

Another Concept of Cancer Interpretation in View of the Interaction between Plasma Radiation and DNA

Abstract

Several studies included our preceding works on different types and doses of plasma sources on both normal and cancerous cells. All previous research tried to discuss and interpret cancer treatment concepts given plasma and cell interaction. Plasma has an effective role and specification character on cancerous cells outcome via many investigations (genetically, immunologically, and biochemically measurements) lead us to numerous paths of different theories which may create a new approach for considerations. This study was designed using a plasma jet powered by a Tesla coil used for generating Cold Atmospheric Pressure Plasma Jet (CAPPJ) from dielectric barrier discharge. The subject samples were categorized into three groups, the first was the cancer cell line. The second was normal blood samples whereas, the third one was non-exposed blood cells cultivated in CAPPJ-exposed cultures. The Cytokinesis Blocked Micronucleus Test (CBMN), a cyto assay, the protein expression of the P53 and Bcl2 genes, the interleukins (IL-1 β , IL-6, and IL-10), and tumor necrosis factor (TNF- α) were the variables used in the current investigation. Results indicated that the direct interaction between cells and CAPPJ is more efficient than cells cultivated in CAPPJ-exposed cultures. Cell viability and protein expression levels of Bcl2 and P53 genes in CAPPJ irradiated Breast Cancer Cell lines (BCC) were remarkably valuable. CAPPJ affects cells via not only free radicals and enhancement of several important pathways but may be via direct interaction with DNA.

Keywords: CAPPJ, Epigenetic, Cyto assay P53, Bcl2, Apoptosis

Introduction

World Health Organization (WHO) defined cancer as broad spectrum of diseases which can affect almost each and every system of the body when abnormal cells grow and divide in an uncontrollable manner, move away from their usual limitations to infiltrate adjacent parts of the body, and/or travels to other organs. The later progression is called metastasizing and it is the prime causes of death from cancer.^[1]

Another definition said that “most cancer is a complex and heterogeneous set of diseases without a simple definition”. In addition, it is believed that between 5 and 10% of cancer cases are brought on by mutation and up to 15% by inflammation; a few 80% of cancer cases are "sporadic" tumors, the cause of which is yet unclear. Even mutations are late events, or epiphenomena, in a complex chain of circumstances that can explain the genesis of the majority of cancer form.^[2]

Cancer primarily develops as a result of

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chromosomal instability. The CBMN cyto assay, which Fenech^[3] demonstrated to be a multi-endpoint cytogenetic approach, allowed assessments of a variety of nuclear anomalies suggestive of a variety of chromosomal instabilities. Chromosomal aberrations include structural/numerical chromosome abnormalities, chromosome mal-segregation occurring throughout mitosis and manifested as micronuclei (MNI), anaphase bridge formation manifested as nucleoplasmic bridges (NPBs), and gene amplification or elimination of unresolved DNA complexes manifested as nucleoplasmic buds (NBUDs).

Plasma medicine is an interdisciplinary research field that combines plasma physics and chemistry with biology and clinical medicine to bring new modalities of cancer treatment to market. Application of plasma to cellular structures has been found to generate abundant reactive species. Their interaction with cells manipulates cellular redox signaling, ultimately resulting in changes in surface receptor function, initiation of cell cycle arrest, activation of

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p53 by DNA damage, and subsequent p53-dependent apoptosis and other effects on various cells.^[4]

According to another viewpoint, The goal of cancer therapy is to encourage the death of malignant cells while sparing healthy cells from too much harm (selectivity).^[5] As well, the absence of functionality of tumor-suppressor gene or the overexpression of an anti-apoptotic protein are both important pathways in cancer development^[6] P53 referred to as "the guardian of the genome."^[7] In addition, The Bcl2 family of proteins is widely studied for its role in apoptosis.^[8] Even the absence of apoptosis leads to cancer.^[9]

All regulation and communication between our body and mind go in a bidirectional flow of neuropeptides, cytokines, and hormones.^[10] Cytokines are the major mediators of communication, particularly regarding inflammation and infection.^[11] Cytokines play a highly critical position in system homeostasis at some point of immune challenge.^[12]

Additionally, they claimed that DNA damage responses could be in charge of the start of senescence after inflammatory mediators released a variety of cytokines, including IL-1, IL-6, IL-8, and TGF- β .^[13] Pro-inflammatory cytokines like TNF- α , IL-1, and IL-6, as well as the anti-inflammatory IL-10 family, cause up- and down-regulation of cellular activity.^[14]

CAPPJ efficacy has been estimated in a variety of medical conditions such as osteosarcoma, glioblastoma, melanoma, pancreatic, ovarian, breast, and cervical cancer ...etc. it's entirely believed that the major mechanism of the plasma's efficacy is facilitated through the stimulation of debauchery of energy via radiation and/or chemical energy.^[15]

The goal of the current study is to try to explain how plasma affects cells accurately through its roles in oxidative stress, changes in redox state pathways, or direct impact on DNA (epigenetically), Then, it tries to interpret the interaction between plasma and DNA and its reflection on the realization of the concept for cancer definition factors and its treatment.

Materials and Methods

Experimental design

The presented work is designed to clarify the following: Direct and indirect (irradiated cultures) effects of CAPPJ exposure on the normal whole blood samples and breast cancerous cells. harmless and effective exposure doses of CAPPJ for normal and cancerous cells respectively and the relationship between genetic damage and triggering of the immunological cytokines.

Characters of the plasma jet supplied by the tesla coil

A cold plasma jet's characteristics may be crucial for having a better impact in the biological application, according to Elaragi^[16] who created a CAPPJ powered by a Tesla coil. As a result of the breakdown of a brief spark gap, the capacitor in Tesla's coil transformer connected to a coil with a few turns,

creating a resonant circuit with an oscillation frequency that was typically between 20 and 100 kHz and was controlled by the capacitance of the capacitor and the inductance of the coil. A plasma jet generator, gas flow circuit, and high voltage, low current, and high frequency AC make up the experimental system as shown in (Figure 1).

