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Cytogenetic and Biochemical Investigations of Cultured Leukemia Cells Exposed to Gliding Arc Discharges

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ABSTRACT: One of the most important sources of plasma radiation, gliding arc discharge (GAD), has the potential to combine advantages of both thermal and nonthermal plasmas with destructive physical and biochemical properties. Thus, GAD may be effective when applied to the local microenvironment of leukemia cells, especially in the extracellular matrix (ECM). Blood samples from chronic myelogenous leukemia (CML) patients were studied and compared with matched control samples. Triple blood cultures for each case were exposed to GAD for different time periods (20, 40, and 60 s). Cytogenetic and biochemical parameters were investigated before and after irradiation. We observed significant consequences in ECM structure and cytome assay parameters, such as increased frequencies of dispersal cells, micronuclei, and necrotic and apoptotic cells. In addition, results showed improved values of vascular endothelial growth factor and interleukin-10 and decreased levels of collagen IV. The most effective exposure period was 40 s, which presented highly significant results. This type of energy can stop the progress and development of CML via lyses, cell spreading, and eradication of ECM, especially with the use of GAD for a period of 40 s.

KEY WORDS: gliding arc discharge, cytome assay, cytokine, collagen IV, VEGF

I. INTRODUCTION

Cold atmospheric plasma radiation is gaining extensive interest as a promising addition to antitumor therapy, primarily due to its ability to generate and control delivery of electrons, ions, excited molecules, ultraviolet (UV) photons, and reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) to a specific site. The heterogeneous composition of cold atmospheric plasma offers the opportunity to mediate several signaling pathways that regulate tumor cells. Consequently, the array of cold atmospheric plasma–generated products has limited the identification of the mechanisms of action on tumor cells.[1] Gliding arc discharge (GAD) plasma has demonstrated the advantages of combining both thermal and nonthermal plasmas for certain applications in optimized regimes. The device used for GAD plasma can be used in future for vital and important applications including sterilization and gas cleaning and biomedical applications such as plasma healing, blood coagulation, and cancer treatment.[2]

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The use of argon as the working gas results in strong OH emission, and emission intensities of nitrogen emission bands from the surrounding air are more than three times higher than those of helium.[3] In addition, for argon the Townsend first ionization coefficient is larger and increases more quickly with electric field than that for helium, although much less than that for nitrogen. Nitrogen leads to the presence of negative ions in nitrogen plasma and less electrons, which leads to increases in the discharge voltage of nitrogen in comparison to that of argon plasma. The plasma nonequilibrium is much higher in atomic argon gas than in diatomic nitrogen.

The extracellular matrix (ECM) is comprised of a large collection of biochemically distinct components including proteins, glycoproteins, proteoglycans, and polysaccharides with different physical and biochemical properties.[4,5] The ECM limits the diffusive range, accessibility, and signaling direction of ligands to their cognate receptors.[6,7] Additionally, the ECM can also directly initiate signaling events, particularly by functioning as a precursor of biologically active signaling fragments.[8]

A rapidly growing area in ECM biology is the study of its biomechanical properties, including ECM elasticity that ranges from soft and compliant to stiff and rigid, and how they may contribute to development and disease.[9] Together with the cytoskeleton and nuclear matrices, nuclear envelope, and chromatin, these constitute sophisticated mechanosensing machinery that determine how cells react to forces from the ECM.[10] An abnormal ECM affects cancer progression by promoting transformation and metastasis. Indeed, abnormal ECM dynamics are one of the most ostensible clinical outcomes in diseases such as tissue fibrosis and cancer; however, ECM anomalies also deregulate the behavior of stromal cells that facilitate tumor-associated angiogenesis and inflammation, which leads to tumour progess.[11]

Collagens, the principal proteins of the ECM, are structural proteins that provide tissues with strength and flexibility and serve other essential roles as well. Collagens do not simply provide filler for tissues. Both fibrillar and basal lamina collagens interact with other ECM proteins and play important parts in regulating activities of the cells with which they interact. Cells associate with collagen via cell-surface receptors, and through such interactions, collagens may have a profound impact on cell proliferation, migration, and differentiation.[12]

Recent research has added new strategies for the initiation and maintenance of chronic myelogenous leukemia (CML) through the study of hematopoietic stem cells, leukemic stem cells (LSCs), the ECM, and adhesion properties.[13] From a clinical point of view, LSCs are of fundamental interest because they resist most of our current cancer treatments such as irradiation and chemotherapy and probably more targeted therapies such as tyrosine kinase inhibitors and immunotherapy.[14] In fact, LSCs are the main reason treatment failure and disease relapse.[15]

