Asian Journal of Current Research in Clinical Cancer
ISSN: 3062-4444
2022, Volume 2, Issue 1, Page No: 34-46
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Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Alternative Perspective on Cancer Based on the Interaction between Plasma Radiation and DNA

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Received: 27 February 2022; **Revised:** 23 May 2022; **Accepted:** 27 May 2022

ABSTRACT

Numerous studies, including our previous research, have investigated the effects of different plasma sources and their respective doses on both healthy and cancerous cells. These studies aimed to interpret the potential mechanisms behind cancer treatment involving plasma and its interactions with cells. Plasma has been shown to play an important role in the outcomes of cancer cell treatment through several investigations, including genetic, biochemical, and immunological analysis. This body of work has led to a variety of theories, suggesting possible new strategies for cancer treatment. The present study used a cold atmospheric pressure plasma jet (CAPPJ) generated by a Tesla coil in a dielectric barrier discharge system. The samples were divided into three groups: the first group consisted of cancer cell lines, the second group included normal blood samples, and the third group consisted of blood cells cultivated in CAPPJ-exposed environments without direct exposure. The research focused on variables including the cytokinesis blocked micronucleus test (CBMN), protein expression levels of P53 and Bcl2 genes, interleukins (IL-1β, IL-6, IL-10), and tumor necrosis factor (TNF-α). The findings showed that direct exposure to CAPPJ had a more significant effect on cell viability and protein expression levels compared to cells grown in CAPPJ-exposed cultures. In particular, the expressions of Bcl2 and P53 protein were significantly altered in CAPPJ-irradiated breast cancer cell lines (BCC). These results suggest that CAPPJ affects cellular behavior not only through the generation of free radicals and modulation of key signaling pathways but also by interacting directly with DNA.

Keywords: Apoptosis, CAPPJ, Cytome assayP53, Epigenetic, Bcl2

How to Cite This Article: Ahmed MM, Montaser SA, Elhadary A, Elaragi GGM. Alternative Perspective on Cancer Based on the Interaction between Plasma Radiation and DNA. Asian J Curr Res Clin Cancer. 2022;2(1):34-46. https://doi.org/10.51847/gqTzBQfMB1

Introduction

The World Health Organization (WHO) characterizes cancer as a broad range of diseases that can affect nearly every system in the body. It occurs when abnormal cells proliferate uncontrollably, bypass their normal boundaries to invade neighboring tissues, and spread to other organs, a process known as metastasis, which is a leading cause of cancer-related death [1].

Another perspective emphasizes that cancer is a complex, heterogeneous set of diseases that resist a simple definition. It is estimated that between 5% and 10% of cancers are due to mutations, and up to 15% result from inflammation. The remaining 80% are considered “sporadic” cases, with unclear causes. Even mutations are often late-stage events or epiphenomena in a complex sequence of factors leading to cancer development [2].

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Cancer commonly arises from chromosomal instability. The cytokinesis-blocked micronucleus (CBMN) cytome assay, developed by Fenech [3], is a multi-endpoint method for assessing various nuclear anomalies indicative of chromosomal instability. These include structural and numerical chromosomal abnormalities, chromosome mis-segregation during mitosis, micronuclei (MNi), anaphase bridges (NPBs), and nucleoplasmic buds (NBUDs) caused by unresolved DNA complexes.

Plasma medicine, an interdisciplinary field combining plasma physics, chemistry, biology, and clinical medicine, is emerging as a promising cancer treatment modality. Plasma application to cells generates numerous reactive species that interact with cellular structures, influencing cellular redox signaling. This leads to changes in surface receptor activity, cell cycle arrest, DNA damage-induced activation of p53, and subsequent p53-dependent apoptosis, among other effects on cells [4].

One of the main goals of cancer treatment is to promote the death of cancerous cells while minimizing harm to healthy ones (selectivity) [5]. The dysfunction of tumor-suppressor genes or the overexpression of anti-apoptotic proteins are key mechanisms in cancer progression [6]. P53, often referred to as "the guardian of the genome," plays a pivotal role in this context [7]. Additionally, the Bcl2 family of proteins is crucial in regulating apoptosis [8]. Impaired apoptosis is a hallmark of cancer [9].

The communication between the body and mind occurs through a bidirectional flow of neuropeptides, cytokines, and hormones, all of which regulate various physiological processes [10]. Cytokines are key mediators in this communication, particularly concerning inflammation and infection [11]. They are critical to maintaining homeostasis, especially during immune challenges [12].

It has been suggested that DNA damage responses can trigger senescence, particularly after inflammatory mediators release a variety of cytokines, including IL-1, IL-6, IL-8, and TGF-β [13]. Pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, as well as the anti-inflammatory IL-10 family, play important roles in regulating cellular activity [14].