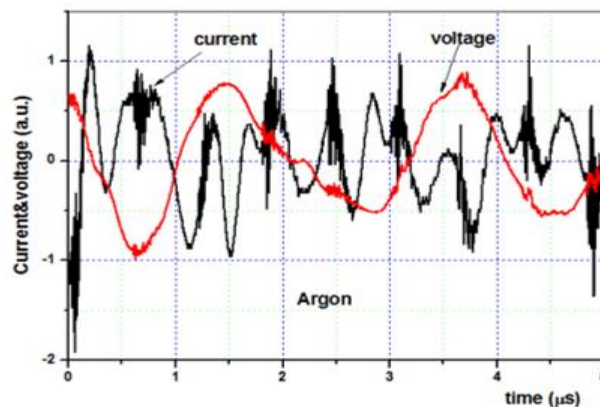


Figure 1. Typical voltage-current waveforms of argon plasma jet

Chemicals

Chemicals for blood culture were purchased from Gibco (Carlsbad, CA, USA) and heat-inactivated fetal calf serum (FCS) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Determination of IL-1 β : Human (IL-1 β) ELISA Kit was purchased from CUSABIO with Catalog Number: CSB-E08053h. IL-6: Human (IL-6) ELISA Kit was purchased from RayBio with Catalog Number: ELH-IL6-001. IL-10: Human (IL-10) ELISA Kit was purchased from CUSABIO. TNF- α : Human TNF- α ELISA Kit was purchased from ALPCO with Catalog Number: 45-TNFHU-E01. Finally, anti-P53 primary antibody was purchased from Abcam, ab131442, and anti-Bcl2 primary antibody from Abcam, ab131442.

Subjects and blood sampling

Subjects

Normal human blood samples: Eight healthy volunteers (4 males and 4 females) with blood samples were collected in order to reduce any potential inter-individual heterogeneity in treatment response. The donors matched for age, smoking, and environment.

Breast cancer cell line: it was purchased from the Genetic Engineering Unit, National Research Centre, Egypt.

All human blood samples had been drawn under sterile conditions and placed in a heparinized vacutainer tube ($v = 5$ ml; Becton Dickinson, USA).

Exposure doses

Normal whole blood samples were taken from everyone and split into two categories: the first was for direct CAPPJ exposure to four dose groups (20 (BI-D1), 40 (BI-D2), 60 (BI-

D3), and 120 (BI-D4) sec, respectively) at a distance of 3 cm from the blood surface, and the second was for an unexposed group (control group). The second exposure was indirect and involved normal blood that was grown on CAPPJ-exposed media with the same 4 dosages (20 (CI-D1), 40 (CI-D2), 60 (CI-D3), and 120 (CI-D4) sec, respectively). The identical doses were administered directly after conception to the Breast Cancer Cell Line (BCC).

Blood culture

Before beginning culture and immunological investigations, blood samples incubated for 20 hours at 37 °C. For each sample, triple blood cultures established for 72 hours in accordance with the Evans and O’Riordan methodology.^[17]

Cytokinesis-block micronucleus cytochrome assay, immunological parameters, gene expressions, and cell viability assay

Cytokinesis-block micronucleus cytochrome assay was carried out as described by Fenech.^[3, 18] The assay evidenced mitotic alert (mono-, bi-, tri-, and quadrinucleated cells), cytotoxicity (MNI

frequencies, NPBs, and nuclear buds), necrotic and apoptotic cells. An enzyme-linked immunosorbent assay for the quantitative measurement of human IL-1β, IL-6, IL-10, and TNF-α concentrations in cell culture supernatants and plasma. P53 and Bcl2 gene expressions were observed using electrophoresed proteins on SDS-PAGE in accordance with Horiuchi *et al.*^[19]

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS) software version 20 for Windows, a one-way analysis of variance (ANOVA), and Tukey multiple comparison tests (P values of 0.05 were considered significant).^[20]

Results and Discussion

Table 1 represents the cytogenetic data of the direct effect of CAPPJ on the normal whole blood; **Table 2** indicates indirect CAPPJ effects on the normal whole blood.

Table 1. The incidence of mono-, bi-, tri-, quadrinucleated, apoptotic, necrotic cells, and the frequencies of micronuclei, NPBs, and buds in normal whole blood and CAPPJ irradiated groups (counts in 1000 cells).

| Groups | Control | BI-D ₁ | BI-D ₂ | BI-D ₃ | BI-D ₄ |
|-----------------------|-------------|--------------------|----------------------|------------------------|-------------------------|
| Mononucleated cells | 796.66±2.99 | 806.13±2.70 | (a,b) 892.88±3.12 | (a,b,c) 869.75±6.44 | (a,c,d) 810.50±4.13 |
| Mono+ 1 MN | 2.38±0.12 | 1.61±0.18 | (a,b) 0.57±0.04 | (a,b) 0.39±0.05 | (a,b,c,d) 14.75±0.75 |
| Mono+ 2 MNi | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | (a,b,c,d) 1.44±0.06 |
| Binucleated cells | 194.20±3.13 | 185.93±2.65 | (a,b) 100.76±3.12 | (a,b,c) 122.10±6.44 | (a,b,d) 109.71±3.47 |
| Bi+ 1 MN | 2.55±0.11 | (a) 1.33±0.11 | (a,b) 00.00±00.00 | (a,b) 00.00±00.00 | (a,b,c,d) 5.03±0.20 |
| Bi+ 2 MNi | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| Bi+ 3 MNi | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| Trinucleated cells | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| Quadrinucleated cells | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| NPBs | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| NBUDs | 0.10±0.07 | (a) 00.00±00.00 | (a) 00.00±00.00 | (a) 00.00±00.00 | (a) 00.00±00.00 |
| Necrotic cells | 2.90±0.23 | 2.85±0.18 | 3.16±0.12 | 2.45±0.15 | (a,b,c,d) 49.68±1.18 |
| Apoptotic cells | 1.22±0.06 | (a) 2.04±0.03 | (a) 2.64±0.13 | (a,b,c) 5.31±0.29 | (a,b,c,d) 8.74±0.36 |

a: significant when compared with control gp
 b: significant when compared with BI-D1 gp
 c: significant when compared with BI-D2 gp
 d: significant when compared with BI-D3 gp

