

Study of Smoking Effects on Total WBCs Count and Phagocytic Activity of Clinical Laboratories Workers Blood in Al-Muthana Governorate

2nd Conference on Environment and Sustainable Development 28-29-Oct-2015

Dr. Weam S. Al-Hamadany

Dean Assitant for Scientific Affairs, College of Pharmacy, Al-Muthana University, Al-Muthana, Iraq

Email: dr.Weam2013@yahoo.com or akweam@yahoo.com

Shaymaa M. Jabbar

Biology Department Ccollage of Science, Al-Muthana University, Al-Muthana, Iraq.

Huda T. Ali

Biology Department, Collage of Science, Al-Muthana University, Al-Muthana, Iraq.

Asmahan Y. Faram

Biology Department, Collage of Science, Al-Muthana University, Al-Muthana, Iraq.

Abstract

Clinical Laboratory workers undergo a periodic checkup as biosafety management's procedures; due to that, they are in continuous exposure to pathogens during such work. Smoking is a problem for healthy peoples, but as laboratory technicians, this subject was not under seeking before. Total WBCs count is a test listed in the clinical form depended by Ministry of Health, while phagocytosis activity is not. These two clinical parameters were estimated in this research in healthy volunteers Blood; working in Al-Muthana Governorate clinical laboratories, NBT test was used for Neutrophils activity evaluation. Results showed elevation in WBCs Total Counts and decrease in phagocytic activity; smoking caused more elevation in total count and lower percentages of active phagocytic Neutrophils comparing with the non-smokers obtained values. Conclusion: Periodic checkup in Iraq should be updated and smoking status must be included.

Keywords: Smoking; Clinical laboratories workers; NBT; Total WBCs count; Periodic checkup; Phagocytic activity

دراسة تأثيرات التدخين على العد الكلي لكريات الدم البيضاء والفعالية البلعية لدم العاملين في المختبرات السريرية لمحافظة المثنى

الخلاصة

يخضع العاملون في مختبرات التحليلات المرضية الى فحص دوري ضمن اجراءات السلامة البيولوجية المتبعة وذلك لتعرضهم المستمر الى العوامل المرضية اثناء اداء عملهم. ويعد التدخين مشكلة للناس الأصحاء. اما بالنسبة للعاملين في المختبر فالموضوع لم يتم تسليط الضوء عليه. أن العد الكلي لكريات الدم البيض هو جزء من الاستمارة الخاصة بالفحوصات الدورية للعاملين في المختبرات ومعتمد من قبل وزارة الصحة، أما قياس فعالية الخلايا البلعية فإنه غير مدرج ضمنها. تم قياس هذين المؤشرين في دم المتطوعين من العاملين بالمختبرات السريرية لمحافظة المثنى. وتم اعتماد اختبار

NBT (Nitroblue-Tetra Zolium Test) لتحديد فعالية الخلايا البلعمية العدلة. أظهرت النتائج ارتفاع قيم العد الكلي لكريات الدم البيضاء وأنخفاض فعالية الخلايا البلعمية العدلة في دم المتطوعين, وسبب التدخين زيادة في ارتفاع قيم العد الكلي وانخفاض أكثر في فعالية الخلايا العدلة للمدخنين مقارنة بأقرانهم من غير المدخنين. الأستنتاج يحتاج الفحص الدوري في العراق الى تحديث وينبغي أدرج التدخين ضمن أستمارة الفحص.

الكلمات المرشدة: التدخين، العاملين بالمختبرات السريرية، NBT، العد الكلي لكريات الدم البيضاء، الفحص الدوري، الفعالية البلعمية

INTRODUCTION

Smoking is an important pollution factor, it is a fact that it is a public health problem; it affects both smoker and non-smoker peoples via passive smoking. Clinical laboratories employees are in continuous danger to Laboratory-associated infections or laboratory-acquired infections, which suggests that unique risks are associated with the laboratory work site due to contact with patients as biological hazard, chemical and physical materials, from that point several tests for workers in laboratory are periodically done to ensure their safety [1].