The efficacy of cold atmospheric pressure plasma jets (CAPPJ) has been evaluated in various medical conditions, including osteosarcoma, glioblastoma, melanoma, pancreatic, ovarian, breast, and cervical cancers. The primary mechanism underlying plasma's effectiveness is believed to be the release of energy through radiation and/or chemical interactions [15].

This study aims to explore how plasma affects cells, particularly through oxidative stress, alterations in redox state pathways, and potential direct impacts on DNA (epigenetically). It further seeks to understand the interaction between plasma and DNA and its implications for redefining cancer and improving cancer treatment strategies.

Materials and Methods

Experimental design

This study aimed to assess both the direct and indirect effects of cold atmospheric pressure plasma jet (CAPPJ) on normal blood cells and breast cancer cells. It sought to identify optimal, non-harmful CAPPJ exposure levels for both cell types and to explore how genetic damage correlates with the activation of immunological cytokines.

Plasma jet characteristics from the tesla coil

The properties of a cold plasma jet are essential for its biological efficacy, as emphasized by Elaragi [16]. In this study, a plasma jet was generated using a Tesla coil. The Tesla coil operates by generating a resonant circuit, with an oscillating frequency typically between 20 and 100 kHz, which is influenced by both the capacitor's capacitance and the coil's inductance. The experimental setup includes the plasma jet generator, a gas flow system, and a high-frequency AC power source, as displayed in **Figure 1**.

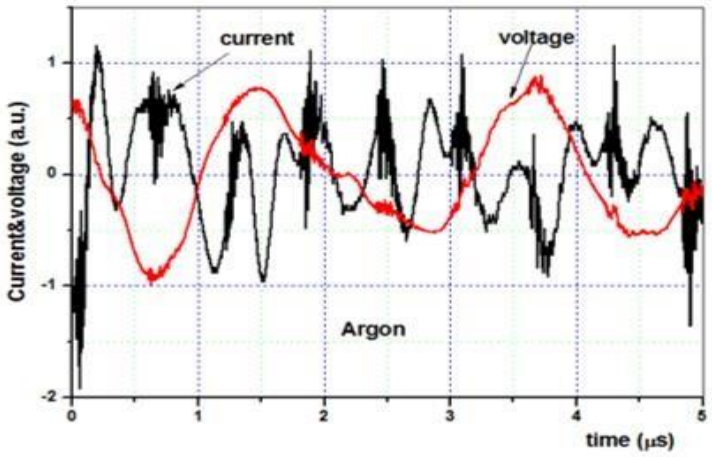


Figure 1. Typical voltage-current waveforms of argon plasma jet

Chemicals

The blood culture reagents were sourced from Gibco (Carlsbad, CA, USA), with heat-inactivated fetal calf serum (FCS) obtained from Sigma-Aldrich (St. Louis, MO, USA).

To measure IL-1β, a human (IL-1β) ELISA kit was acquired from CUSABIO (Catalog Number: CSB-E08053h). For IL-6, a human (IL-6) ELISA kit from RayBio (Catalog Number: ELH-IL6-001) was used. The IL-10 human (IL-10) ELISA kit was also from CUSABIO. TNF-α was quantified using the human TNF-α ELISA kit from ALPCO (Catalog Number: 45-TNFHU-E01). Additionally, an anti-P53 primary antibody (Abcam, ab131442) and an anti-Bcl2 primary antibody (Abcam, ab131442) were employed for protein detection.

Subjects and blood sampling

Subjects

Human blood samples: To minimize inter-individual variability in treatment outcomes, blood samples were gathered from eight healthy participants (4 males, and 4 females). The individuals were selected to ensure similar age, smoking habits, and environmental exposures.

Breast cancer cell line: The cell line was sourced from the Genetic Engineering Unit at the National Research Centre, Egypt.

All blood samples were collected using sterile techniques and transferred into heparinized vacutainer tubes (five ml; Becton Dickinson, USA).

Exposure doses

Blood samples from healthy individuals were categorized into two groups. The first group was exposed directly to CAPPJ for varying durations (20 seconds (Bl-D1), 40 seconds (Bl-D2), 60 seconds (Bl-D3), and 120 seconds (Bl-D4)) at a distance of three cm from the surface of the blood. The second group served as the unexposed control. For the second exposure type, normal blood was cultured in media previously treated with CAPPJ, receiving the same four exposure durations (20 seconds (Cl-D1), 40 seconds (Cl-D2), 60 seconds (Cl-D3), and 120 seconds (Cl-D4)). The identical exposure durations were administered directly after the breast cancer cell line (BCC) was established.