Table 2. The incidence of mono-, bi-, tri-, quadrinucleated, apoptotic, necrotic cells, and the frequencies of micronuclei, NPBs, and buds of normal whole blood in CAPPJ irradiated cultures (count in 1000 cells).

| Groups | Control | CI-D ₁ | CI-D ₂ | CI-D ₃ | CI-D ₄ |
|-----------------------|-------------|--------------------|----------------------|------------------------|--------------------------|
| Mononucleated cells | 796.66±3.0 | 798.0±2.32 | (a,b) 714.38±4.27 | (a,b,c) 682.60±1.51 | (a,b,c,d) 761.91±1.02 |
| Mono+ 1 MN | 2.28±0.11 | (a) 7.0±0.36 | (a) 7.0±0.27 | (a,b,c) 11.38±0.18 | (a,b,c,d) 16.38±0.75 |
| Mono+ 2 MNi | 00.00±00.00 | 00.00±00.00 | (a,b) 1.00±0.19 | (a,b,c) 3.19±0.07 | (a,b,d) 0.77±0.04 |
| Binucleated cells | 194.20±3.13 | (a) 177.63±2.55 | (b) 193.4±4.68 | (a,b,c) 156.04±0.99 | (a,b,c,d) 68.13±3.44 |
| Bi+ 1 MN | 0.00±0.00 | (a) 3.0±0.46 | (a,b) 0.97±0.19 | (a,b) 0.80±0.03 | (a,c,d) 2.75±0.31 |
| Bi+ 2 MNi | 00.00±00.00 | 00.00±00.00 | (a,b) 0.29±0.14 | (c) 00.00±00.00 | (c) 00.00±00.00 |
| Bi+ 3 MNi | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| Trinucleated cells | 00.00±00.00 | 00.00±00.00 | 0.39±0.15 | (a,b,c) 4.39±0.11 | (a,b,c,d) 5.63±0.38 |
| Quadrinucleated cells | 00.00±00.00 | 00.00±00.00 | (a,b) 0.29±0.14 | (a,b,c) 0.78±0.01 | (a,b,c) 0.86±0.4 |
| NPBs | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| NBUDs | 0.10±0.07 | (a) 0.75±0.16 | 0.39±0.15 | (a,b,c) 1.68±0.05 | (a,b,c) 1.63±0.18 |
| Necrotic cells | 2.90±0.23 | (a) 8.25±0.53 | (a,b) 65.63±1.28 | (a,b,c) 117.38±1.56 | (a,b,c,d) 124.75±0.86 |
| Apoptotic cells | 1.22±0.06 | (a) 5.38±0.42 | (a,b) 16.75±0.59 | (a,b,c) 21.43±0.57 | (a,b,c) 20.58±0.69 |

a: significant when compared with control gp

b: significant when compared with CI-D1 gp

c: significant when compared with CI-D2 gp

d: significant when compared with C-D3 gp

Samples exposed to CAPPJ with different doses (Control, BI-D1, BI-D2, BI-D3, and BI-D4 groups) illustrated in **Table 1**. The same previous parameters were recorded for normal human blood cultivated in irradiated culture with the same CAPPJ doses (CI-D1, CI-D2, CI-D3, and CI-D4) in **Table 2**.

Table 1 showed that, a significant decrease in the genetic damage for both doses (BI-D2 and BI-D3) groups (mono + 1 Mn, bi+ 1Mn, and nuclear buds) when compared with control and BI-D1, where the BI-D4 group recorded significantly high incidences of Mni in mono- and binucleated cells.

Table 1 illustrated a gradually significant increase in the incidence of apoptotic cells from group BI-D1 to BI-D4 in comparison with the control group. There are no significant differences for mono+2MN, bi+2MN, bi+3MN, trinucleated, quadrinucleated, and nucleoplasmic bridge when its values are compared between all groups. Necrotic cells in BI-D4 recorded a high frequency of incidence in comparison with the other groups.

There is a significant difference between BI-D2, BI-D3, and BI-D4 in mononucleated and binucleated cell counts. The data in **Table 2** is opposed to that obtained in **Table 1**. There is a high incidence of frequencies of Mni in both mono- and binucleated cells and nuclear buds. Also, necrotic and apoptotic cells recorded high significant frequencies, especially CI-D4 when compared to control and CI-D1 groups. But, CI-D3 and CI-D4 groups displayed significant differences in trinucleated and quadrinucleated counts when compared with control and other groups.

Table 3 illustrated BCC viability percentages and protein expression of Bcl2 and P53 genes. The BCC - Control group is considered a reference for cell viability with a value of 100%. There are gradual decreases in the cell line from the BCC-D1 group to the BCC-D4 group (82%, 64%, 51%, and 10%) respectively. While results recorded a decrease in Bcl2 protein expression there are increases in P53 gene expressions.

