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ORIGINAL ARTICLE

Respiratory Burst Activities of Peripheral Blood Neutrophils in Leukaemic Subjects in Northern Nigeria

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Abstract

Introduction: Polymorphonuclear neutrophils (PMN) are the only leucocytes that are competent to produce large amounts of reactive oxygen species (ROS) to kill phagocytized bacteria. In leukaemia, we hypothesized this bactericidal function might be hampered. The goal of this study was to assess respiratory burst activity of PMN in leukaemia compared with non-leukaemic control subjects. We assessed the respiratory burst function of PMN as an effective defence against pathogens in leukaemia and control subjects.

Materials and Methods: Peripheral blood samples were collected from leukaemia and control subjects (30 in each case) in lithium heparin anticoagulant containers. Our study cases were 30 leukaemia belonging to different subtypes (AML=12, CML=12, ALL=4 and CLL=2), 22 males and 8 females age range from 2.5–63 years (M±SEM, 28.0±3.4 years); the controls were 26 males and 4 females, age range from 17–53 years (M±SEM, 31.1±1.5 years), respectively. The respiratory burst activity was assessed using the nitroblue tetrazolium (NBT) dye reduction test on stimulated and un-stimulated PMN in leukaemia and control groups.

Results: The cells purity in leukaemia was >99% using Turk's solution and cells viability was >95% by Trypan blue dye exclusion test. The respiratory burst activity of PMNs showed a statistically significant increase ($P<0.05$) in controls compared with leukaemia subtypes. Similarly, comparison within leukaemia subtypes indicates a

statistically significant increase (P<0.05) RBA in CML compared with AML, ALL and CLL, respectively.

Conclusion: The respiratory burst activity of PMNs in leukaemia is variable with enhanced activity in CML subjects, while depressed in AML, ALL and CLL subtypes; suggesting impaired bactericidal capacities of PMNs in these diseases.

Keywords: Blood, Leukaemia, Neutrophils, Northern Nigeria, Respiratory Burst activity.

INTRODUCTION

Protection of the human host against disease-causing pathogens is mediated by many cellular and acellular factors. The professional phagocytic cells of peripheral blood in humans, polymorphonuclear neutrophils (PMN) and macrophages comprise the first line of immune defence against invading pathogens. It has become clear that PMN chemotaxis, diapedesis, phagocytosis, and eventually microbicidal activity each contribute to the ability of PMN to provide an effective first line of immune defence for the host organism. PMN are the only leucocytes that are competent to produce large amounts of reactive oxygen species (ROS) to kill phagocytosed bacteria (1,2). ROS are generated by a variety of intracellular mechanisms, although the predominant mechanism, referred to as respiratory burst, is based upon the assembly and activation of nicotinamide Adenine dinucleotide phosphate (NADPH) oxidase which then catalyzes the univalent reduction of molecular oxygen (O_2) to O_2- (3). This functional activity, referred to as oxidative or respiratory burst contributes to the host defence, but it can also result in collateral damage to host tissues and is thought to be a key factor in the development of pathologies such as acute lung injury (ALI) in its most severe form, acute respiratory distress syndrome (ARDS), as well as the multiple organ failure characteristics of sepsis (4). Any condition that jeopardizes PMN functions adversely affects the host's resistance to invasive infections.

In leukaemia, there exists a disturbance of the delicate balance between self-renewal and differentiation in haematopoietic stem cells (HSCs), resulting in partial differentiation block around the blast/promyelocyte stage (5,6). Normally, blasts constitute 5% or less of healthy bone marrow. In leukaemia, however, these blasts remain immature and multiply continuously, eventually constituting between 30 - 100% of the bone marrow. These malignant blast cells fill up the bone marrow and prevent the production of healthy red cells, platelets, and mature white cells (leucocytes). They spill out of the marrow into the bloodstream and lymph system and can travel to the brain and spinal cord (the central nervous system). As the number of normal cells declines, dangerous symptoms develop, which if untreated become lethal (7).

According to pathological features, there are four major types of leukaemia. Acute leukaemia is divided into acute myeloid leukaemia (AML)

and acute lymphoblastic leukaemia (ALL). Chronic leukaemia is divided into chronic myeloid leukaemia (CML) and chronic lymphocytic leukaemia (CLL) (8). The most important treatment choices include chemotherapy, radiotherapy, and haematopoietic stem cells transplantation (HSCT) (9).