Blood culture

Before proceeding with culture and immune assays, blood samples were incubated at 37 °C for 20 hours. Each sample was cultured in triplicate for 72 hours, following the procedure described by Evans and O'Riordan [17].

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

The cytokinesis-block micronucleus cytome assay was conducted as outlined by Fenech [3, 18]. This assay evaluated various aspects of mitotic activity (such as mono-, bi-, tri-, and quadrinucleated cells), cytotoxicity (measured through MNi frequencies, NPBs, and nuclear buds), as well as necrotic and apoptotic cell markers. The

enzyme-linked immunosorbent assay (ELISA) was used to quantify human IL-1β, IL-6, IL-10, and TNF-α levels in both plasma and cell culture supernatants. P53 and Bcl2 gene expressions were analyzed by protein electrophoresis on SDS-PAGE, following the protocol by Horiuchi *et al*. [19].

Statistical analysis

The statistical analysis was performed using SPSS software (version 20 for Windows), with one-way ANOVA and Tukey's multiple comparison tests applied. A P-value of 0.05 was considered statistically significant [20].

Results and Discussion

The cytogenetic data related to the direct influence of CAPPJ on normal whole blood is presented in **Table 1**, while **Table 2** outlines the indirect effects of CAPPJ on 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	Bl-D1	Bl-D2	Bl-D3	Bl-D4
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
Apoptic 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 Bl-D1 gp, c: significant when compared with Bl-D2 gp, and d: significant when compared with Bl-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	Cl-D1	Cl-D2	Cl-D3	Cl-D4
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
Apoptic 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 Cl-D1 gp, c: significant when compared with Cl-D2 gp, and d: significant when compared with C-D3 gp.

The data regarding the effects of CAPPJ exposure at different doses (control, Bl-D1, Bl-D2, Bl-D3, and Bl-D4) are displayed in **Table 1**, while **Table 2** presents the results for normal human blood cultured in media treated with CAPPJ at the same doses (Cl-D1, Cl-D2, Cl-D3, and Cl-D4).

In **Table 1**, a notable reduction in genetic damage was observed in the Bl-D2 and Bl-D3 groups, as evidenced by a decrease in mono + 1 MNi, bi + 1 MNi, and nuclear buds compared to the control and Bl-D1 groups. In contrast, the Bl-D4 group exhibited a significantly higher frequency of MNi in mono- and binucleated cells.

Table 1 also showed a clear increase in apoptotic cell counts from the Bl-D1 to Bl-D4 groups, which was statistically significant when compared to the control group. No significant differences were found in the counts of mono + 2 MNi, bi + 2 MNi, bi + 3 MNi, trinucleated, quadrinucleated cells, or nucleoplasmic bridges across all groups. The Bl-D4 group displayed a notable rise in necrotic cells compared to other groups. Significant differences were observed in the mononucleated and binucleated cell counts between Bl-D2, Bl-D3, and Bl-D4.

On the other hand, **Table 2** showed opposite trends, with high frequencies of MNi observed in mono- and binucleated cells, as well as nuclear buds. Additionally, necrotic and apoptotic cells were significantly higher, particularly in the Cl-D4 group, compared to the control and Cl-D1 groups. Notable differences in trinucleated and quadrinucleated cell counts were recorded between Cl-D3, Cl-D4, and the control group.

Table 3 highlights the viability percentages of the BCC cells and the expression of Bcl2 and P53 proteins. The BCC-Control group served as the baseline, with 100% cell viability. Cell viability progressively decreased in the BCC-D1 to BCC-D4 groups (82 percent, 64 percent, 51 percent, and 10 percent, respectively). While Bcl2 expression decreased, P53 gene expression exhibited an upward trend.

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-D1**	**BCC-D2**	**BCC-D3**	**BCC-D4**
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

Table 4 highlights that the Bl-D2 group exhibited the strongest effect on Bcl2 gene expression, with notable differences observed across all exposed groups when compared to the control. On the other hand, a significant increase in P53 gene expression was found in the Bl-D2, Bl-D3, and Bl-D4 groups relative to both the control and Bl-D1 groups.

Regarding protein expression, Bcl2 showed a consistent decline from the Cl-D1 to Cl-D4 groups in comparison to the control, whereas P53 protein expression increased steadily in these groups compared to the control.

In **Figure 2a**, the Bl-D1 group did not show a significant change in IL-1β and TNF-α levels compared to the control group, although significant increases were observed in IL-6 and IL-10 levels. For the Bl-D2, Bl-D3, and Bl-D4 groups, there was a notable and consistent rise in IL-1β and IL-6 concentrations when compared to both the control and Bl-D1 groups. IL-10 levels were higher in Bl-D2 but decreased in Bl-D3 and Bl-D4. TNF-α levels were elevated in Bl-D2 and Bl-D3 but reduced significantly in Bl-D4. A similar pattern was observed in **Figure 2b**, except for IL-1β values.