The clinical staging of CML is divided into three phases: chronic, accelerated, and blast crisis. Once the disease is at blast crisis, patients become insensitive to treatment and chances for survival are reduced.[16] The study of CML pathogenesis can help to impede initiation and development of the disease and lead to discovery of novel thera-

peutic strategies.[17,18] The cytokinesis-block micronucleus (CBMN) cytome assay is a comprehensive system for measuring DNA damage, cytostasis, and cytotoxicity. With CBMN, cytostatic effects are measured via the proportion of mononucleated, binucleated (BN), and multinucleated cells and cytotoxicity via necrotic and/or apoptotic cell ratios.[19] Vascular endothelial growth factor (VEGF) has an important role in initiation and development of CML, so monitoring serum VEGF assists in guiding treatment and predicting a prognosis. VEGF, a protein that is produced by various types of cells including macrophages, keratinocytes, tumor cells, platelets, and mesangial cells in the kidney, promotes the formation of blood and lymphatic capillaries.[20] Interleukin (IL)-10 is an important immunoregulatory cytokine produced by many cell populations. Its main biological functions are thought to be limitation and termination of inflammatory responses and regulation of differentiation and proliferation of several immune cells such as T, B, natural killer, antigen-presenting, and mast cells as well as granulocytes. Numerous investigations, including expression analyses in patients *in vitro* and animal experiments, suggest a major impact of IL-10 on inflammatory, malignant, and autoimmune diseases. IL-10 overexpression was found in certain tumors such as melanomas and several lymphomas and is thought to promote further tumor development.[21] Both VEGF and IL-10 are critical cytokines in the microenvironment of a tumor, with roles in immune suppression.[22]

The present study reviews the effect of GAD exposure on the ECM via the decline of cell division rate and the spread. We also aim to verify the control of ECM components that have an essential role in the progress and treatment of CML.

II. MATERIAL AND METHOD

A. Character of the Exposure Device

EL-Zein et al.[2] designed the GAD experiment to consist of two identical diverging electrodes made of copper with a length of 44 mm, width of 2 mm, thickness of 15 mm, arc (the deviation of the electrode) angle of 120 degrees, and a gap of 1 mm between the two electrodes. The electrodes were connected to an alternating current (AC) power supply (6.6 kV) via resistor (R = 200 kΩ) to avoid high current. The input voltage was controlled with variable autotransformers (Slidacs). The AC power supply was a step-up transformer with a ratio of 1:30, and gas was injected from a narrow tube located in a thin gap between the two electrodes (Fig. 1). The gases used for the experiment were Ar and N_2. A pressure regulator controlled the gas flow rate. Discharge voltage was controlled by the voltage slide regulator and was increased with the use of a high-voltage transformer (ration 1:30). High voltage was then applied to the electrodes, and frequency of the electric discharge was 50 Hz. We measured discharge current using a clamp digital meter (for a digital value) and a Rogowski coil connected to an oscilloscope (to follow the current via oscillogram).

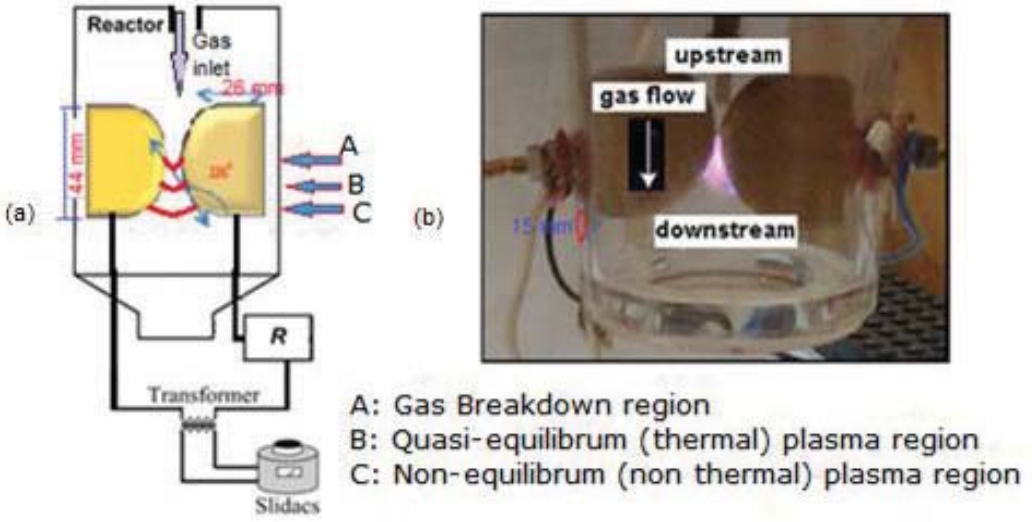

FIG. 1: (a) Schematic of gliding arc and electric scheme showing regions of (A) gas breakdown (B) quasiequilibrium (thermal) plasma, and (C) nonequilibrium (nonthermal) plasma. (b) GAD plasma photo.