To study the role of the oxidative burst in host protection, it is germane to develop efficient, simple, and highly reproducible techniques to quantify ROS generation by PMN. The PMN reactive oxygen species (ROS) production can be quantified following stimulation with soluble agents or with particles. Different methods have been optimized to measure respiratory bursts of isolated PMN and PMN suspended in heparinized whole blood. We have designed this study to compare the respiratory burst activity of PMN in leukaemia subjects with that of controls.

Materials and Methods

Subjects

The subjects of this present study were patients with different leukaemia subtypes attending Haematology, Medicine and Pediatrics Oncology Clinics of Aminu Kano Teaching Hospital, Kano Nigeria; together with healthy non-malignant controls. They comprised 30 in each case. The study was approved by the Medical Ethics Committee of this hospital and all subjects gave their consent before inclusion in the study. Leukaemia subjects with a recent history of whole blood transfusion of less than one week were excluded from the study.

Sampling and cell preparation

Blood samples were collected from peripheral veins of subjects in lithium heparin (100 IU/ml) plastic containers for leucocytes functional assay. This heparinized whole blood was used to assess respiratory burst activity of PMN following stimulation with formyl- leucine-methionine- phenylalanine (fLMP) (Sigma-Aldrich, USA) as a bacterial peptide.

Respiratory burst activity test: The NBT reduction test in stimulated (fLMP) and unstimulated cells was performed within one hour after specimen collection, according to the method of Park *et al.,* (10) and its modifications (11,12). Two tubes were arranged, in one tube 100 µl of whole blood, 50 µl phosphates buffered saline (PBS) (pH 7.2), 100 ul of 0.2% NBT (Sigma-Aldrich, USA) were then mixed (un-stimulated) test. In the stimulated test in the second tube, 100 µl of whole blood, 25 µl PBS (pH 7.2), 25 µl fLMP and 100 µl of 0.2% NBT (Sigma-Aldrich, USA) were also mixed. After 15 minutes of incubation at 37°C in a water bath (coloured NBT compound is converted to the purple-blue formazan precipitate), a drop of these mixtures was placed on each microscope slide. Blood films were made and stained with Leishman's stain. The percentage of cells containing reduced blue formazan precipitates was counted microscopically using oil immersion objectives. PMN showing formazan deposits were recorded as NBT positive cells, and the result of the test was obtained by subtracting the fLMP stimulated positive cells from those un-stimulated tests.

Statistical analysis

The data in this study were reported mean±SEM). The results of control subjects were compared with patients' results in each group by performing a rank-sum test for non-parametric data and considered significant if the p-value is less than or equal to 0.05. The data were also correlated by Spearman's rank correlation. Analysis was done using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA 92108, USA) and statistical significance is indicated in all tables and figures by a p-value lower than 0.05 notation.

Results

In this study, leukaemia subjects were diagnosed based on clinical diagnosis and peripheral blood evaluations. Thirty leukaemia subjects (8 females and 22 males, ages at diagnosis ranged from 2.5 to 63 years M±SEM = 25.80±3.38 years) were studied (Table 1, Figure 1). Twelve leukaemia subjects (2 female and 10 male) had AML, 1 female and 3 males were ALL, 12 (4 females, 8 males) were CML, with 1 female and 1 male CLL subjects, respectively. The oldest age groups were subjects with CLL followed by CML subjects. The youngest being those with ALL then AML subjects (Figure 1).

The respiratory function test was also compared with cells from thirty healthy non-malignant (age and sex-matched as controls). A statistically significant difference was observed in total white blood cell count between subjects with leukaemia and its subtypes compared with controls ($P<0.05$) (Table 2 Figure 2). The NBT dye-reduction test on stimulated and un-stimulated leucocytes in cases and controls is presented in Table 1, Figure 3. Overall, there was no statistically significant difference ($P>0.05$) in RBA in Leukaemia subjects compared with controls. However, a statistically significant decrease ($P<0.05$) in RBA was observed in ALL and CLL subjects compared with controls, coupled with an increase in RBA particularly in CML subjects compared with controls (Table 2, Figure 3). No statistically significant difference ($P>0.05$) in RBA in AML subjects compared with the controls group.

There was no strong positive and statistically significant relationship ($P>0.05$) between TWB counts and RBA in leukaemia and its subtypes (Figure 4-6).

Table 1: Comparison of age, total white blood cells count and respiratory burst activity in controls and subjects with leukaemia.

