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Fluorescent Estimation of Pollutants in workers blood sample of Industrial and Urban Areas

بحث التخرج للمرحلة الخامسة

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Abstract

Introduction: Blood and serum samples from 35 workers in both industrial and urban areas were collected in two areas in Basrah city, Iraq. analyzed by fluorescent microscopy and automated hematology analyzer. The objective was to determine the levels of polution elements in blood and serum from the workers and to check for blood pathological changes and to investigate the correlation between the pollutans in blood and hematological parameters.

METHODS: The EDTA anti-coagulated blood samples were kept in a cool box until arrival to university campus for complete blood picture (CBP) assay andfor fluorescent viability assays study. RBCs parameters included total RBC count (RBC) in millions per litre, hemoglubin concentration (HGB) in gram per deciliter (gm/dL), hematocrite value in percentage (%), mean corpuscular volume (MCV) in femtolitre (fL), mean corpuscolar hemoglobin (MCH) in pecogram per litre (pg/L), mean corpuscular hemoglubin concentration (MCHC) in gram per deciliter (gm/dL), and RBC distribution width (RDW-CV) in percentage (%). Fluorescence method is straightforward to perform with only one step.

RESULTS: The percentage of apoptotic cells detected by dual acridine orange/ethidium bromide (AO/EB) staining of urban and industrial workers was significant for WBCs ($P<0.05$), Cells appeared to be in the process of disintegrating. Statistical analysis showed that an extremely significant decrease ($P\leq 0.001$) is present in urban workers hemoglobin concentration and hematocrite number than industrial workers. The study showed also a highly significanct decrease ($p<0.01$) in urban total RBC number than industrial workers. Mean corpuscular hemoglobin concentration (MCHC) decreased significantly ($p<0.05$) in urban workers than in industrial ones. A general trend of decrease which is present in RBCs mean corpuscular volume (MCV), mean corpuscular hemoglubin (MCH) and RBCs distribution width of urban workers but with no significance ($P> 0.05$).

CONCLUSION:Our results suggest that fluorescent staining is an economic and convenient method to test cyto-toxicity and appototic changes of blood cell membranes due to exposure to pollutants using dual acridine orange/ethidium bromide (AO/EB) fluorescent staining, visualized under a fluorescent microscope that can be reinforced with blood parameters assays like CBP tests.

1. Introduction

Air pollution can cause number of health hazards to human beings, animalsand birds, in addition to causing number of deleterious effects on earth's atmosphere as well as vegetation and even on some buildings. It is a global malady causing innumerable irreversible changes to our planet. The effects of air pollution have no limitations or country boundaries. They can extend to any adjoining country or even far off country from the country of origin.

Pollution is the result of many activities - which are as normal as human diet in life, but effects of such pollution are far reaching and irreversible. The dangerous effects of some important air pollutants on human health are discussed.Gaseous ammonia is a pungent, irritant gas often causing effects like skin irritation, eye irritation and irritation to throat. It can cause number of allergies. SO_2 gas is often present in industrial places where its excess levels in air can lead to some consequences like: lead to acid rain - which is the result of that is SO_2 is converted into sulphite in presence of atmospheric oxygen.

Also SO_3 (sulphite) can mix with rain water to bring mild sulphuric acid to earth's surface during its movement.Inhalation of sulphur dioxide damages the respiratory tract very badly causing inflammation, irritation, ulcers as well as disruption of alveoli activity. On the other handSO_2 + atmospheric humidity (H_2O) can result into fine particles of sulphites and H_2SO_4. They are often attached to dust or fine carbon particles released out of vehicular pollution ,when inhaled they have capacity to damage delicate tissue linings to eyes, nose, respiratory tract, inhibit cilial action in nose.

Highly dangerous gases are often released out of leakages in chemical industries. They are also released in metal finishing processes like electroplating, case hardening activities. They are fatal in their action causing instant deaths if exposed to such gas for 3 - 5 minutes continuously. In milder form, it can cause damage cells of bronchi, alveoli, nasal tract. It can also lead to many heart problems and vision problems.

Fluorescence Microscopy of cells is considered a promising method to discriminate in vivo normal tissue from pathological tissue at various sites including blood (Lualdi et. Al., 2007). However, only few studies have been reported on the feasibility of exploiting fluorescence spectroscopy of blood to characterize pathological changes usable in diagnosis. In this study, the fluorescence characteristics of human blood cells have been studied in the visible spectral range in an attempt to discriminate blood cells with pathological changes from workers in industrial and urban populations.

