.001$).

Oxidative stress parameters

In cord blood, in Groups 1 and 3, the levels of 8-OHdG, MDA, PCO, TOS, OCI were increased and TAS was decreased compared to the control ($p < 0.001$; Table 4). However, in Group 2, there were significantly lower levels of 8-OHdG, MDA and PCO ($p < 0.001$) as compared to the other groups.

In the placenta, in Groups 1 and 3, the values of 8-OHdG, MDA and PCO where higher than those in the control group ($p < 0.001$; Table 5). Moreover, the values of 8-OHdG, MDA and PCO were higher in Group 3 compared to Group 2 ($p < 0.001$). However, in Group 2, a significant decrease was found in the values of 8-OHdG, MDA and PCO compared to the Group 1 ($p < 0.001$). In Group 2, the level of TAS was higher

Table 3. Comet assay findings in lymphocytes from umbilical cord blood.

Lymphocytes	Control (*n*: 20)	(Mobile phone) Group 1 (*n*: 48)	(Wi-Fi) Group 2 (*n*: 17)	(Mobile phone + Wi-Fi) Group 3 (*n*: 64)	*p*
Tail intensity	24.72955 ± 2.79449	46.13002 ± 11.50257[a]	23.76200 ± 4.45289[b]	46.86076 ± 11.47941[a,c]	**<0.001**
Tail moment	41.3229 ± 10.09878	106.9553 ± 71.78970[a]	38.1318 ± 7.87293[b]	127.8285 ± 81.01049[a,c]	**<0.001**

[a]Significant difference from control group.
[b]Significant difference from Group 1.
[c]Significant difference from Group 2.

Table 4. 8-OHdG, MDA, PCO, TAS, TOS and OSI findings in cord blood.

Cord blood	Control (*n*: 20)	(Mobile phone) Group 1 (*n*: 48)	(Wi-Fi) Group 2 (*n*: 17)	(Mobile phone + Wi-Fi) Group 3 (*n*: 64)	*p*
8-OHdG (ng/mL)	81.63550 ± 9.87941	96.11158 ± 13.84800[a]	80.43941 ± 11.44760[b]	99.77741 ± 15.12398[a,c]	**<0.001**
MDA (nmol/mL)	10.35460 ± 7.86654	25.45302 ± 15.47587[a]	11.87518 ± 7.55306[b]	27.48351 ± 12.03887[a,c]	**<0.001**
PCO (ng/mL)	62.54700 ± 16.28469	82.92677 ± 17.01314[a]	68.46571 ± 11.93756[b]	86.68276 ± 13.22829[a,c]	**<0.001**
TAS (mmol/L)	1.280050 ± 0.488033	0.894771 ± 0.377831[a]	1.097294 ± 0.435418	0.919175 ± 0.417570[a]	**<0.001**
TOS (μmol/L)	9.08855 ± 1.940754	11.30110 ± 2.116981[a]	10.18394 ± 1.869079	10.96379 ± 2.157406[a]	**<0.001**
OCI	8.29960 ± 3.954663	14.65458 ± 5.619678[a]	10.45606 ± 3.676169	14.62333 ± 7.444033[a]	**<0.001**

[a]Significant difference from control group.
[b]Significant difference from Group1.
[c]Significant difference from Group 2.

Table 5. 8-OHdG, MDA, PCO, TAS, TOS and OSI findings in placenta.

Placenta	Control (*n*: 20)	(Mobile phone) Group 1 (*n*: 48)	(Wi-Fi) Group 2 (*n*: 17)	(Mobile phone + Wi-Fi) Group 3 (*n*: 64)	*p*
8-OHdG (ng/ml)	68.39710 ± 9.14412	90.59917 ± 13.58303[a]	68.14447 ± 12.27792[b]	94.56463 ± 14.66638[a,c]	**<0.001**
MDA (nmol/ml)	12.88065 ± 7.64414	30.75248 ± 16.64219[a]	17.05176 ± 7.41858[b]	34.76579 ± 12.79696[a,c]	**<0.001**
PCO (ng/ml)	56.92160 ± 15.63014	76.29560 ± 14.98233[a]	62.59476 ± 12.55616[b]	81.00340 ± 13.82716[a,c]	**<0.001**
TAS (mmol/L)	1.146900 ± 0.456885	0.897771 ± 0.399989	1.329176 ± 0.466903[b]	0.883730 ± 0.418490[c]	**<0.001**
TOS (μmol/L)	10.39555 ± 2.203754	11.24592 ± 2.075945	10.25771 ± 2.288172	11.54397 ± 2.245704	0.067
OSI	10.25130 ± 4.048792	14.89819 ± 6.703951[a]	8.71618 ± 3.753837[b]	15.54092 ± 6.568665[a,c]	**<0.001**

[a]Significant difference from control group.
[b]Significant difference from Group 1.
[c]Significant difference from Group 2.

and OCI was lower compared to those in Group 1 (respectively $p = 0.001$, $p < 0.001$).

The use of wireless Internet and mobile phones has been increasing rapidly. Nowadays, access points to the internet are present in workplaces, public places, houses, and schools. These sources of RFR are raising the public concern regarding the potential health effects. Possibly, these systems are particularly popular with children and the young population. However, exact proof is not present on whether these RFR systems are harmful or not for humans. Therefore, people, especially pregnant women and children should be cautious around such equipment until there are sufficient data. Some animal studies have recently indicated that RFR exposure during pregnancy induced some adverse effects on infants [13–15]. On the other hand, in a human pregnancy study, it is reported that RFR exposure during pregnancy caused significant changes in some biochemical parameters of cord blood [16].