	Age	TWBC x 10^9/l	RBA
Controls	31.1±1.5	5.72±0.27	1.2±0.1
Subjects	28.3±1.8	15.94±2.45	1.1±0.1
P value	0.5118	0.0003*	0.4132

Key: TWBC= total white blood cells, RBA = respiratory burst activity,
*= statistically significant

Table 2: Total leucocytes count and respiratory burst activity of controls and subjects with leukaemia

	Controls (n=30)	Subjects (n=30)	AML (n=12)	CML (n=12)	ALL (n=4)	CLL (n=2)
			Mean ±SEM			
TWBC x 10^9/l	5.72±0.27	15.94±2.45	14.68±5.35	15.26±1.49	22.65±8.87	14.20±1.90
RBA	1.20±0.07	1.1±0.14	1.0±0.17	1.7±0.14**	0.00*	0.00*

Key: *p value = statistically significant, TWBC= total white blood cells, RBA = respiratory burst activity, AML= acute myeloid leukaemia, CML- chronic myeloid leukaemia, ALL= acute lymphocytic leukaemia, CLL= chronic lymphocytic leukaemia.

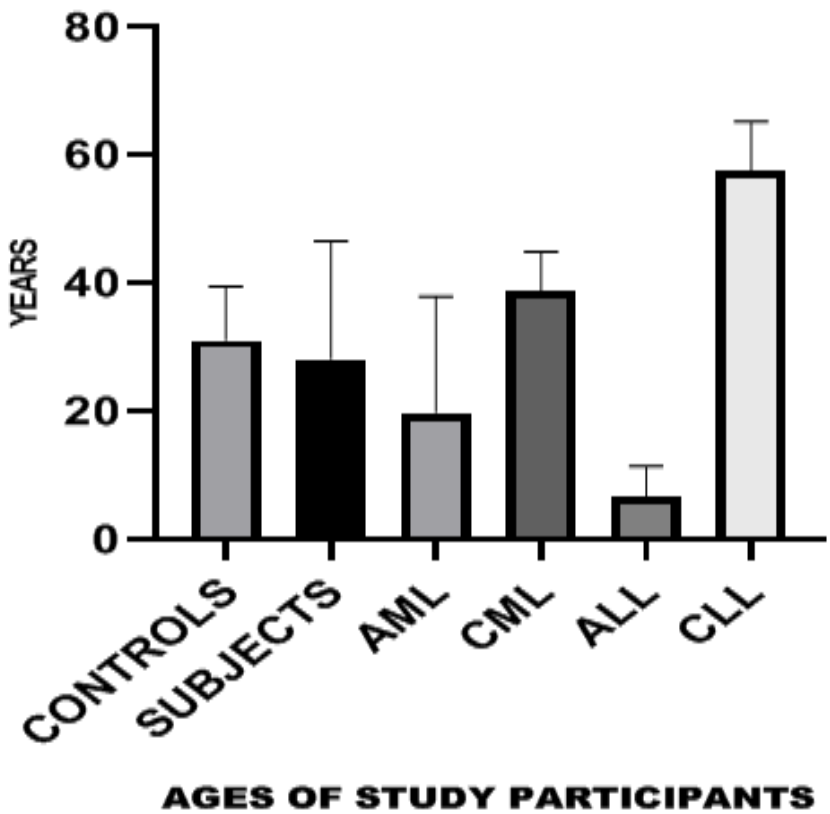

Figure 1: Distribution by age of study participants

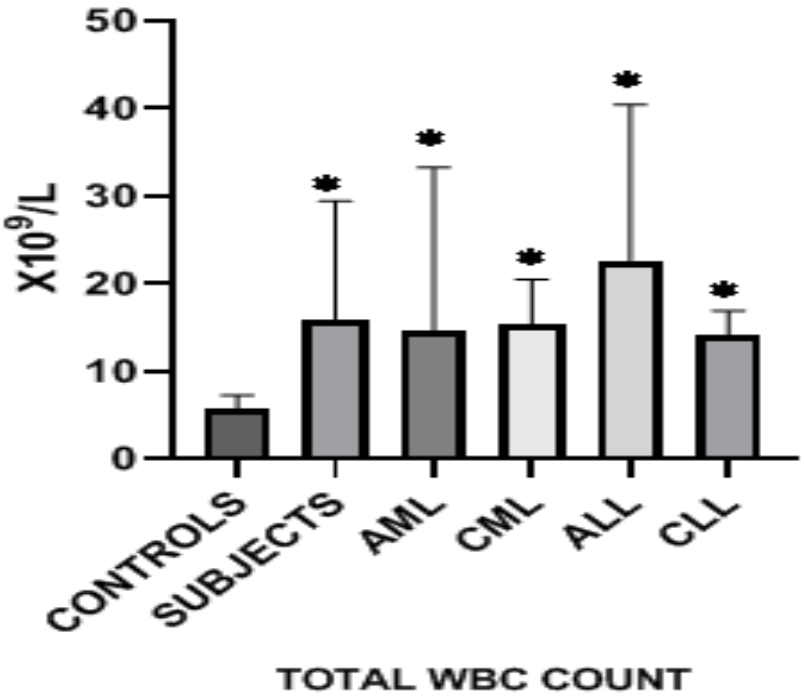

Figure 2: Comparison of total white blood cell count among study participants

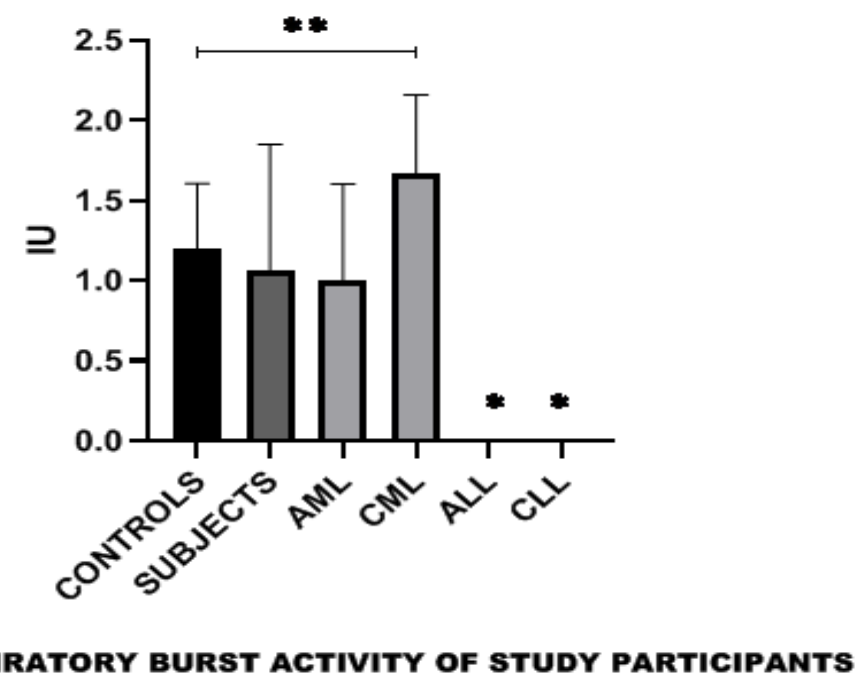

Figure 3: Comparison of respiratory burst activity in study participants

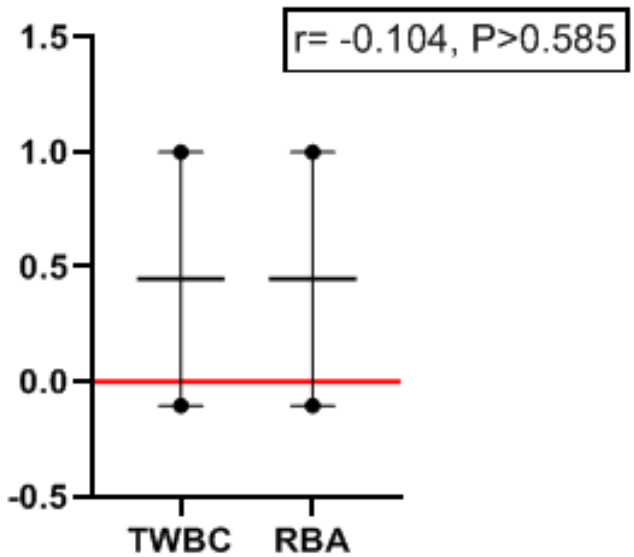

Figure 4: Scatterplot of TWBC count and RBA in Leukaemia subjects. The correlation coefficient is negative and not statistically significant (P>0.585).

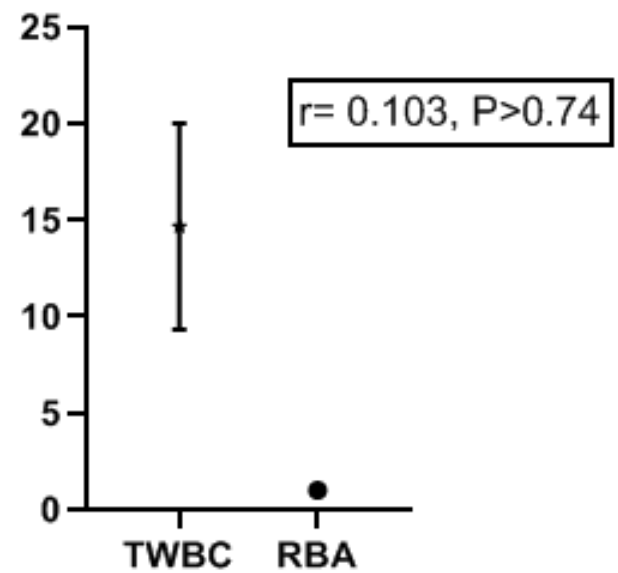

Figure 5: Scatterplot of TWBC count and RBA in AML subjects. The correlation coefficient is weakly positive and not statistically significant (P>0.05):