2. Materials and Methods

The EDTA anti-coagulated blood samples were kept in a cool box until arrival to university campus for complete blood picture (CBP) assay, then part of blood was transferred to gel tubes and centrifuged for serum collection.

2.1. Fluorescence StainingTechnique

The blood collected using EDTA tubes, was used for fluorescent viability assays study. Fluorescence method is straightforward to perform with only one step, it can also be easily combined with other 96-well-plate-

based assays within one experiment, such as cell viability assay (MTS), cell death assay (LDH), or certain activity assays. Therefore, multiple endpoints of cell death and apoptosis can be measured in a single experiment with very small amounts of cells. This could be very valuable for the cells difficult to grow in large amounts (e.g., the short term cultures of patient or animal samples).Dual fluorescent staining solution (1 µl) containing 100 µg/ml Acridine Orange (AO) and 100 µg/ml Ethedium Bromide (EB) (AO/EB, Sigma, St. Louis, MO) was added to each (20 µL)blood sample, and then covered with a coverslip. The morphology of apoptotic cells was examined and 100 cells were counted within 20 min using a fluorescent microscope

2.2. Hematological Study

All study subjects had complete blood picture (CBP) test for hematology parameters concerning red blood cells (RBCs) using Automated Hematology Analyzer COUNT-60, U.S.A (Fig. 1).

Fig.(1) Genex Laboratories Hematology Analyzer

RBCs parameters included total RBC count (RBC) in millions per litre, hemoglubin concentration (HGB) in gram per deciliter (gm/dL), hematocrite value in percentage (%), mean corpuscular volume (MCV) in femtolitre (fL), mean corpuscolar hemoglobin (MCH) in pecogram per litre (pg/L), mean corpuscular hemoglubin concentration (MCHC) in gram per deciliter (gm/dL), and RBC distribution width (RDW-CV) in percentage (%).

3. Results

3.1. Fluorescence Examination

Normal cells, early and late apoptotic cells, and necrotic cells were examined using fluorescent microscopy. Early-stage apoptotic cells were marked by crescent-shaped or granular yellow-green acridine orange nuclear

staining. Late-stage apoptotic cells were marked with concentrated and asymmetrically localized orange nuclear ethidium bromide staining. Necrotic cells increased in volume and showed uneven orange-red fluorescence at their periphery. Cells appeared to be in the process of disintegrating. The percentage of apoptotic cells detected by dual acridine orange/ethidium bromide (AO/EB) staining of urban and industrial workers was significant for WBCs ($P<0.05$).The resultant Percentage of apoptotic cells identified using AO/BR method is presented in Table (1).

Table (1). Percentage of apoptotic cells identified using AO/EB method

Blood Cells	Urban(%)± SD.	Industrial(%) ± SD.	p-value
WBCs	6.97±1.5	10.33±1.7	0.02 (*)

(Not Significant N.S., Significant *, Highly Significant **, n=35)

3.2. Hematological Study

Urban and Industrial workers RBCs parameters calculated for Means ±SD are presented in figure (2)