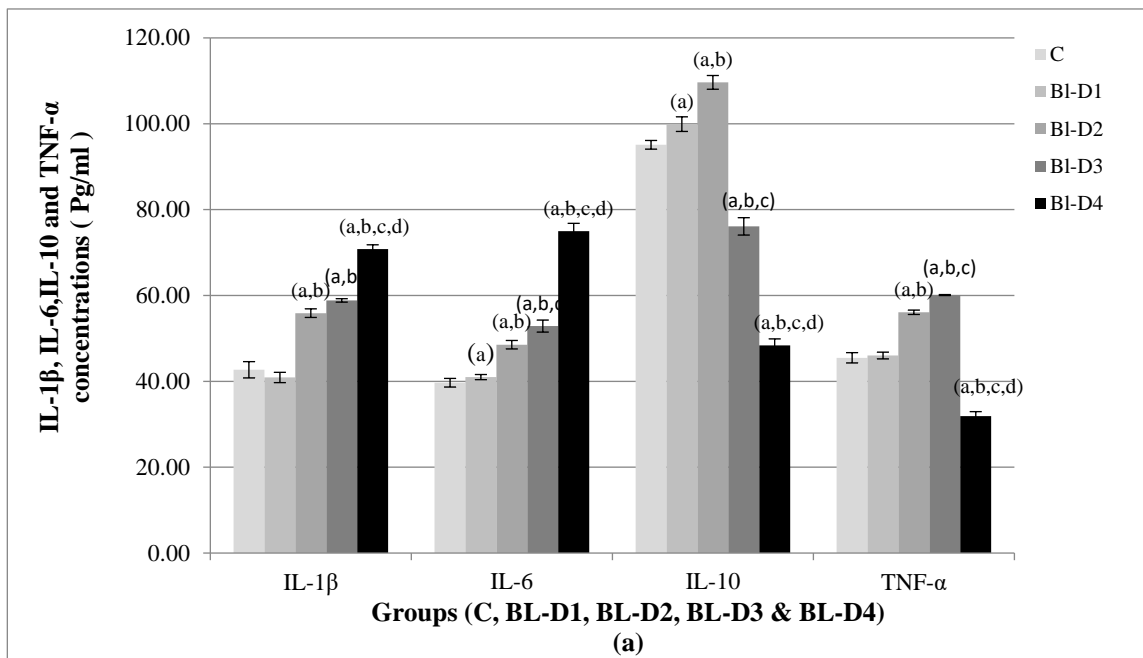
Table 3. Cell viability and protein expression levels of Bcl2 and P53 genes in CAPPJ irradiated and non-irradiated Breast Cancer Cell lines (BCC).

| Groups | BCC-Control | BCC-D ₁ | BCC-D ₂ | BCC-D ₃ | BCC-D ₄ |
|----------------|-------------|--------------------|--------------------|--------------------|--------------------|
| Cell Viability | 100% | 82% | 64% | 51% | 10% |
| Bcl2 | 86.10 | 85.90 | 76.16 | 68.25 | 50.25 |
| P53 | 76.76 | 80.29 | 91.34 | 99.92 | 99.92 |
| B-actin | 100.0 | 100.0 | 99.92 | 99.92 | 99.94 |

In **Table 4** the most effective dose was observed in the BI-D2 group for the Bcl2 gene expression and their significant difference between all exposed groups when compared with the control group. On the other hand, there is a significant increase for (BI-D2, BI-D3, and BI-D4) when compared with control and BI-D1 groups for P53 gene expression.

It is also displayed a regular decrease in protein expression of Bcl2 genes from CI-D1 to CI-D4 groups when compared with the control group, which opposes the regular elevation of protein expression of P53 from CI-D1 to CI-D4 groups when compared with the control group.

(**Figure 2a**) illustrated that the first dose group BL-D1 had a non-significant difference in the concentrations of IL-1 β and TNF- α compared to the control group. While there are differences in levels of IL-6 and IL-10 that were significantly increased. At BL-D2, BL-D3, and BL-D4, there is a progressive and significant increase in concentrations of IL-1 β and IL-6 compared to controls and BL-D1. Whereas, IL-10 levels were raised in the BL-D2 group and gradually decreased in the BL-D3 and BL-D4 groups. TNF- α recorded increasing levels through BL-D2 and BL-D3 groups but significantly decreased in the BL-D4 group. The corresponding attitude occurs for the same rating data from (**Figure 2b**) except for the IL-1 β values.



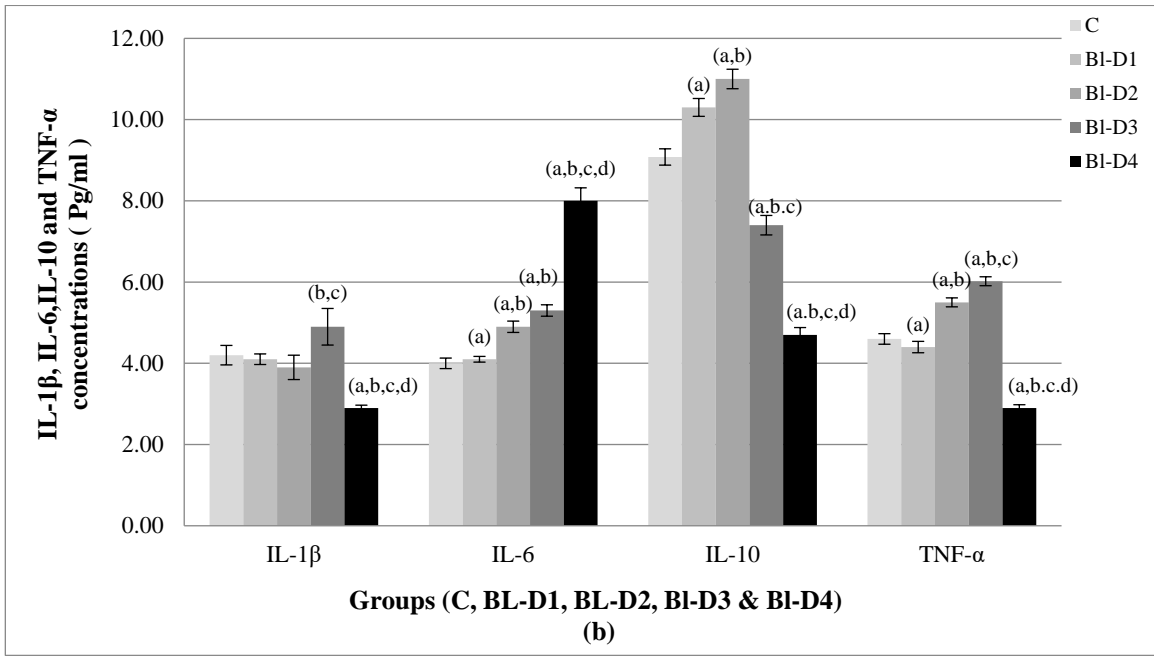


Figure 2. IL-1β, IL-6, IL-10 and TNF-α levels. (a) in serum of CAPPJ irradiated and non-irradiated normal whole blood groups and (b) in cultures of CAPPJ irradiated and non-irradiated normal whole blood groups.