B. Chemicals

Chemicals for blood culture were purchased from GibcoBRL and heat-inactivated fetal calf serum (FCS) from Sigma-Aldrich (St. Louis, MI). The collagen IV enzyme-linked immunosorbent assay (ELISA) kit was purchased from ALPCO Diagnostics (Salem, NH), the human VEGF ELISA kit from Eagle Biosciences, Inc. (Nashua, NH), and the IL-10 kit from RayBiotech, Inc. (Norcross, GA).

C. Blood Sampling

Blood samples were obtained from six volunteer patients, whose CML diagnoses were determined by clinical examination and laboratory evaluation. Healthy control subjects were matched with patient groups for ethnicity, gender, age, and nicotine use. All cases were clinically diagnosed with a mean duration of 6 mo during the chronic phase. The mean white blood cell count in CML patients was 280,000 cells/mm^3 and the mean value of BCR-ABL was 3.84 before sampling. All subjects gave informed consent for participation in this study. The donors were selected according to current International Program on Chemical Safety guidelines for the monitoring genotoxic effects of carcinogens in humans.[23] Venous blood was collected under sterile conditions in heparinized vacationer tubes (v = 5 mL; Becton Dickinson, Franklin Lakes, NJ) containing the coagulant lithium heparin.

D. Experimental Design

Blood samples were divided into four groups for each case. One was unexposed (CML group), and the other three were applied directly to GAD plasma at a distance of 3 cm from the blood surface. For each group, three time-period groups were called CML-D1 (20 s), CML-D2 (40 s), and CML-D3 (60 s), respectively. All previous groups were matched with healthy-subject groups, which represented the negative control group (C group). After GAD exposure, blood sample groups incubated for 20 hr at 37ºC before initiation of cultures and biochemical investigation.

E. Cytogenetic and Biochemical Analysis

Triple blood cultures were set up for 72 hr to undergo a blocked micronucleus assay according to the protocol described by Evans and O'Riodran[24] and its modification by Fenech.[25] For biochemical estimation, collagen IV in human plasma was measured using an ELISA kit according to Obata et al.[26] IL-10 levels were assessed with the ELISA kit according to RayBiotech methodology (catalog number ELH–IL-10). Human VEGF levels were quantified using a human VEGF ELISA kit according to Ferrara et al.[27]

F. Statistical Analysis

Data were presented as distribution analyses, percentages, and means ± standard error, and then analyzed using a two-way analysis of variance F test according to Abramowitz and Stegun.[28] The level for statistical significance was $p < 0.05$.

III. RESULTS

Table 1 shows that the control group had a high incidence of BN cells and low frequencies of mononucleated, trinucleated, and quadrinucleated cells; nucleoplasmic bridges (NPBs); and apoptotic and necrotic cells. In contrast, the CML group was characterized by low percentages of binucleated (BN), apoptotic, and necrotic cells but increases in NPB levels and mononucleated, trinucleated, and quadrinucleated cells. In addition, there was a significant increment of micronuclei (MN) frequency in the CML group in comparison to controls.

After exposure to GAD, the parameters of CML-D1, -D2, and -D3 groups changed to reflect increased expression of MN and mononucleated, trinucleated, quadrinucleated, apoptotic, and necrotic cells, whereas BN cells significantly decreased. The most important effect of GAD exposure was incidental increments of apoptotic and necrotic cells and the amounts of spreading cells outside of ECM clusters.