From another point, bad treatment for medical wastes and low hygiene play critical role in transmission of infection even among workers themselves. Smoking cause many clinical parameters to be imbalanced, total WBCs count can be affected and function of these blood cells too. White blood cells are the major component of the body's immune system, when their number or function is affected; the immune system rigidity will be broken and infections will occur easily [2]. Smoking is a major health hazard as stated by [3], and always non-smokers are healthier than smokers more over saving money, nonsmoking would save money spent on health care too. Tobacco smoke contains over 4000 chemicals in the form of particles and gases. Many potentially toxic gases are present in higher concentrations inside stream smoke than in mainstream smoke and nearly 85 % of the smoke in a room results from side stream smoke [3].

Biosafety is an important issue in laboratory settings worldwide and especially in developing countries where standard operating procedures (SOP) are weak. There is a periodic checkup applied usually on clinical laboratory workers in Iraq as biosafety management, but smoking is not listed in that checkup form. This study will focus light on being clinical laboratory workers and smokers at the same time, and what consequences can be recorded on Immune system WBCs health [4-5].

Total WBCs count and phagocytic activity evaluation are two parameters of innate immunity efficiency. Phagocytosis serves a dual role: as an innate immune effector as well as a bridge between the innate and acquired immune responses. It is an important link between specific and nonspecific immune responses, characterized by the establishment of immunological memory made possible by clonal expansion of effector lymphocytes and somatic mutation and germ line recombination of antigen receptors [6].

Neutrophils are the microphagocytes; they are the first cells that arrive upon any inflammation induction, hence their activity is linked with good and healthy immune system since they are the guardians for human body from any outer invasion. When the immune system is healthy, the neutrophils are with good relative number, effective, that means they are able to generate phagocytosis properly, and effectively [7].

Estimation of phagocytic cells activity to eliminate foreign bodies give a view about clinical case even when there is no clinical signs appear on individuals .There are many ways and methods used for that purpose, using Nitro-Blue Tetrazolium test (NBT) is one of the most common tests used to evaluate phagocytic activity of neutrophils [8].

Materials and Methods

Materials

Samples: 20 blood samples were collected during a study, they were collected randomly from adult healthy volunteers, males, with no clinical signs. Laboratory workers aged from (24 - 46 years), they were all working in the clinical laboratories of Al-Muthanna Governorate hospitals, this research was performed during (October 2014 to April 2015).

Collection and Treatment of Blood Samples: Four milliliters (4 ml) of blood were taken by venipuncture from all individuals involved in this study. Samples were put in heparinized tubes and gently mixed; heparin concentration was 10 – 20 % Iu/ml; heparin was used instead of EDTA, due to EDTA toxic effects on phagocytes viability [9].

Data collection: In a record used during this research each data about the person involved in the study was listed. Data included age, marital status, residency, either smoker or not, history record of health and any other information can serve the research, Appendix A.

Appendix A

(Data Collection Form)

Name:
Career:
Hospital:
Age:
Gender:
Marital status:
Health record:
Employment period:
Smoker; Yes ----- No.-----
Others:

Solution:

- **Diluting fluid for total WBCs count**, this fluid was prepared as recommended by [9]. Solution was kept in dark condition until use.
- **Leishman's Stain**, This stain (Fluka; Germany) was prepared as [9], and kept in a glass bottle inside the incubator at 37°C, till it was used.
- **Nitro-blue Tetrazolium Preparation (NBT)**, this dye was manufactured by Sigma (USA), and prepared as [10]. This dye solution must be freshly made for each batch

of tests. This step was made carefully since this dye is highly toxic and its powder is volatile.

Methods

- Total WBCs count: Total WBCs count performed using prepared WBCs diluting fluid and the Neubauer Haemocytometer chamber (Germany), test was done as [8].

The following equation was performed in calculation values which were obtained in cells/ml:

$$\text{Total WBCs} = \text{Total cells count} / 4 * \text{dilution factor (DF)} * 10.$$

- **Nitro-blue tetrazolium (NBT) Test**

This test is one of standard neutrophils function tests, it involves the detection of respiratory oxidative burst activity in neutrophils via the ability to reduce NBT dye after mixing with heparinized blood with equal volumes. This test was used to evaluate neutrophils activity. This test operated as [10]. Whereas 100 cells of neutrophils were randomly selected and identified whether they were positive or negative (concerning the presence of Formazan particles), results recorded as percentages.