(Figure 3) indicated data of irradiated culture media before cultivation and demonstrated that IL-1β, IL-6, IL-10, and TNF-α concentrations values were opposed to those recorded for all groups in (Figures 2a and 2b) that there are gradually decreasing through the four doses groups.

(Photomicrographs illustrated the different forms of the cell types of cytochrome assay) (Figure 4).

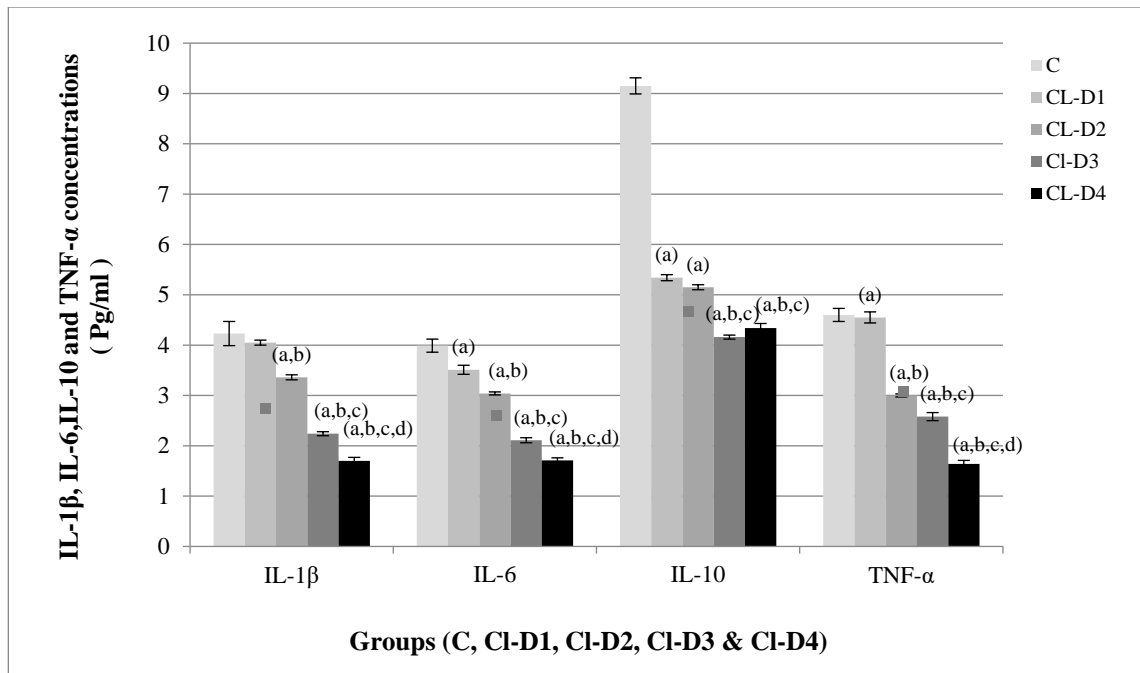
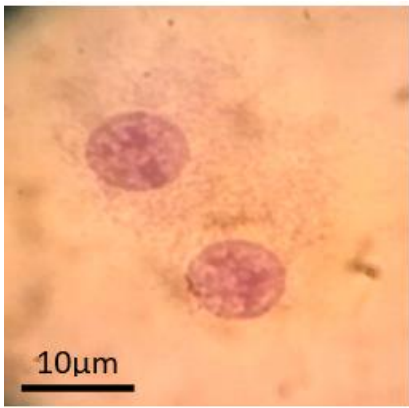
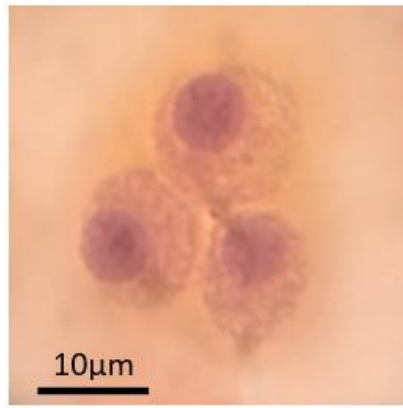


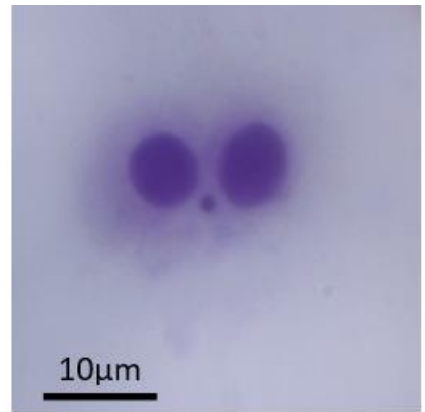
Figure 3. IL-1β, IL-6, IL-10 and TNF-α levels of normal whole blood groups cultivated in CAPPJ irradiated and non-irradiated cultures.



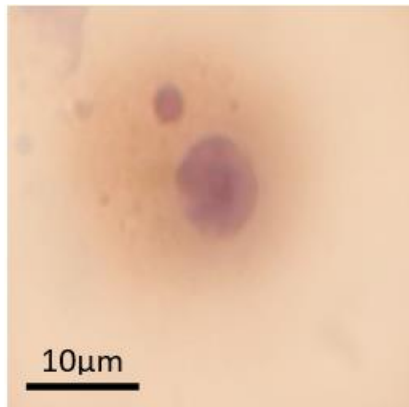
(a)



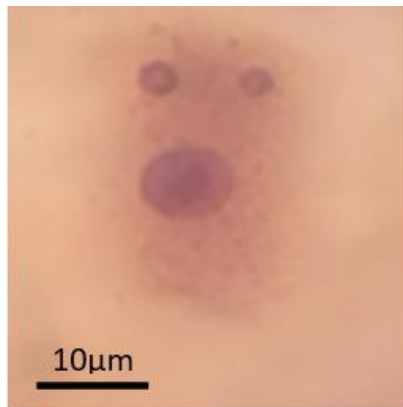
(b)