Table 2 shows a significant increase in collagen IV levels in the CML group by approximately 4.6-fold in comparison to the control group. After plasma exposure, collagen IV concentrations significantly decreased in CML-D1, -D2, and -D3 groups compared to the unexposed CML group. Additionally, the CML group showed significant inhibition of IL-10 levels compared to those of the control group. Plasma exposure

TABLE 1: Incidence of mononucleated, BN, trinucleated, quadrinucleated, apoptotic, and necrotic cells

	Control		CML		CML-D1		CML-D2		CML-D3	
Groups	**%**	**Mean ± SE**	**%**	**Mean ± SE**	**%**	**Mean ± SE**	**%**	**Mean ± SE**	**%**	**Mean ± SE**
MN	24.65	247.5 ± 6.5	38.25	382.5 ± 7.6[a]	27.57	275.7 ± 8.1[a,b]	31.68	316.8 ± 12.5[a–c]	37.52	375.2 ± 3.3[a,c,d]
MN + 1 Mn	0.12	1.2 ± 0.5	1.20	12.0 ± 0.4[a]	1.03	10.3 ± 2.1[a,b]	1.65	16.5 ± 0.7[a–c]	1.62	16.2 ± 0.8[a–c]
MN + 2 Mn	0.20	0.2 ± 0.2	0.15	1.5 ± 0.3[a]	0.75	7.5 ± 1.7[a]	0.85	8.5 ± 1.7[a,b]	0.95	9.5 ± 0.4[a,b]
BN	74.17	741.7 ± 7.2	52.83	528.3 ± 10.2[a]	49.67	496.7 ± 3.1[a,b]	32.47	324.7 ± 6.9[a–c]	39.73	397.3 ± 2.2[a–d]
BN + 1 Mn	0.10	1.0 ± 0.3	1.31	13.1 ± 0.7[a]	1.28	12.8 ± 1.1[a]	1.55	15.5 ± 1.9[a]	1.37	13.7 ± 0.1[a]
BN + 2 Mn	0.0	0.0 ± 0.0	1.32	13.2 ± 0.7[a]	0.75	7.5 ± 0.99[a,b]	0.8	8.0 ± 2.1[a,b]	0.92	9.2 ± 0.7[a,b]
NPBs	0.15	1.5 ± 0.7	3.32	33.2 ± 4.8[a]	2.4	24.0 ± 0.8[a]	3.02	30.2 ± 1.7[a,c]	2.72	27.2 ± 1.7[a]
TN	0.32	3.2 ± 0.5	1.63	16.3 ± 1.4[a]	6.6	66.0 ± 1.6[a,b]	4.87	48.7 ± 2.8[a–c]	4.52	45.2 ± 2.04[c]
QN	0.33	3.3 ± 0.8	0.86	8.6 ± 1.5[a]	5.05	50.5 ± 2.6[a,b]	2.73	27.3 ± 5.2[a–c]	3.45	34.5 ± 1.9[a–c]
Apoptotic cells	0.05	0.5 ± 0.3	0.32	3.2 ± 0.5[a]	2.7	27.0 ± 0.9[a,b]	9.37	93.7 ± 2.6[a–c]	3.42	34.2 ± 1.9[b–d]
Necrotic cells	0.0	0.0 ± 0.0	0.08	0.8 ± 0.3[a]	2.2	22.0 ± 0.8[a,b]	11.02	110.2 ± 3.5[a–c]	3.80	38.0 ± 2.1[a–d]
Average cell count	--	7666.7 ± 378.2	--	72866.7 ± 2145.4[a]	--	21250.0 ± 1181.2[a,b]	--	16216.7 ± 682.2[a–c]	--	19016.7 ± 341.9[a,b,d]

	Mean of percentages				
Group	**Control**	**CML**	**CML-D1**	**CML-D2**	**CML-D3**
ECM Clusters Percentage	0.8 ± 0.3	17.4 ± 0.7[a]	13.9 ± 0.6[a,b]	7.4 ± 0.3[a–c]	14.5 ± 0.8[a,b,d]

Frequencies of micronuclei (MN) and nucleoplasmic bridges (NPBs) and mean of percentages of ECM in exposed and unexposed CML and control groups (counts in 1000 cells). $p < 0.05$.

[a]The p value is significant when compared with the control group.

[b]The p value is significant when compared with the CML group.

[c]The p value is significant when compared with the CML-D1 group.

[d]The p value is significant when compared with the CML-D2 group.

BN, Binucleated cells; CML, chronic myelogenous leukemia; ECM, extracellular matrix; IL, interleukin; MN, mononucleated cells; NPBs, nucleoplasmic bridges; QN, quadrinucleated cells; SE, standard error; TN, trinucleated cells.