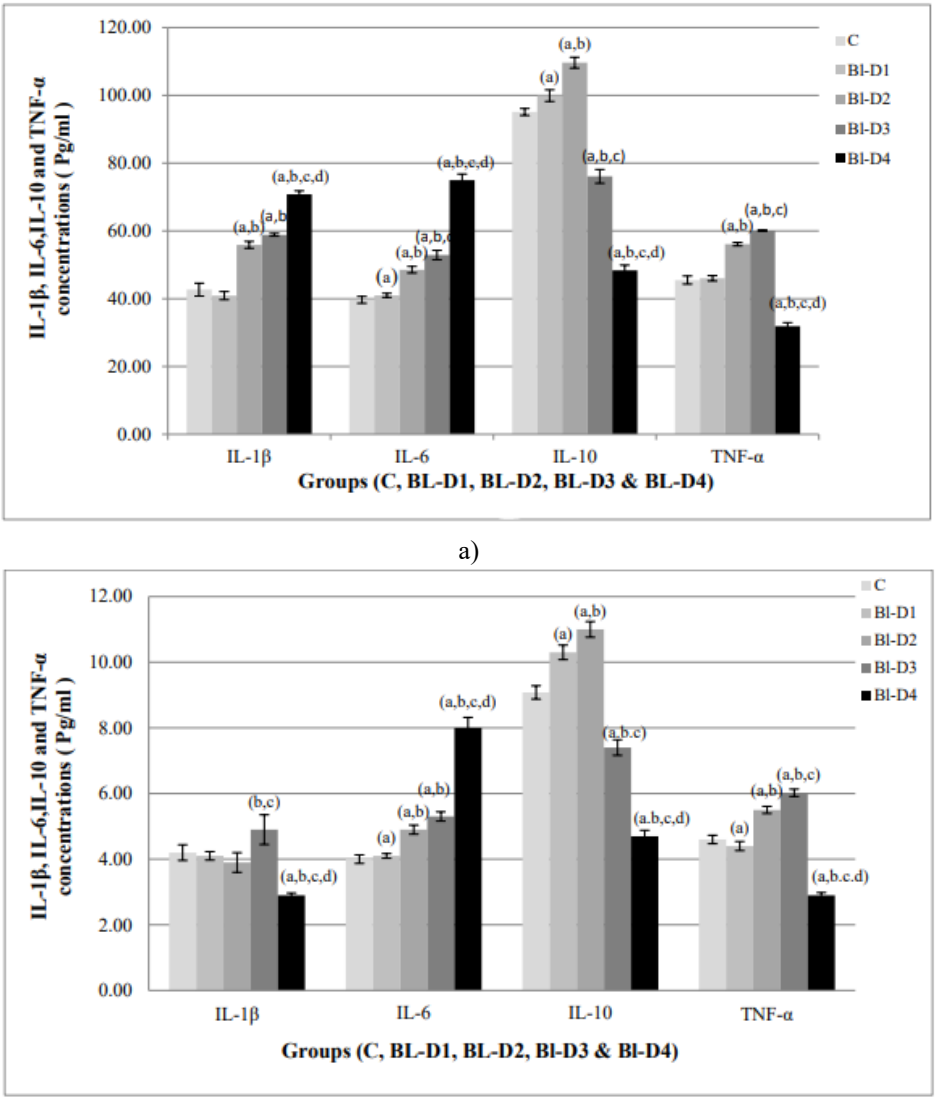


Figure 2. IL-1β, IL-6, IL-10, and TNF-α levels: (a) in serum of CAPPJ irradiated and non-irradiated normal whole blood groups, and (b) in cultures of CAPPJ irradiated and non-irradiated normal whole blood groups.

Figure 3 displays the results for the culture media that was irradiated before cultivation, revealing that the concentrations of IL-1β, IL-6, IL-10, and TNF-α showed an opposite trend to those presented in **Figures 2a and 2b**, with a progressive decline observed across the four dosage groups. Additionally, **Figure 4** provides photomicrographs illustrating the different cell types observed in the cytome assay.