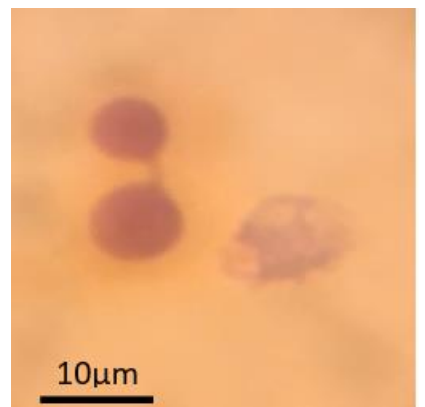
(c)



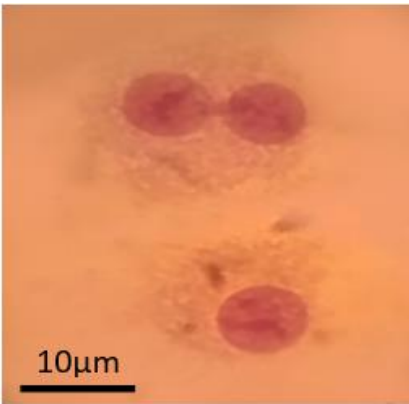
(d)



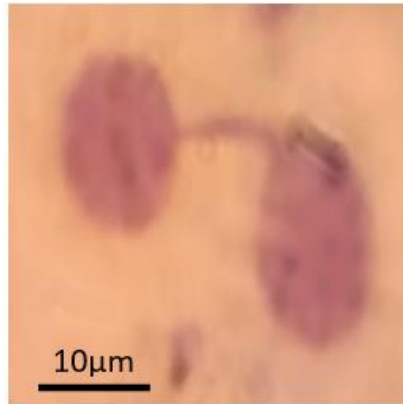
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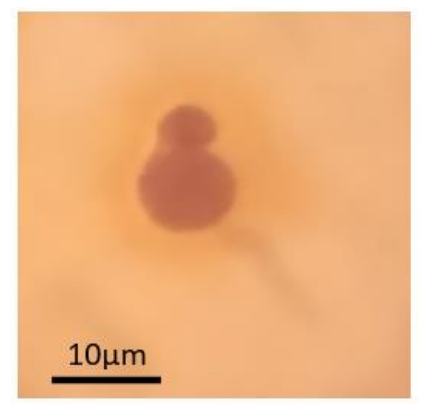
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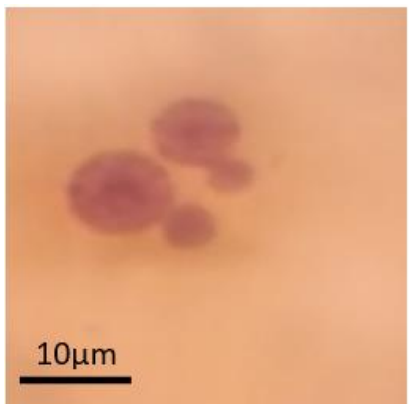
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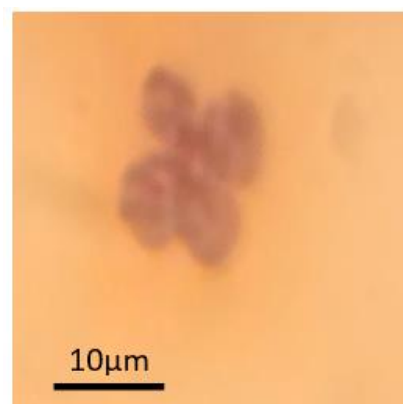
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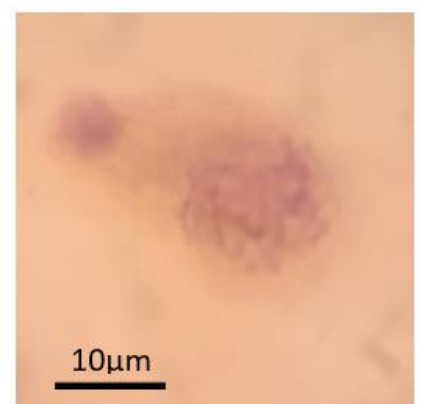
(i)



(j)



(k)



(l)