TABLE 2: Levels of IL-10 (pg/mL), collagen IV (μg/mL), and VEGF (pg/mL) in exposed and unexposed CML and control groups

Group	Control Mean ± SE	CML Mean ± SE	CML-D1 Mean ± SE	CML-D2 Mean ± SE	CML-D3 Mean ± SE
Collagen IV (μg/mL)	38.3 ± 0.8	174.6 ± 0.8[a]	105.3 ± 0.9[a,b]	86.3 ± 0.8[a–c]	94.6 ± 0.9[a–d]
IL-10 (pg/mL)	114.3 ± 0.6	42.3 ± 0.6[a]	74.5 ± 0.9[a,b]	81.7 ± 0.6[a–c]	69.8 ± 1.0[a–d]
VEGF (pg/mL)	45.5 ± 0.9	164.4 ± 0.9[a]	111.7 ± 0.9[a,b]	101.3 ± 0.8[a–c]	92.6 ± 0.8[a–d]

[a]The *p* value is significant when compared with the control group.
[b]The *p* value is significant when compared with the CML group.
[c]The *p* value is significant when compared with the CML-D1 group.
[d]The *p* value is significant when compared with the CML-D2 group.
CML, chronic myelogenous leukemia; IL, interleukin; SE, standard error; VEGF, vascular endothelial growth factor.

induced significant elevations in IL-10 levels in CML-D1, -D2, and -D3 groups compared to that in the CML group. VEGF levels significantly increased in the CML group compared to the control group, and VEGF levels in the CML-D1, -D2, and -D3 groups declined in comparison to those of CML patients. Remarkably optimal results occurred in the CML-D2 group with an exposure period of 40 s, the most effective dose of all investigated data for the CML group.

IV. DISCUSSION

Cytogenetic, immunological, and molecular analyses have become obligatory for exact diagnoses and to predict and monitor responses to newer molecular-targeted treatment modalities. In fact, the presence of a solid bulky ECM prevents leukemic cell sensitivity to chemotherapy[8] and CML characterized by inhibition of apoptosis.[29] An important area for future cancer research will help to determine whether an abnormal ECM could be an effective cancer therapeutic target. To achieve this goal, it is important to understand how ECM composition and organization are normally maintained and regulated and how they may be deregulated in cancer. A daunting task in this regard will be to determine the kind of ECM changes that result in disease progression and how these changes of the ECM, alone or in combination, may affect cancer cells and cells in the stromal compartment. Additionally, with the growing available documentation of diverse ECM functions in development and cancer, a major challenge is to understand the molecular basis of these functions, for example, whether they involve only receptor signaling, rearrangements of the cytoskeleton, changes in gene expression, or other aspects of cell behavior and how such changes are integrated with conventional signaling cascades that are known to have a role in these processes.[4]

The role of an abnormal ECM in tumor angiogenesis is based mainly on the various functions of ECM components in blood vessel formation during normal development. For example, many ECM fragments, including endostatin, tumstatin, canstatin, arresten,

and hexastatin, all of which are derived from collagens IV and XVIII, have potent stimulatory or inhibitory effects on angiogenesis. They are likely to collaborate with other proapoptotic or antiangiogenic factors, including VEGF, to determine where to initiate vascular branching and the final branch pattern.[30]

The adhesion of cells to adjacent cells or to the surrounding ECM is fundamentally important for the maintenance of normal adult tissue structure and function. Ordered hematopoietic development is believed to depend not only on the interaction of the most primitive hematopoietic progenitors, with specific cellular and extracellular components of the bone marrow microenvironment, but also on the trafficking of less primitive hematopoietic progenitors to specific sites of differentiation within the bone marrow. At the end of the process, mature blood elements are released from the bone marrow into the peripheral blood. Such processes require multiple, discrete recognition events that occur in a cell type– and developmental stage–specific fashion. CML is characterized by the premature release and circulation of vast numbers of malignant, primitive, and more differentiated hematopoietic progenitors from the bone marrow microenvironment into the peripheral circulation.[31] From this viewpoint, adhesion to stroma negatively regulates cell proliferation, but CML cells escape this regulation by virtue of their perturbed adhesion properties. Self-renewal deregulation has been recognized as an important event in disease progression.[32]

The main characteristic of plasma radiation sources in medical therapy is its direct application on or in the human body. *In vitro* tests to characterize plasma–cell interactions should include basic cellular parameters such as morphology, viability, proliferation, and characterization of special cellular responses, including their influence on DNA or cellular proteins.[33] The effect of cold plasmas is due to charged particles, ROS, RNS, UV photons, and intense electric field. To obtain a more efficient action on mammalian cells (useful in cancer therapy), activated cold plasma (with an He/O_2 gas mixture) is recommended. The effect of chemically activated cold-plasma apoptosis or necrosis depends on gas mixture and treatment period. Taking into account that ROS density in the cell microenvironment is related to the percentage of O_2 in the gas mixture as well as treatment period, it can be presumed that cell death is due to ROS produced in the plasma jet.[34] It is also known that a major cause of antitumor chemotherapy failure is the development of multidrug resistance of tumors. Cold atmospheric plasma–induced apoptosis of cancer cells that are chemotherapy resistant could eliminate this problem.[35] Barekzi and Laroussi[36] stated that leukemia results from an overabundance of white blood cells; thus, controlling the number of white cells (by destroying them with plasmas exposure) would restore the balance in the blood and result in a partial solution for a cure.