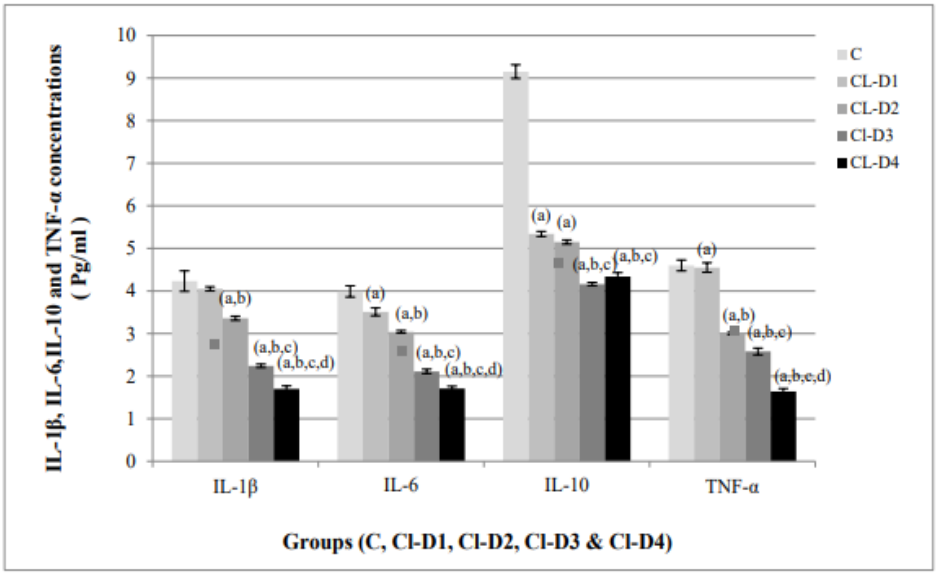
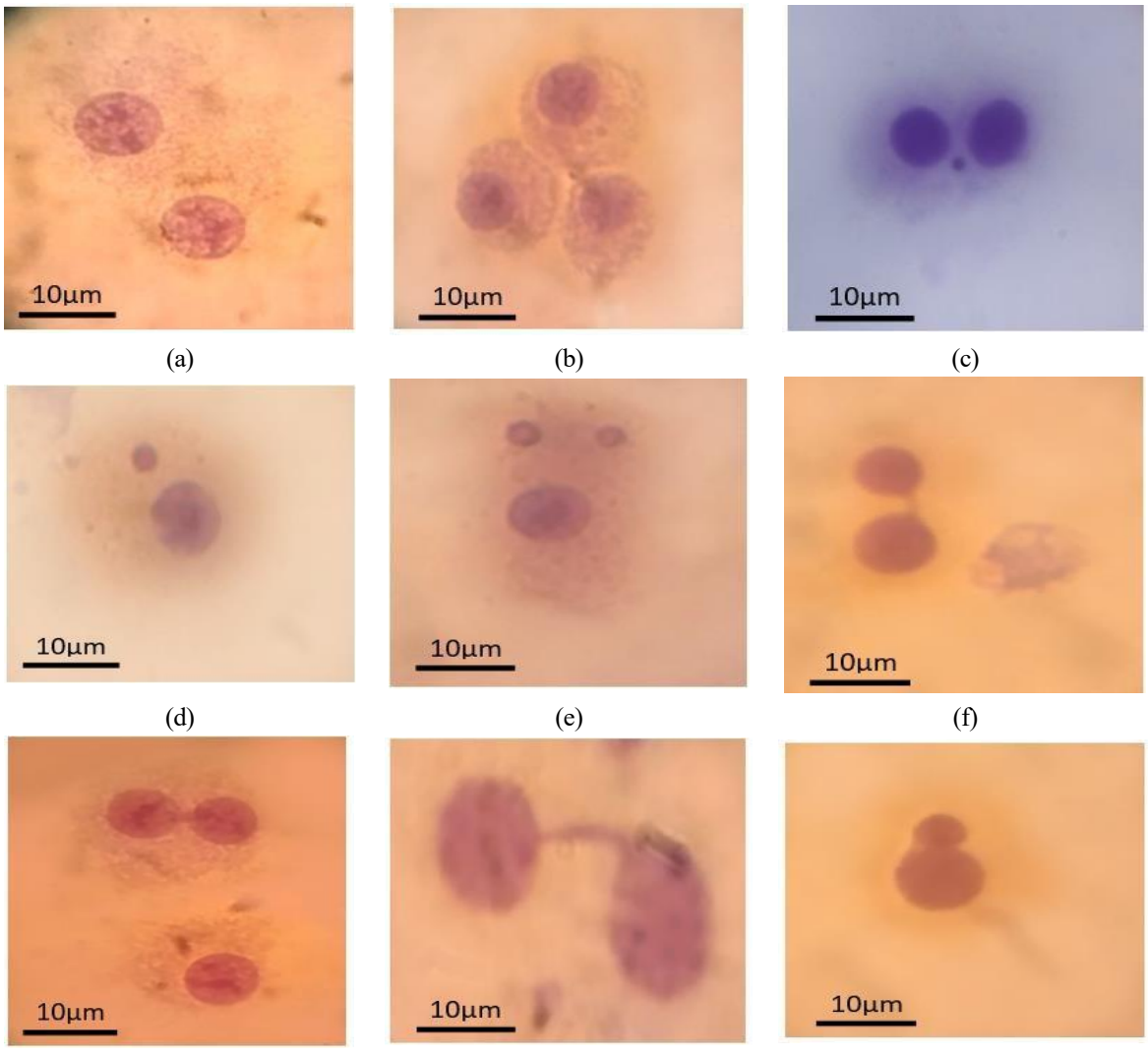