Statistical Analysis, Mean value calculations was the only statistical test used in this research results, this test was applied on the (20) relative value of both positive and negative results [11].

Results

From (20) individuals included in this study, recorded data cleared that there were (12) individuals from Al-Hussien Hospital ,and (8) were from Gynecology and Pediatric Hospital. Also there were (11) smoker (55 %) and, (9) non smoker (45 %).Residency outcomes showed that there were (11) workers from Al-Sammawa town and (2) workers from Al-Rumaytha town, and , (3) workers from Al-Khader town and (4) workers from Al-Muthanna country sides (villages). Ages ranged (24 - 46) with an average (30.5) years. Employment period ranged (3 - 14) years.Clinical parameters results, Appendix B Table 1,

Appendix B

Table (1). Total WBCs results for smoker and non-smoker employees

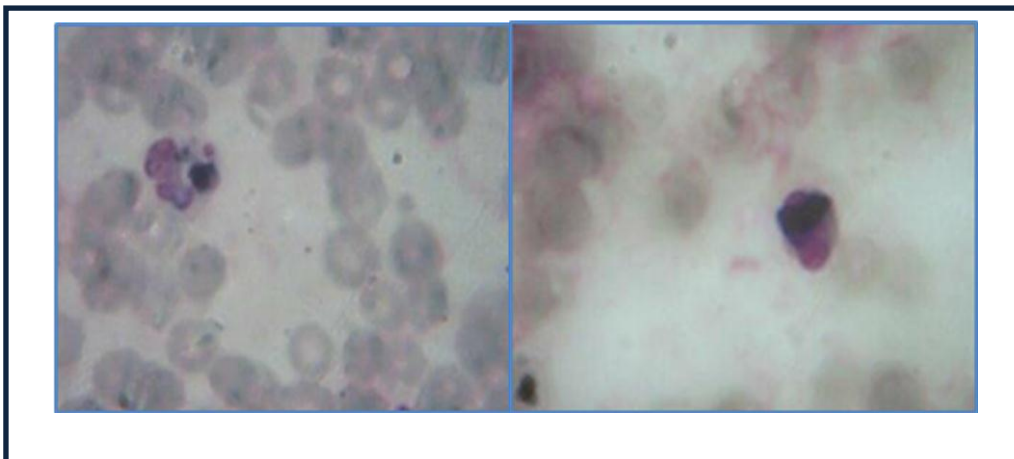
NO.	Case status	No. of cases	Percentage	Total WBCs average (cell/ml)	NBT +%
1	Smoker	11	55 %	10325 *	58 %**
2	Non-Smoker	9	45 %	9375	64 %
Total		20	100 %	9850	61 %

*: normal reference range depended in this study was (4,500 to 10,000 cells/ml); [9].

** : normal value depended in this study was higher than (60% +); [12].

The total WBC values obtained range was (17000 – 2700 cell/ml) with an average of (9850 cell/ml), the average of non smoking worker values is (9900 cell/ml) , and for smoking employees is (10325 cell/ml).The normal reference values that depended in this study was (4,500 to 10,000 cells/ml) [9].Concerning phagocytic activity estimation using NBT test; normal reference range is higher than (60 % +); [12]. Accordingly, there were (11) individuals recorded with decreased values of positive reaction and were lower than standard reference value with a percentage mean (58%). While values recorded in non- smokers (9) persons from the total 20

volunteers involved in this study were obtained normal with an average percentage (64 %), Picture 1.



Picture (1). Blood film for healthy laboratory workers under oil immersion X100, shows Neutrophils with positive reaction to NBT- Test (black crystals inside Neutrophils are the Formazan particles).

Discussion

First of all , our results restricted with male gender inorder to avoid Menstruation period effects on the total WBCs count because this factor influence on the results as recommended by [8].

Our findings showed that total WBCs mean value that obtained was (10150 cell/ml) for all samples was elevated compairing with normal range. Regarding mean values for smokers and non smoker. Outcomes showed that, there was an increase in most of the values gained, this outcome is inagreement with the results of [13], wherease they found a significant difference between total WBCs count values of males working as employees in laboratories and the general population.