GAD, a simple and inexpensive way to generate nonthermal plasma, can produce both thermal and nonthermal plasma and involve relatively high electric power compared to corona discharge. Such powers are quite reactive and often have a high selectivity for chemical processes (Fig. 1). The main reason to enlist GAD is to provide plasma with useful properties both from thermal plasmas (large electron densities, currents, and

power) and nonthermal plasmas (low gas temperature).[2] For these reasons, we chose GAD as the source of plasma to test CML treatment in the present study.

The present study investigated the effects of GAD plasma exposure on early diagnosed cases of CML. Cytogenetic analysis (using the CBMN cytome assay) pointed to the presence of ECM clusters within blood cells in the culture (see Table 1 and Fig. 2) and reflected a high rate of cell division and low expression of MN, apoptotic, and necrotic cells. In addition, the biochemical examination of collagen IV, the main protein supporting ECM and VEGF levels, showed significant increases, whereas levels of IL-10, which demonstrates immune response, decreased before GAD exposure (Table 2).

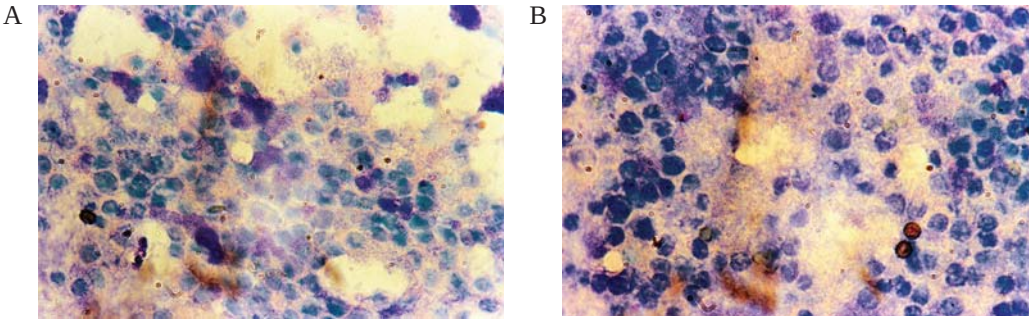

FIG. 2: Images (a & b) showed how CML cells clustered were bounded and surrounded with crowded ECM during 72 h in blood cultures.

The frequency of MN is extensively used as a biomarker for genomic instability, genotoxic exposure, and early biological effect in human biomonitoring studies. Moreover, the test detects both clastogens and aneugens and, simultaneously, mitotic delay, apoptosis, chromosome breakage, chromosome loss, and nondisjunction.[37]

The Micronucleus Assay Working Group of the International Workshop on Genotoxicity test procedures concluded that demonstration of cell proliferation in both control and treated cells is required for test acceptance, because MN expression is compromised when the proportion of dividing cells is reduced by cytostatic or poor tissue culture conditions.[38] It is evident that the best way to eliminate any possible confounding effects of altered cell division kinetics is to use the CBMN cytome assay, regardless of the cell type used, by scoring MN in BN cells, which, in addition, allows measurement of NPBs as well as nuclear buds, cytotoxicity, and cytostatic effects. The cytome approach in the CBMN cytome assay is important because it allows genotoxic (MN and NPBs in BN cells), cytotoxic (proportion of necrotic and apoptotic cells), and cytostatic (proportion and ratios of mononucleated, BN, and multinucleated cells) events to be captured within one assay (Figs. 3 and 4). In conclusion, the CBMN method has evolved into an efficient cytome assay of DNA damage and misrepair, chromosomal instability, mitotic abnormalities, cell death, and cytostasis, enabling direct and/or indirect measurement of