Figure 3. IL-1β, IL-6, IL-10, and TNF-α levels of normal whole blood groups cultivated in CAPPJ irradiated and non-irradiated cultures.



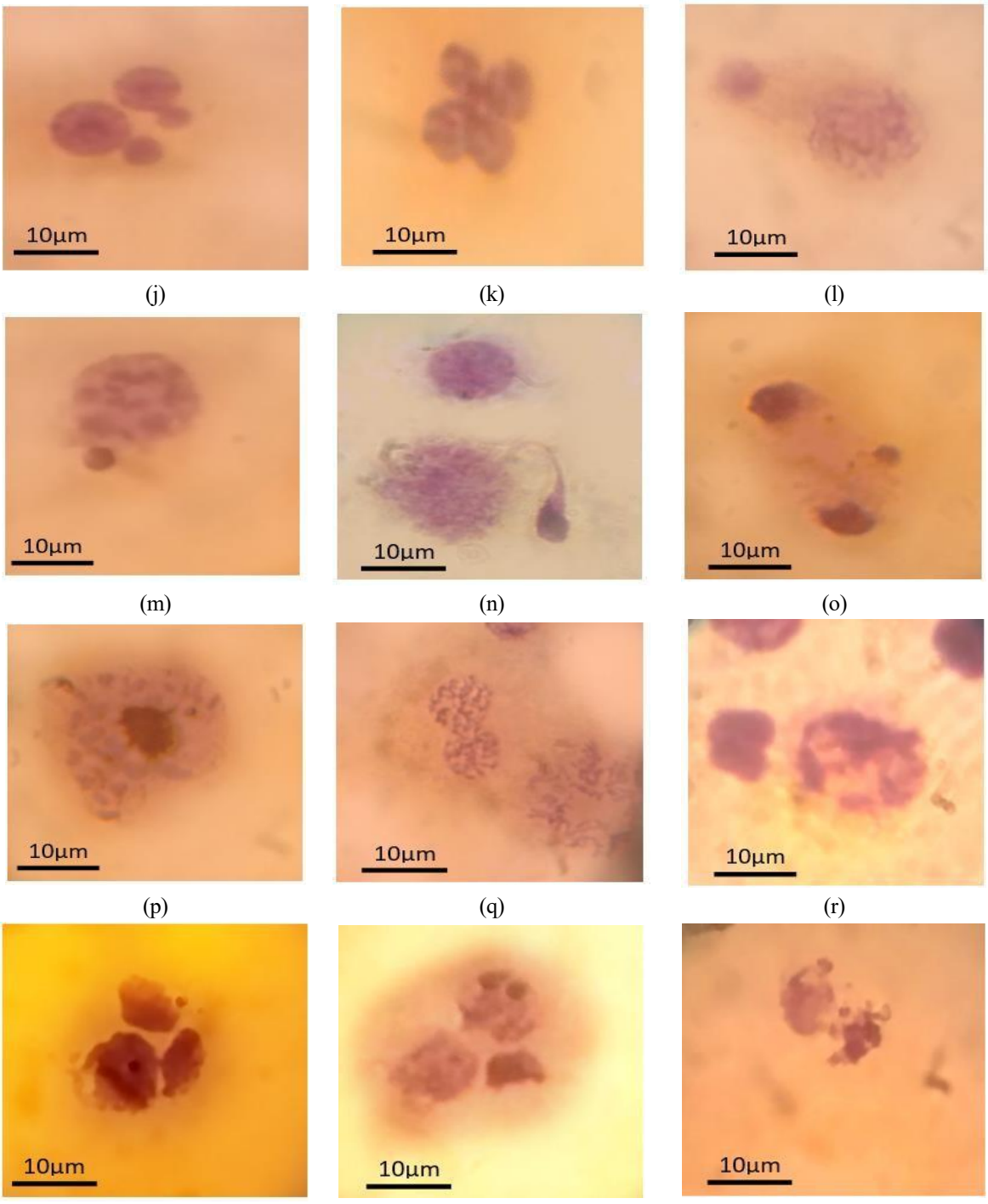


Figure 4. Photomicrographs of the cells scored in the CBMN ‘‘cytome’’ assay: a) normal binucleated cell, b) normal mononucleated cells, c) binucleated cell with one MN, d) mononucleated cell with one MN, e) mononucleated cell with two MNi, f, g, and h) binucleated cells with NPBs, i and j) mononucleated 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) apoptic cells (scale bar, 1.25 cm: 10 µm).