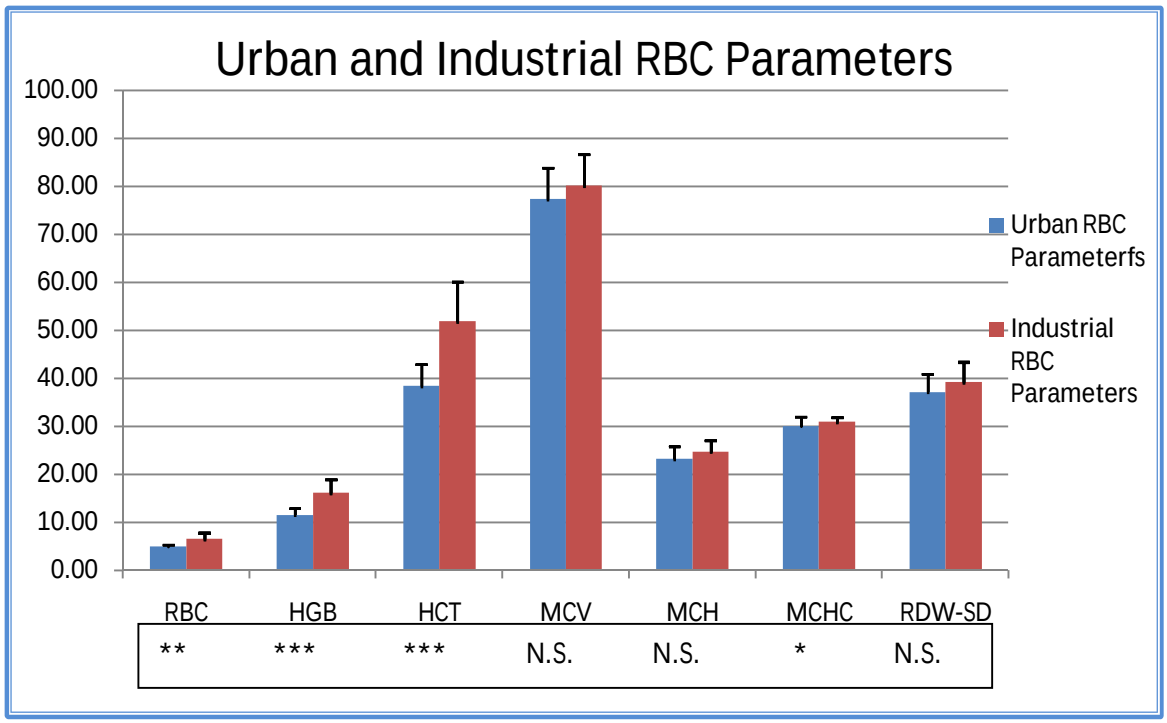

Fig (2) Urban and Industrial workers RBCs parameters Means ±SD

(Not Significant N.S., Significant *, Highly Significant **,Extremely Significant *, n=35)**

Statistical analysis showed that an extremely significant decrease ($P \leq 0.001$) is present in urban workers hemoglobin concentration and hematocrite number than industrial workers. The study showed also a highly significanct decrease ($p<0.01$) in urban total RBC number than industrial workers. Mean corpuscular hemoglobin concentration (MCHC) decreased significantly ($p<0.05$) in urban workers than in industrial ones.A general trend of decrease which is present in RBCs mean corpuscular volume (MCV), mean corpuscular hemoglubin (MCH) and RBCs distribution width of urban workers but with no significance ($P> 0.05$).

4. Discussion

Olanrevaju et al., (2008) has reported that ammonia can reduce the capturing of oxygen by the hemoglobin due to its impact on the blood pH. oxidative stress alters plasma membrane redox system (transfer of electrons from intracellular substrates to extracellular electron acceptors) in red blood cells (Pandey and Rizvi, 2011). In addition, ammonia-induced oxidative stress inflicts tissue damage. Same results yielded in this study where RBCs percentage of appoptotic cells increased significantly ($P<0.05$).

This study revield that an extremely significant decrease ($P\leq 0.001$) is present in urban workers hemoglobin concentration and hematocrite number than industrial workers. Also Subash and Subramanian (2008), showed that oxidative stress increases hemoglobin oxidation, membrane proteins and membrane lipids oxidation in red blood cells. Acute ammonia intoxication also increases the formation of NO, another free radical that contributes to ammonia toxicity On the contrary,Dyavolova et al., (2013) mentioned that erythrocyte concentrations declined slightly ($P>0.05$). His data suggested that the increased level of hematocrit was most probably due to ammonia-induced change in erythrocyte volume rather than to increase in erythrocyte concentrations.

Hematocrit level after exposure to stress could also be influenced by NO induced deformability of erythrocytes as revealed byBor-Kucukatay et al., 2003), since ammonia stimulates NO production (Monfort et al., 2002), thus increasing the exposure of red blood cells to external NO in addition to the NO synthesized within erythrocytes.

The observed fluctuation of respiratory rate may be related to ammonia-induced change in blood pH (Olanrevaju et al., 2008). Respiratory compensation has been reported to correlate with change in pH (Roller, 1967). The pH of the blood is maintained within a very narrow range during exposure to ammonia and fluctuates along with partial pressure of CO2, O2, hematocrit and hemoglobin (Olanrevaju et al., 2008).

Mohammed et al., (2015) reported that chronic exposure to petroleumfumes has adverse effects on human hematopoetic system, leading to bonemarrow depression and resultant pancytopenia.Benzene, an aromatic hydrocarbon that is anatural component of crude oil and naturalgas, is toxic to the blood and blood-formingorgans. Findings showed that mean values ofhaemoglobin %, RBC count and Total leucocyte counts weresignificantly decreased in the test groupwhen compared with the control group. Theresults obtained were statisticallysignificant, p-value being less than 0.05 incase of Red and White cell counts and lessthan 0.01 in case of haemoglobin values.

Chronic hematotoxic effects ofbenzene exposure, including reduce lymphocyte, neutrophil and platelet countsin peripheral blood, have been detected atoccupational exposure below a level that hadpreviously been considered not to cause anyhealth effects. Whether these abnormalitiesrepresent bone marrow damage and/or initialevents in the development of a trueneoplastic disease is not known (Jorunn *etal.*, 2008).