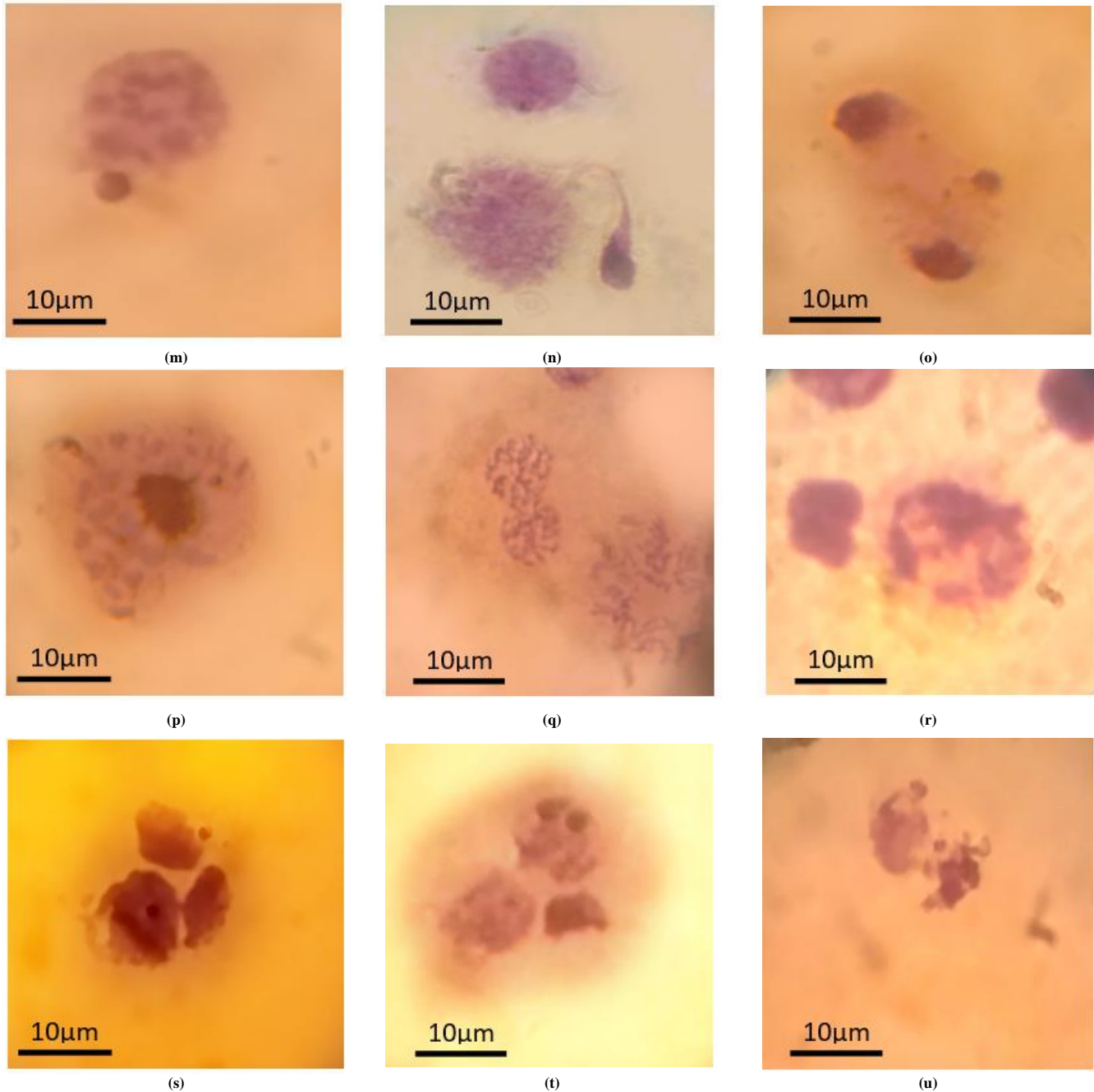


Figure 4. Photomicrographs of the cells scored in the CBMN “cytome” assay. a) Normal binucleated cell; b) Normal mononucleated cells; c) Binucleated cell with one MN; d) Mononucleated cell with one MN; e) Mononucleated cell with two MNI; f, g and h) Binucleated cells with NPBs; i and j) Mononucleated cells with NBUDs; k) Quadrinucleated cell; l, m, n and o) Necrotic cells with MNI; p and q) Necrotic cells; r, s, t and u) Apoptotic cells (Scale bar, 1.25cm:10µm).

Muresanu and Khalchitsky^[21] concluded that cancer is a metabolic, multifactorial, multistage disease in 2022 after presenting an integrative review from various sources to comprehend the cancer concept. They concentrate on telomere variations as indicators of underlying genetic/environmental interactions. Physical agents and predisposing elements like nutrition and lifestyle are among the many genetic and epigenetic factors that are considered. In addition, ideal cancer treatments specifically target and destroy tumor cells without affecting healthy cells. Therefore, new strategies must be created to achieve this target but most anti-cancer drugs work

by causing oxidative stress in cancer cells, which is thought to be the cause of most macromolecular modifications throughout the cell.^[22-25]

CAPPJ and gliding arc plasma exposure can be utilized to repair tissues, treat some diseases, and treat tumors, especially drug-resistant forms, according to evidence from several studies on various types of plasma and their effects on both healthy and malignant cells.^[26, 27] The most significant improvements in the halting of chromosomal instabilities were achieved by stimulation of several mitotic checkpoints,

followed by apoptosis and necrosis processes, which were plasma's most effective roles. The same events were also found to increase immunological responses (interleukins and TNF- α) and the transcription of anti-tumor genes (P53, caspase-3, and Bcl2).^[28]

All the data included in **Table 1** suggested amelioration for genetic damages in most exposed groups moreover, there are significant differences between numbers of mono- and binucleated cells that may be attributed to the plasma effect on the mitotic index of division.

Whereas, **Table 2** showed an opposite tendency for its cytogenetic data when compared with that recorded in **Table 1**. There are significant genetic damages represented with high frequencies of MNi in both mono- and bi-nucleated cells and NBUDs. In addition, there are remarkably significant increments of necrotic cell counts correlated with increasing exposure doses.

According to the scored data in **Tables 1 and 2**, the direct exposure of blood cells to CAPPJ originates good effects and enhancement for all cytogenetic parameters while there are destructive effects for the CAPPJ when exposure occurred to the culture media before blood cell cultivation. These records impressed that the plasma effect not only depends on the free radical effects theory according to many interpretations of the previous studies but also may be on other component factors

of plasma as the spin electrons that initiate resonance directly interfere with DNA as discussed by Kang *et al.*^[29]

Furthermore, CAPPJ is characterized by its safety effects and amelioration of genetic damages in normal cells with the enhancement of apoptosis pathways and immune activation, especially for doses (40 & 60 sec).^[30,31]

Table 3, explained that the cytotoxicity of plasma exposure is directly proportional to dose value. Cell viability was recorded (100%, 82%, 64%, 51%, and finally 10%)for doses (0, 20, 40, 60, and 120 Sec) respectively. These trends are accompanied by a gradually significant increase in P53 protein expression levels in cell line culture with the ascending values of CAPPJ exposure doses. On the contrary, Bcl2 recorded reducing of its values with the increase of CAPPJ exposure doses.

In **Table 4** the data discussed that the effects of CAPPJ exposure on normal blood cultures were in the same pattern as in (**Table 3**) for P53. But, the protein expression of Bcl2 displayed a significant decrease in its values when compared with the control group, the most decreased value was at 40 Sec which gradually increased at 60 and 120 Sec but still recorded the lowest value when compared with the control and 20 Sec. The same model of results occurred for protein expression levels of both Bcl2 and P53 genes for normal blood cells cultivated in irradiated cultures (**Table 4**).