Most of the studies on the effects of RFR concern mobile phone exposure. However, the studies on the health effects of Wi-Fi are limited. In this study, we compared the potential adverse effects associated with oxidative stress associated with RFR emitted from Wi-Fi and mobile phones on placenta and cord blood. This study is one of a few human studies to examine the bio-molecular effects of exposure to RFR emitted from mobile phones and Wi-Fi.

Both Wi-Fi devices and mobile phones emit RFR when used. There are some structural and functional differences between the exposures to RFR emitted from mobile phones and Wi-Fi. Whereas mobile phones emit 900, 1800 or 2100 MHz RFR, Wi-Fi devices emit 2.45 GHz. On the other hand, mobile phones cause a near field exposure to RFR but Wi-Fi emitters cause a far field exposure to RFR.

Some studies indicate that 1800 MHz RFR causes oxidative stress and DNA damage in the brains and livers of pregnant rabbits and their offspring [17–20]. Another study reported that the exposure to 900 MHz RF during the prenatal period induced oxidative stress and pathologic changes in the liver tissues of rats [21]. Ozarak et al. [22] reported that the exposure to 2450, 900 and 1800 MHz RFR during the prenatal period induced oxidative damage in the kidneys of newborn rats. On the other hand, Ferreira et al. [23] reported that the exposure to 834 MHz RFR during the prenatal period did not cause any change in some parameters of oxidative stress in the blood and liver tissues of infant rats.

Additionally, many studies have reported that 1800 MHz [17–19,24–28] and 900 MHz RFR [29,30] cause oxidative stress and DNA damage in animals. Moreover, some studies indicate that 900 MHz [6,31–38] and 1800 MHz RFR [17,26,39–41] induce oxidative stress with an increase in MDA levels. There are other reports that 900 MHz [6,42], 1800 MHz [24] and 2450 MHz [43] RFR cause protein oxidation.

Exposure to 2450 MHz RFR reportedly induced DNA damage in both the brain and plasma of rats, caused protein oxidation in the plasma and decreased the activities of some antioxidant enzymes in male rats' brain [43,44]. In another study, 2.45 GHz RFR caused oxidative injury in the kidneys and testes of the offspring of rats [22].

In our study, the tail moment and tail intensity, which are DNA damage indicators, were found higher in Group 1 (mobile phone only) than in the control group. However, no significant change was observed in the Wi-Fi group (Group 2) in terms of the parameters under discussion. The 8-OHdG level, which is also another oxidative DNA damage indicator, was higher in Group 1 than in the Control. On the other hand, no significant alteration was observed in Group 2 as compared to the control. We also observed differences in the 8-OHDG levels, tail moment and tail intensity between the experimental groups ($p < 0.001$). Therefore, we suggest that RFR emitted from mobile phones was more effective in inducing oxidative DNA damage than RFR emitted from Wi-Fi. On the other hand, the results from our study indicated that Wi-Fi has the potential to contribute to the detrimental effects of mobile phones.

In cord blood, the values of TOS and OSI in Groups 1 and 3 were higher than those in the control group ($p = 0.001$), whereas the TAS levels were lower than that in the control ($p < 0.05$). Alterations in TAS, TOS, OSI were not observed in the Wi-Fi exposed group. In placenta, TAS in Group 2 was higher than that in Group 1 ($p < 0.001$), OSI in Group 2 were acquired lower than Group 1. The results obtained from our study showed that the exposure to mobile phones during the gestation period might be able to induce oxidative stress, but exposure to Wi-Fi does not appear to cause oxidative stress. The reason behind this difference may be attributed to absorption and attenuation of RFR emitted from Wi-Fi devices by walls and materials in the vicinity. In addition, mobile phone users are exposed to more RFR in the near field. Known harmful effects of RFR on living organisms depend on some physical characteristics of radiation [7]. The body RF penetration decreases when RF frequency increases [8].

The power density of RF declines in proportion to $1/d^2$ (where d is the distance to source) as it moves away from the source. The maximum power density of laptops and access points (15 laptops and 12 access points operating at 2.4 GHz) at a distance of 0.5 m was 22 and 87 mWm^{-2} respectively; these values decreased to 4 and 18 mWm^{-2} at a distance of 1 m [45]. In another study, the highest value of the localized SAR originating from Wi-Fi equipment (2.4 GHz, 100 mW) was 5.7 mW kg^{-1} in the heads of adults and children. This value is also less than about 1% of the value of SAR originating from talking on mobile phone [46]. In our study, Wi-Fi contributed to increase the harmful effects of the mobile phone exposure on placenta and cord blood in human pregnancies. We speculate that there might be a cumulative effect of RFR emitted from mobile phones and Wi-Fi devices.

There are some limitations to this study. For example, although all efforts were made to carefully select the participants enrolled in this study, the study design could not possibly exclude all potential effects of other factors on the pregnant women that may have affected the levels of the parameters in this study. However, the cofounding factors presented in Tables 1 and 2 indicated that external factors were found statistically insignificant ($p > 0.05$).

Conclusions

In this study, the exposure of mobile phones during the gestation period was associated with increased levels of some oxidative stress parameters such as 8-OHdG, MDA, PCO, TOS and OCI) and caused DNA damage in cord blood and placenta. We did not observe that Wi-Fi alone could be as effective as the use of a mobile phone. However, we observed that Wi-Fi could contribute to the harmful effects of mobile phones when Wi-Fi is used along with a mobile phone. Further investigations on the biological effects of RFR emitted from wireless communication devices on pregnant women and foetuses may help us to better understand the pathogenesis of many diseases which have been seen in recent years in children.

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No potential conflict of interest was reported by the author(s).

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