Decrease in haemoglobin contentcould be due to decrease in red blood cellsor impaired biosynthesis of heme in bonemarrow (Ali and Sahb, 2011). Decreasedhaemoglobin and red blood cell could alsobe attributed to insufficiency of proteinsynthesis that mainly induces decrease ofessential

amino acids and shortage of theenergy source of protein synthesisincorporated in haemoglobin production.The decrease in red blood cell count wasobserved in the exposed population (Gautamand Chowdhury, 1987).

Another study done in Nigeria on fuelattendants showed similar results, with aglobal reduction in the mean values of totalleucocyte count, red blood cell count,Packed Cell Volume and other red bloodcell indices in exposed individuals (Okoro *etal.*, 2006).

Shumeiet.al. (2004) measured the fluorescence spectra of the whole blood, the red blood cell (RBC) and the hemoglobin using U.V.spectrofluorometer.In his work, he found that the fluorescence spectra of the whole blood and the RBC have much similarities in the intensity, the emission peaks and the emitting region, and abundant peaks can be found. But for the hemoglobin, fluorescence could only be found in the wavelength range 580-650 nm. It was concluded that in the wavelength range of 650-850 nm, the fluorescence spectra were emitted by the new fluorophores generated by the breakdown of some weak bonds on the RBC membrane, such as the C-C bond and the C-N bond. In the wavelength range of 590-650 nm, the fluorescence spectra are mainly emitted by the hemoglobin, but the hemoglobin solution of cracked RBC has a strong quencher effect on the fluorescence spectrum. The experimental result and the theoretical analysis are meaningful for the medical diagnostics and the therapy. (Zhu et. Al., 2006): (Zhu et. Al., 2008a&b).

Conclusion

Our results suggest that fluorescent staining is an economic and convenient method to test cyto-toxicity and appototic changes of blood cell membranes due to exposure to pollutants using dual acridine orange/ethidium bromide (AO/EB) fluorescent staining, visualized under a fluorescent microscope that can be reinforced with blood parameters assays like CBP tests.

References:

Ali, A., Sahb, A. 2011. Hematologicalassessment of gasoline exposureamong petrol filling workers inBaghdad. *J. Fac. Med. Baghdad,*53(4).

Bor-Kucukatay, M., R. B. Wenby, H. J. Meiselman and O. K. Baskurt. 2003. Effects of nitric oxide on red blood cell deformability.*American Journal of Physiology*, 284(5): H1577-1584.

Close, L. G., F. I. Catlin and A. M. Cohn. 1980. Acute and chronic effects of ammonia burns of the respiratory tract. *Arch. Otolaryngol*., 106 (3): 151-158.

Dyavolova, M., I. Yanchev, D. Gudev and P. Moneva, 2013. Effect of high ammonia level on stressinduced hematological changes in rabbits: preventive effect of pyridoxine. *Bulg. J. Agric. Sci.*, 19: 828-834.

Gautam, A.K., Chowdhury, A.R. 1987. Effect of lead on erythropoietic system of intact and splenectomizedrats. *Indian J. Physiol. Pharmacol.*,31(2): 117 124.

Jorunn, K., Trond, R., Tore, G.B., *et al.,* 2008. Effects of benzene on humanhematopoiesis. *Open Hematol. J.,* 2:87 102.

Lan X-F, Liu J-G, Liu Y, Luo X-S, Tang X-G, Lu S-Y, Ni X-W (2006). spectroscopy research on cholesterol in hypercholesterolemia serum, spectroscopy and spectral analysis, 26(3): 467-470.

Li D-T, Zhang C-J, Wan GJ, Cheng C-G (2006). Application of wavelet transform to infrared analysis, spectroscopy and spectral analysis, 26(11): 2024-2026.

Lualdi M, Colombo A, Leo E, Morelli D, Vannelli A, Battaglia L, Poiasina E, Marchesini R. Natural fluorescence spectroscopy of human blood plasma in the diagnosis of colorectal cancer: feasibility study and preliminary results. Tumori. 2007 Nov-Dec;93(6):567-71.

Mohammed Aleemuddin, M.Girish Babu, M.L.Manjunath and Shireen Swaliha Quadri, 2015. Effect of chronic inhalation of petroleum products on hematological parameters. Int. J. Curr. Res. Aca. Rev. Vol. 3 (4): 196-201

Monfort, P, E. Kosenko, S. Erceg, J. J. Canales and V. Feligo, 2002. Molecular mechanism of acute ammonia toxicity: role ofNMDA receptors. *Neurochem. Int.,* 41 (2-3): 95-102.