In 2022, Muresanu and Khalchitsky [21] characterized cancer as a complex, multistage, and metabolic disease after conducting an integrative review across multiple sources to clarify its nature. They focused particularly on telomere alterations as indicators of genetic and environmental factors interacting with one another. The authors also highlighted the role of lifestyle, nutrition, and physical agents as potential predisposers, in addition to genetic and epigenetic influences. The ideal cancer treatment strategy should be one that selectively targets and eliminates

tumor cells, sparing healthy cells. However, many existing anti-cancer drugs work by inducing oxidative stress in cancer cells, which is believed to contribute to extensive cellular damage and macromolecular alterations [22-25]. Research has demonstrated that various forms of plasma, such as CAPPJ and gliding arc plasma, can aid in tissue regeneration, disease management, and even treat drug-resistant tumors [26, 27]. These studies showed that the most notable improvements in mitigating chromosomal instability came from stimulating multiple mitotic checkpoints, followed by apoptosis and necrosis, the primary mechanisms through which plasma exerts its most effective actions. Additionally, plasma exposure was found to enhance immune responses (interleukins and TNF-α) and induce the expression of anti-tumor genes such as P53, caspase-3, and Bcl2 [28].

The findings in **Table 1** showed a reduction in genetic damage in most groups exposed to plasma, with significant differences observed in the frequency of mono- and binucleated cells, potentially due to plasma's influence on the mitotic division index.

Conversely, **Table 2** illustrated a contrasting pattern of cytogenetic data, revealing substantial genetic damage with higher frequencies of MNi in mono- and binucleated cells and NBUDs. Additionally, a considerable increase in necrotic cells was observed with rising exposure doses.

Comparing the results from **Tables 1 and 2**, it was evident that direct plasma exposure to blood cells improved cytogenetic outcomes, while exposure to plasma-treated culture media before blood cell cultivation resulted in damaging effects. This suggests that the plasma's action may not solely depend on the free radical theory but could also involve other plasma components, such as spin electrons, which may directly interact with DNA, as proposed by Kang *et al*. [29].

Furthermore, CAPPJ has been found to enhance the repair of genetic damage in normal cells, promoting apoptosis and activating immune responses, particularly at 40 and 60-second exposure times [30, 31].

In **Table 3**, plasma exposure's cytotoxicity was shown to correlate with dose, as evidenced by the progressive reduction in cell viability with exposure times of 0, 20, 40, 60, and 120 seconds, yielding 100%, 82%, 64%, 51%, and 10% viability, respectively. Alongside these trends, P53 protein expression increased significantly with higher plasma exposure doses, while Bcl2 protein levels showed a decrease.

In **Table 4**, the effects on normal blood cultures were similar to those in **Table 3** regarding P53 expression. However, a marked decrease in Bcl2 expression was observed when compared with the control group, with the most significant drop occurring at the 40-second exposure, followed by a gradual increase at 60 and 120 seconds, though still lower than the control and 20-second groups. Similar trends in protein expression of Bcl2 and P53 were observed in blood cells cultured in plasma-treated media **(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-D1	CL-D1	BL-D2	CL-D2	BL-D3	CL-D3	BL-D4	CL-D4
		a	a	ab	ab	abc	abc	abcd	abcd
Bcl2	97.85± 0.39	94.17± 0.31	95.16± 0.46	54.74± 0.42	65.31± 1.46	64.88± 0.25	68.47± 0.76	87.48± 0.71	42.76± 0.53
M wt.	30.0	30.0	30.0	30.0	30.0	30.67	30.00	31.42	30.0
				ab	ab	ab	abc	ab	abcd
P53	66.92± 1.29	70.08± 0.82	67.48± 0.97	76.79± 1.12	85.16± 1.0	77.92 ± 1.16	92.05± 1.54	79.36± 1.23	97.46± 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

a: significant when compared with control gp, b: significant when compared with Cl-D1 gp, c: significant when compared with Cl-D2 gp, and d: significant when compared with C-D3 gp.

Future research in cancer therapies is likely to focus on enhancing the synergistic effects that both inhibit cell proliferation and promote apoptosis [32]. In normal cells, the P53 protein is present at low levels, but it can be induced by signals such as DNA damage or cellular stress. This protein plays an essential role in regulating DNA repair, controlling cell growth, and initiating apoptosis. It is primarily involved in halting the cell cycle to prevent the replication of damaged DNA [33].