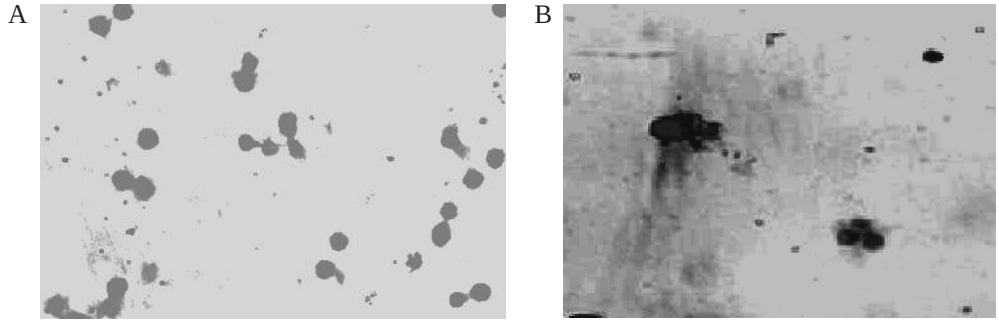

FIG. 3: (A) Control blood cells; (B) necrotic and apoptotic cells spread after GAD exposure

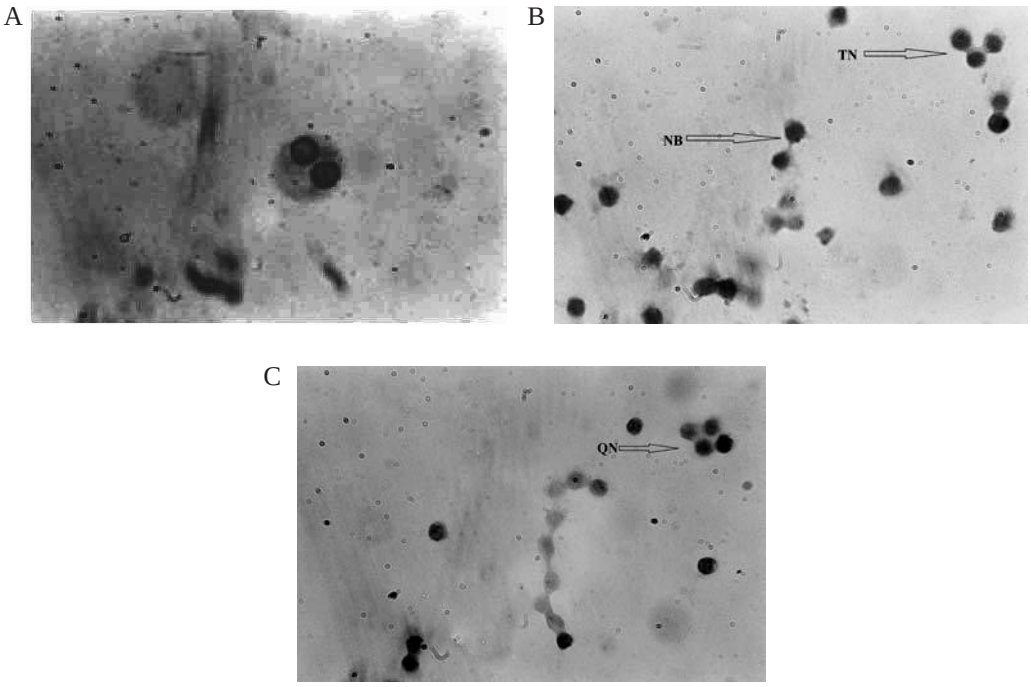

FIG. 4: CBMN cytome assay. (A) BN cells, (B) nucleoplasmic bridge (NB) and trinucleated (TN) cell, and (C) quadrinucleated (QN) cell.

The present results show that the control group had a high incidence of BN cells, and this may be attributed to the regulatory effect of cytochalasin B on the cultured blood cells. But the low frequencies of mononucleated, trinucleated, and quadrinucleated cells; NPBs; and apoptotic and necrotic cells are the result of normal cytostatic and cytotoxic proceedings. In contrast, the CML group was characterized by low percentages of BN, apoptotic, and necrotic cells and increased levels of NPBs and mononucleated, trinucleated, and quadrinucleated cells. These results reflected uncontrolled division and

of BN, apoptotic, and necrotic cells and increased levels of NPBs and mononucleated, trinucleated, and quadrinucleated cells. These results reflected uncontrolled division and interrupted apoptosis. In addition, there is a significant increment of MN frequencies in CML-group BN cell rate and MN formation in diseases such as cancer, as stated by Shimizu.[39]

However, after exposure to GAD, the measured parameters of CML-D1, -D2, and -D3 groups revealed increasing expression of MN, mononucleated, trinucleated, quadrinucleated, apoptotic, and necrotic cells, whereas BN cells significantly decreased. The most significant effect of GAD exposure was the induction of the apoptosis process and increased spreading of cells counts and degradation of ECM clusters (Fig. 3). These findings imply that cell spreading may be an important factor for propagating apoptosis, matching the results of Fuortes et al.[40] and Kettritz et al.[41]