Okoro, A.M., Ani, E.J., Ibu, J.O.,Akpohomeh, B.A. 2006. Effect ofpetroleum products inhalation onsome hematological indices of fuelattendants in Calabar metropolis,Nigeria. *Niger. J. Physiol. Sci.,* 21(1-2): 71 75.

Olanrevaju, H. A., J. P. Thaxton, W. A. Dozier III, J. Purswell, S. D. Collier and S. **L.** Branyon, 2008. Interactive effects of ammonia and light intensity on hematochemical variables inbroiler chickens. *Poult. Sci*., **87** (7): 1407-1414.

Pandey, K. B. and S. I. Rizvi, 2011.Biomarkers of oxidative stress in red blood cells.*Biomed Pap Med Fac Univ Palacky OlomoncCzech Repub*., 155 (2): 131-136.

Qin S-P, Din GJ-H, Li J-D (2006). Research on fluorescence spectra of liver pathological serum of mice with wavelet transforms method, J. Optoelectronics. Laser, 17(5): 634-637.

Rochkind S, Nissan M, Alon M, Shamir M, Salame K (2001). Effects of laser irradiation on the spinal cord for the regeneration of crushed peripheral nerve in rats, Laser Surg. Med. 28(3): 216-219.

Rohleder D, Kocherscheidt G, Gerber K (2005). Comparison of midinfrared and Raman spectroscopy in the quantitative analysis of serum, J. Biomed. Opt. 10(3): 1-10

Roller, M. H., 1967. The effect of acute ammonia toxicity on certain blood parameters in cattle. *Dissertation abstracts*: B 1966,27:1603B-1604B, Record number 1967, 1407707.

Runger T-M, Thomas M (2007). How different wavelengths of the ultraviolet spectrum contribute to skin carcinogenesis: the role of cellular damage responses, J. Invest. Dermatol. 127(9): 2103-2105

Shumei Gao, Xiufeng Lan, Ying Liu, Zhonghua Shen, Jian Lu, and Xiaowu Ni. (2004) Characteristics of blood fluorescence spectra using low-level, 457.9-nm excitation from Ar+ laser Vol. 2,Issue 3,pp. 160-161

Subash, S. and P. Subramanian, 2008. Effect of morin on the level of liver markers and redox status in experimental chronic hyperammonaemic rats. *Singapore Medical Journal*, 49 (8): 650-655.

Tang GC (1989). Pulsed and CW laser fluorescence from cancerous, normal and chemically treated normal human heart and lung tissues, Appl. Opt. 28(12): 2337-2343.

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Wang L-X, Zhao Z-M, Yao H-B, Chen Y-M, Shi L, Gao Y (2002). Infrared Absorption Spectrum Analysis and Its Application to Blood, Spectroscopy and Spectral Analysis, 22(6): 980.

Wang Y-T, Li Y-C (2006). Application of wavelet threshold denoising method in the fluorescence analysis of pesticides. J. Appl. Opt. 27(3): 192-194.

Zhao Z-M, Chen Y-M, Yu X-L, Xin Y-J (2003). Characteristic analysis and application of blood fluorescence spectra, spectroscopy and spectral analysis, 23(5): 922.

Zhao Z-M, Xin Y-J, Wang L-X, Zhu W-H, Zheng M, Guo X (2008). Measurement and analysis of absorption spectrum of human blood, spectroscopy and spectral analysis, 28(1): 726-728

Zhu et al. 899 Shi X-F, Ma J, Mao W-Z, Li Y, Zheng R-E, Meng J-W (2006). Applying patial least squares discriminant analysis on autofluorescence spectra to identify gastric cancer, spectroscopy and spectral analysis, 26(2): 295-298.

Zhu W-H, Zhao Z-M, Zheng M, Xin Y-J, Wang L-X, Guo X (2007). Effect of concentration of glucose of human serum on fluorescence intensity, spectroscopy and spectral analysis, 27(12): 2531-2533

Zhu W-H, Zhao Z-M, Guo X, Wang L-X, Chen H (2008a). Study of glucose concentration based on serum UV absorption spectra, J. Life Sci. USA, 2: 62-66

Zhu W-H, Zhao Z-M, Guo X, Wang L-X, Chen Hui (2008b). Investigation of cholesterol concentration based on serum fluorescence spectra, optics in health care and biomedical optics III, Proc. of SPIE, Vol. 6826, 68262U1-U5