Another study of [14], they found that there was total leukocytes elevated significantly during preventive medical check-ups in work places in both genders and they concluded that smoking can increase WBCs total count and affect lung function . From another point view,contineous exposure of laboratory workers can increase WBCs production and hence their count in peripheral blood elevates markedly, this opinion was supported by [15], they stated that exposure to some pathogens can rise up leukocytes counts due to over production of them.

Concerning smoking, it was obvious from our results that smoking had a bad effect on laboratory Workers, smokers leveles were higher than non smokers , these findings were in agreement with those of [14], they analyzed the relationship between WBCs count and smoking wich found it affecting significantly by elevating WBCs Total Count .

During their research, Valiathan and his colleagues found that smoking make immune profile worse when there is another infection compained with smoking or in another word smoking can accelerate disease progression, these statement can support our results since our cases were indirect contact with pathogens [16] .

This study showed that the results of total WBCs counts for smokers are different from non smoker workers in that they were higher, the scientists Gabriel [17]; stated that total WBCs should be always on the check-up of workers, because it is related to the health status and smoking health hazards. Indications for a WBCs count include infectious and inflammatory diseases; leukemia and lymphoma; and bone marrow disorders. Interfering factors: Acute emotional or physical stress can increase WBCs counts [17].

In Iraq ,total WBCs is one of the most important tests in all check- up and followup profiles, [18], result for workers are usually recorded periodically each season and when the employee showed abnormal value, he/she will be given permission to rest and medication to recover clinically and other tests will be performed to make view clear about his case, there is no item in all checkup lists mentioning either employee is smoker or not, as in the lists of other developed countries periodic check up (WHO) ; hence; it is necessary to include the item of smoker or non-smoker because of its effects on the levels of total WBCs count as cleared from our obtained results.

Nitro-blue tetrazolium reduction, NBT is not usually used in ordinary clinical laboratories. It is mostly used for research purposes since this dye is highly toxic, volatile powder and too expensive as stated by [10]. Formazans have been studied extensively because of their ready accessibility, diverse chemical reactivity, broad spectrum of biological activities and wide range of applications [19].

Our outcomes gave clue that smoking can be considered as influencing factor on phagocytes activity and made them less effective, there were (11) persons recorded as smokers, (6) individuals among them had low values than accepted normal level, hence there is an indicator that smoking affected on phagocytes activity, since (6) from (11) smokers had decreased values in another word (54.56) from the total smokers had decreased values for NBT positive.

Baker and his team suggested that programs to quit smoking should be planned for employees because of health consequences and costs for health care, medication and continuing to smoking [20].

The CDC (center of diseases control in USA) releases reports usually under the name (consequences of smoking), in the report of 2004, they evaluated the harm of smoking by that overall health in smokers is poorer than in nonsmokers mentioning the blood components function [21] .

From another point view, the scientists, [22], investigated the polymorphnuclear phagocytes activity in phagocytosis and killing in workers occupationally exposed to toxic substances, they found that neutrophils activity in engulfment and killing was impaired as their results showed. These observations support our results; since more than a half of our volunteer workers had decreased values [22].

The world health organization (WHO) has listed total WBCs count among the Periodic Medical Examination or screening for Laboratory Employees, as a recommendation in the medical programs of this organization [23].

Conclusions

1. Laboratory worker WBCs Total Count values are elevated in laboratory workers comparing with normal range values .Smoking (active smoking) caused WBCs counts elevated in smokers comparing with non-smoker workers .

2. Laboratory workers were suffering from decreased NBT+ values, and smokers results suggested that there is a big link between smoking and low Neutrophils activity in laboratory workers peripheral Blood.
3. There is a defect in follow –up of periodic preventive check up of laboratory workers in Iraq and it should be updated .