The results summarized in **Figures 2a and 2b** demonstrate that with increasing doses of CAPPJ exposure, there is a noticeable rise in the levels of cytokines IL-1β, IL-6, IL-10, and TNF-α, except for IL-10 and TNF-α at the

60 and 120-second doses, where changes were less evident. **Figure 3** highlights that in cultures exposed to CAPPJ before blood cell cultivation, these cytokine levels were suppressed. Linard *et al*. [34] categorized cytokines into pro-inflammatory types, like IL-1, IL-6, IL-8, and TNF-α, and anti-inflammatory ones, including IL-4, IL-10, IL-1ra, and TGF-β.

This data aligns with several studies suggesting that CAPPJ exposure has therapeutic potential in treating various cancers and diseases. This effect is thought to involve molecular actions, regulation of the cell cycle, activation of cell signaling pathways, and interactions with both stromal and immune cells [35-37].

A key topic is the ability of CAPPJ to selectively target and kill cancer cells. Moreover, the interaction between plasma and DNA raises questions about their respective properties and the potential "cross-talk" between them.

Meyl [38] explored longitudinal DNA waves and analyzed how they propagate in alignment with magnetic field vectors. The concept of low-frequency electromagnetic waves influencing biological systems was first proven by Montagnier *et al*. [39], who discovered that these waves could replicate DNA within living cells, opening a new field known as "digital biology."

Furthermore, biophoton radiation frequencies, as well as those predicted from DNA structures, align with findings about magnetic monopoles, establishing a framework for an expanded field theory. A closer examination of DNA waves reveals a combination of radiation and waves, with resonance requiring a field to form. Once this field is established, it enables cellular communication [40, 41].

Biological systems differ from technological devices by using an "autofocus" mechanism, meaning that cells naturally align and synchronize with each other in the presence of scalar waves [39].

Thus, DNA resonance, rather than chemical signals, predominantly controls the communication between cells. This cell-to-cell interaction, often referred to as "the music that the nucleus hears," plays a pivotal role in processes like morphogenesis, cell differentiation, homeostasis, growth, and the overall interaction between cells [42]. When these communications become irregular, it can negatively affect the health of the organism [43].

Additionally, the repetitive sequences in DNA are vital to its resonance capabilities. For example, the Alu repeat, which is over 300 base pairs in length, is the most abundant in the human genome, with approximately one million copies [44].

The DNA of cancer cells also displays unique molecular resonance patterns in the terahertz (THz) range, which can be linked to DNA methylation, an epigenetic modification. This method has been utilized to distinguish between normal and cancerous DNA by analyzing the amplitude of THz resonances [45].

Since 1956, various theories have been proposed to explain the phenomenon of plasma resonance. Wolff's research, based on the Boltzmann transport equation, provided an expression for the rate of change in electron density within gas plasma [46].

Magnetic monopoles, discovered within cold electron plasma, exhibit a frequency that influences the dispersion relation and, consequently, the propagation of electromagnetic waves, as described by Maxwell's equations [47, 48].

Conclusion

To summarize, our findings demonstrate that direct exposure to CAPPJ offers significant biological benefits compared to cells cultured in CAPPJ-exposed media. This indicates that the effects of CAPPJ extend beyond the role of free radicals, involving an interaction between plasma and DNA. Lower doses of CAPPJ enhance genetic repair, boost immune responses, and promote cell division and growth [49]. In contrast, higher doses lead to apoptosis in normal cells, while causing cell cycle arrest and necrosis in cancer cells, indicating selectivity. This suggests that "other factors should be considered when addressing the concept of cancer, its treatment, and diagnosis." The physical characteristics of DNA could potentially influence its expression without altering its sequence (through epigenetic modifications), mediated by the resonance of DNA and its interaction with plasma's unique components. Environmental stressors such as chemical pollution, pathogens (including viral and bacterial infections), environmental conditions, and psychological stress all significantly impact the physical state of DNA, potentially leading to disease and cancer. Moving forward, it is essential to conduct more research in this area, guided by emerging physical theories and advanced technologies.

Acknowledgments: The authors wish to express their sincere gratitude to Dr. Mahmoud R. Morsy, Consultant of Internal Medicine at Military Production Hospital, for his guidance on blood sampling and his valuable medical counsel.

Conflict of Interest: None

Financial Support: None

Ethics Statement: The study was approved by the Research Ethics Committee of the National Centre of Radiation Research and Technology (REC-NCRRT), with approval number 1H/20.

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