Table 4. Protein expression levels of P53 and Bcl2 genes in CAPPJ exposure normal whole blood (Direct and Indirect exposure).

| Groups | Control | BL-D ₁ | CL-D ₁ | BL-D ₂ | CL-D ₂ | BL-D ₃ | CL-D ₃ | BL-D ₄ | CL-D ₄ |
|----------------|------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Bcl2 | 97.85 | a | a | ab | ab | abc | abc | abcd | abcd |
| | ± 0.39 | ± 0.31 | ± 0.46 | ± 0.42 | ± 1.46 | ± 0.25 | ± 0.76 | ± 0.71 | ± 0.53 |
| M wt. | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.67 | 30.00 | 31.42 | 30.0 |
| P53 | 66.92 | 70.08 | 67.48 | 76.79 | 85.16 | ab | abc | ab | abcd |
| | ± 1.29 | ± 0.82 | ± 0.97 | ± 1.12 | ± 1.0 | 77.92 \pm 1.16 | ± 1.54 | ± 1.23 | ± 0.42 |
| M wt. | 52.63 | 52.66 | 52.64 | 52.64 | 52.40 | 52.62 | 53.17 | 52.64 | 52.10 |
| B-actin | 100 | 100 | 99 | 99 | 99 | 98 | 98 | 98 | 98 |

Legends as in **Tables 1 and 2**

Synergistic antiproliferative and proapoptotic effect of cancer therapies may be the goal for the future research.^[32] So, in normal cells, the P53 protein recorded a low level. The production of the P53 protein, which plays three key functions in cellular growth capture, DNA repair, and apoptosis, may be triggered by DNA damage and extra load signals. The development arrest halts the cell cycle's progression and prevents the replication of damaged DNA.^[33]

The overview of the obtained results impresses that (**Figures 2a and 2b**) showed that in blood there are gradually increasing in IL-1 β , IL-6, IL-10, and TNF- α levels through the increasing of the dose exposure of CAPPJ except doses 60 and 120 sec for IL-10 and TNF- α for 120 sec only. (**Figure 3**) displayed inhibition of the investigated cytokines observed in the

irradiated cultures before the cultivation of the normal blood. Linard *et al.*^[34] mentioned that they are functionally grouped into pro-inflammatory cytokines mainly (IL-1, IL-6, IL-8, and TNF- α) and anti-inflammatory cytokines especially (IL-4, IL-10, IL-1 receptor antagonist (IL-1ra), and (TGF- β).

All previous data agreed with many researches and literature which confirmed that CAPPJ exposure can treat some types of cancer and other diseases whether through molecular mechanisms of action, cell cycle regulation, activation of cell signaling pathways^[35] and/or the effect on stromal and immune cells.^[36,37]

Consequently, what is the discussion that describes the ability of plasma especially CAPPJ, in killing cancer cells with high

selectivity? Moreover, what are the characteristic natures of both plasma and DNA, and what is the (cross-talk) between them?

Meyl^[38] described the DNA waves (longitudinal) and calculated their value as it propagates in the direction of the magnetic field vector. The existence of low-frequency electromagnetic waves that may affect the physiology of living things was proven by Montagnier *et al.*^[39] Additionally, they discovered in their lab for the first time that electromagnetic waves may replicate DNA in living cells. This new biology, which is best described as "digital biology".

Furthermore, projected biophoton radiation frequencies and those theoretically estimated from the DNA structure are in agreement with the discovery of magnetic monopoles in 2009 established the fundamental methodology for an expanded field theory. When the DNA wave is examined closely, a collection of waves and radiation can be seen. The underlying idea is that resonance must first build up, which requires a field. Therefore, the discharge of a dispersed field initiates any communication between cells.^[40, 41]

Contrary to technological devices, biological systems employ an "autofocus" function, or, to put it another way, cells exhibit a propensity to enter resonance with one another in the presence of scalar waves.^[39]

Therefore, DNA resonance, rather than chemicals, is primarily used to govern cell-to-cell communication. Cell-to-cell communication is described as "the music that the nucleus hears" and is crucial for morphogenesis, cell differentiation, homeostasis, cell growth, and cell-to-cell interaction.^[42] When aberrant and out of tune, aberrant cell-to-cell communications may be harmful to the organism's health.^[43]

Furthermore, the repetitions in DNA are a crucial component of its resonance function. What do the many repeats in the human genome represent? For instance, the (Alu repeat) has the most copies in the human genome. Alu is over 300 bp long, and there are about one million copies of it in our genome.^[44]

The genomic DNA of cancer cells exhibits the terahertz (THz) region molecular resonance fingerprints of DNA methylation, as an epigenetic component. In addition, they identified the type of cancer cell lines and differentiated between normal and cancer DNA by measuring the THz resonances' amplitudes (a relatively new diagnostic method).^[45]

However, since 1956 there have been numerous competing ideas put forth to explain the plasma resonance phenomenon. An expression for the rate of change of electron density in gas plasma is obtained from the Boltzmann transport equation, according to Wolff.^[46]

The magnetic monopoles were discovered in a cold electron plasma (Maxwell's equations), and the frequency of the magnetic monopole plasma has an impact on the dispersion

relation and, consequently, the propagation of electromagnetic waves.^[47, 48]

Conclusion

In conclusion, our presented results revealed that direct CAPPJ-exposure is biologically improving benefits when compared with cells cultivated in CAPPJ-exposed media. Therefore, CAPPJ effects are not restricted to free radicals and their contents only, but there is another efficient factor, which is plasma and DNA interaction. The lower doses ameliorate the genetic damages and enhance the immunological responses and induce division and proliferation.^[49] Whereas, the higher doses induce apoptosis for normal cells and arrest of cell cycle and necrosis for cancerous cells (selectivity). This leads us to postulate that "It is very important to take into our consideration other factors in dealing with cancer concept and its treatment or even its diagnosis". The physical nature of DNA may regulate its expression without changing its sequences (epigenetic factors) as its resonance and the ability of plasma to interact with it via its unique components. Chemical and physical pollution, pathogens (viral, bacterial ...etc) invasion, environmental circumstances, psychological stress, and many other phenomena and factors are brightly affected in disturbing the physical nature of DNA then disrupting its energy leads to different diseases and cancer. Finally, it is very important to precede more research on this trend in light of the new physical theories and instrumentations.

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Conflict of interest

None.

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Ethics statement

The research is approved by the Research Ethics Committee of the National Centre of Radiation Research and Technology (REC-NCRRT) with No. 1H/20.

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