References

- [1] Sewell, D.L., “Laboratory-associated Infections and Biosafety”, *Clinical microbiology review*, 08, 389-405, 1995.
- [2] Robert, W., Tolan, Jr, MD. “Immunology and Infectious diseases”, 2010.
- [3] Kraev, T.A., Adamkiewicz, G., Hammond, S.K., Spengler, J.D., “Indoor Concentrations of Nicotine in Low-income, Multi-unit Housing: Associations with Smoking Behaviors and Housing Characteristics’ , 18, 438–444, 2009.
- [4] Croock, M.S., “LTE Based E-Health Monitoring System”, *IJCCCE*. 14(2), 37-45, 2014.
- [5] Aksoy, U., Ozdemir, M.H., Usluca, S., & Ergönen, A.T. “A Biosafety Profile of Laboratory Workers at Three Education Hospitals in Izmir, Turkey”, *Mikrobiyoloji Bülteni*, 42, 469-476, 2008.
- [6] Pancer, Z., “Somatic diversification of variable lymphocyte receptors in the Agnathan sea lamprey”, *Nature* 430, 174–180, 2004.
- [7] Al-Hamadany, W.S. “Study of Blood Storage Effects on Neutrophils Total Count, Phagocytic Activity and Morphology”, *IJAR*. 2(10), 537-540, 2014.
- [8] Mckenzie, S.B. “Clinical Laboratory Hematology WBCs count”, Ch. Pearson, 2006.
- [9] Lewis, S.M.; Bain, B.J; Bates, I., Dacie and Lewis: “*Practical Hematology. 10th ed*”, Elsevier Ltd. 2006, Churchill Livingstone. Germany, 2006.
- [10] Al-Hamadany, W.S. “Effect of Irradiation on Neutrophil Activity using α -particles radiation and human blood samples”, *.Int .J .Curr. Microbiol. App Sci* 3(3), 242-245, 2014.
- [11] Statistical Analysis System SAS. “SAS/Stat User Guide for Personal Computer. Release”, 6.12 SAS Institute. Inc. Cary. N.C. USA, 2000.
- [12] Hoffbrand, A.V.; Moss, P.A.; and Pettit, J.E. “Essential Hematology”, 5th ed., Blackwell Pub Ltd. USA, 2006.
- [13] Christian,C.; Werley, B.; Smith, A.; Chian, N. And Grade, D. “Comparison Employees White Blood Cell Counts in a petrochemical plant by Worksite and Race”, *J. Natl . Med .Assoc*, 86(8), 620-623, 1994.
- [14] Fernandez, J, “Systemic Inflammation in 22.841 Healthy Employed Smokers and Non – Smokers: White Blood Cell Count and Relationship to Spirometry”, *J. Tobacco induced diseases*, 10(7), 1617-9625, 2012.
- [15] Delves, P.J.; Martin, S.J.; Burton, D.R.; and Roitt, I.M. “Roitt’s Essential Immunology”, 11th ed., Blackwell publishing Ltd. USA, 2011.
- [16] Valiathan, R.; Miguez, M.; Patel, B.; Arheart, K., and Asthana, D., “Tobacco Smoking Increases Immune Activation and Impairs T-cell functions in HIV Infected Patients on Antiretrovirals: Across- Sectional Pilot Study”, *PLOS. J.* 9(5); 2014.
- [17] Gabriel, V., “Medical Immunology”, Part 1. 6th ed., Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina, U.S.A, 2007.

- [18] Public Health Laboratories, Ministry of Health in Iraq, Baghdad, Public Health Laboratories, References values of checkup tests, 2015.
- [19] Chavan, S.B., Zangade, S.B., Archana, Vibhute, Y., and Vibhute, Y.B., "Synthesis and Evaluation of Antimicrobial Activity of Some New Schiff Bases and Formazans", *J. Chem Pharm Res.*; 3(1), 262-269, 2012.
- [20] Barker, T., "Why People Smoke", Action Paper Number 1, University of Wisconsin, Medical School Prints, 2002.
- [21] Center of Diseases Control, CDC, "Exposure to Blood: What health-care Workers Need to know", US Dept. of Health and Human Services, Centers for Disease Control and Prevention. 1998:1-8. Available at: http://www.cdc.gov/ncidod/hip/blood/exp_blood.htm. Accessed 18 May 2004.
- [22] Queiroz, M., Quadros, M., Valadares, M. and Silveira, J., "Polymorphonuclear Phagocytosis and killing in Workers Occupationally Exposed to Hexachlorobenzene", *J. Immunopharmacology and Immunotoxicology*, 20(3), 447-454, 1998.
- [23] World health organization (WHO). Laboratory Services, Chapter 8/ P.P.6 -14. www.WHO.IntLaboratory Services .pdf 2015.