Cells associate with collagen via cell surface receptors, and through such interactions, collagens may have a deep impact on cell proliferation, migration, and differentiation. Fibers and the meshwork of collagen molecules also act as a repository of growth factors and matrix-degrading enzymes. These are often present in an inactive form but become activated for tissues to undergo remodeling, for example, in development, during cyclical changes in the female reproductive system, and in pathological conditions such as cancer.[15] Both laminin and collagen IV are almost exclusively found in basement membranes, where they comprise the majority of proteins.[42] Malignant transformation of cells is generally accompanied by molecular alterations in the adhesive behavior of tumor cells compared with their nonmalignant counterparts.[43] These alterations generally enable tumor cells to escape from their usual environment and disseminate. During the process of intravasation or extravasation, malignant cells must penetrate subendothelial basement membranes. Malignant tumor cells are therefore likely to acquire the ability to adhere to basement membrane proteins and express adequate numbers of adhesion receptors that interact with collagen IV or laminin.[44] One of the greatest binding abilities of ECM components to leukemic cells is collagen IV. This interaction depends on the matrix composition of the organs, which may facilitate the interaction of leukemic cells with the vessel wall and infiltration into tissues. Increases in collagen IV levels in CML cases in the present work match the results obtained by many researchers, who found that collagen IV increased in different malignant tumors.[15,45] In addition, low collagen IV levels associated with the high incidence of apoptosis after irradiation exposure in the present work may be attributed to the process of loss of cell anchorage, characterized by the dissolution of potentially many different kinds of cell–cell/cell–matrix interactions. Several means of cell anchorage provide survival signals to the cell. Thus, the term anoikis (detachment-induced apoptosis) describes the final outcome once these different means of cell adhesion are disturbed, representing a distinct and single molecular mechanism leading to apoptosis, as explained by Grossmann.[46]

VEGF, as an essential proangiogenic factor, has an important role in the development of CML.[47] Legros et al.[48] revealed that VEGF secretion by bone marrow and its plasma concentration are significantly increased in newly diagnosed CML patients,

paralleling the present study's findings. This elevational increase from phase to phase to an exaggeratingly high level in the blast crisis phase, leading to poor prognostic levels. This elevation is indeed strongly associated with expression of corresponding receptors on the cell surface such as VEGF receptor 1 (VEGFR-1) and VEGFR-2.[49]

Table 2 shows that GAD exposure in CML-D1, -D2, and -D3 groups reduces the intensity of VEGF in the CML group, reflecting a good prognostic level. Kaiafa et al.[50] pointed out the critical role of VEGF in the initiation and development of CML, so VEGF monitoring during chemotherapy can help to predict prognosis after treatment.

The generation of potent, specific, and durable antitumor immunity requires a variety of cytokines that regulate important functions related to the balance between tumor rejection by antigen-specific effector cells and suppressive mechanisms that allow tumors to escape immunologic detection. The cytokines are critical for tumor immunosurveillance and have demonstrated therapeutic antitumor activity in murine models and in clinical treatment of several human cancers.[51]

The role of IL-10 in cancer development as either primarily antitumor, preventing inflammation and tumor angiogenesis, or primarily protumor, encouraging cell survival and suppressing effector regulatory T cells (T-regs), may depend on the conditions of initial carcinogenesis as well as the presence of other cells such as T-regs.[52] In addition, IL-10 is known to increase B-cell lymphoma 2 levels and protect B and T cells, various cell lines, and CD34-positive hematopoietic progenitors from apoptosis.[53] Various tumor cells have been shown to produce IL-10, including cells from non-small-cell lung cancers, melanomas, gliomas, leukemias, and lymphomas.[54] Constitutional IL-10 promoter polymorphisms have been associated with the susceptibility to certain malignancies, suggesting that this cytokine may play a critical part in some aspect of tumor immunosurveillance.[55]

Barekzi and Laroussi[36] believed that an important effect of plasma treatment is that it triggers a biochemical reaction that provokes the cancer cell to self-destruct. The researchers held that targeting cancer cells to induce cell death and leave healthy cells unharmed is the key to developing effective leukemia treatments with plasma. Finally, our results showed that the most effective time for GAD exposure to control ECM and obtain immunological enhancement occurred at 40 s in the CML-D2 group.

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