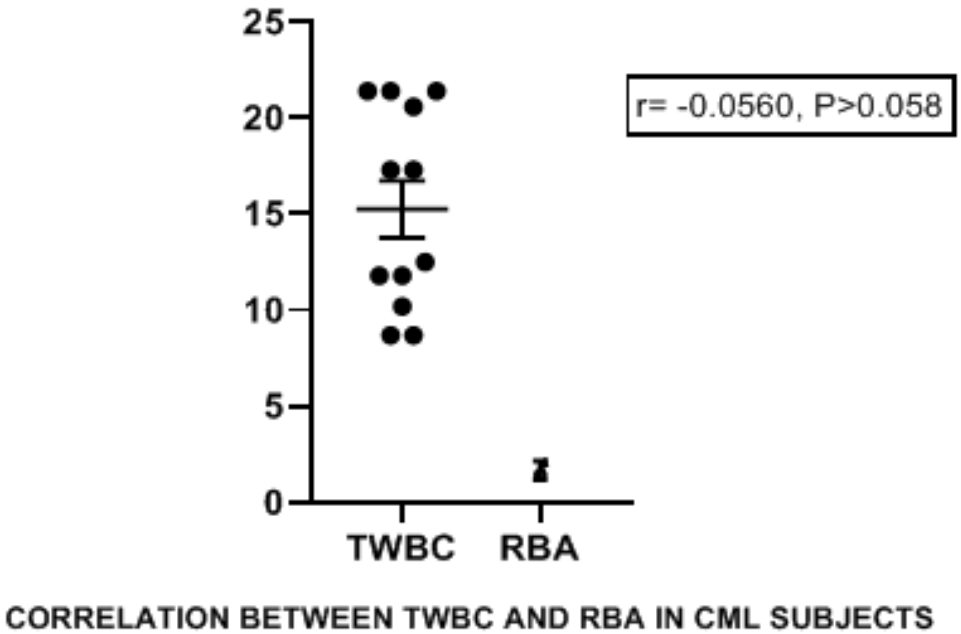

Figure 6: Scatterplot of TWBC count and RBA in CML subjects. The correlation coefficient is negative and not statistically significant (P>0.05)

Discussion and conclusion

In concurrence with chemotaxis and phagocytosis processes, PMN killing of microbe is normally accompanied by a burst of oxidative metabolism resulting in rapid release of high levels of bactericidal reactive oxygen species under the catalysation of NADPH oxidase, myeloperoxidase (MPO), or nitric oxide (NO) synthase. NADPH oxidase is responsible for the generation of ROS, such as superoxide anion (O2–), hydrogen peroxide (H2O2), and hydroxyl radicals (HO-). The generation of these reactive radicals can be demonstrated by the NBT dye reduction test, an important laboratory technique that detects the oxygen-dependent killing activity of PMNs stimulated by microbes (13,14).

In this study, we aimed at assessing the respiratory burst function of PMNs in peripheral blood of subjects with different leukaemia subtypes to ascertain the possible alteration of the physiological function of PMNs.

In this study, the NBT dye reduction test of leukaemia subjects was similar to control subjects with no statistically significant difference. However, comparing the different subtypes with control and each other, we recorded variable functionality. This is in agreement with the study by Mashael and Rasha (15) reports no statistically significant differences between leukaemia subtypes with controls after chemotherapy. Though acute lymphoblastic leukaemia showed a statistically significant reduction compared to the controls, this was also similar to our results. Our report was congruent with that of a study done on subjects in different phases of CML (16) reported that patients who were in remission showed normal intercellular killing and NBT

reduction, whereas the values of patients in chronic and blastic phases were impaired. Also, a study done on CML subjects after imatinib therapy reported that in patients in complete cytogenetic remission, there was no significant difference in NBT test and controls (17). In this our present study, we reported a similar finding in CML subtypes, where the RBA was enhanced compared with controls. Our result of the correlation between total white blood cells counts and the NBT test showed no strong positive with no statistically significant relationship between TWB counts and RBA in leukaemia and its subtypes. The possible explanation is that in leukaemia, the numbers of white blood cells have no relationship with the ability to generate ROS.

Abeer. (17) reported that respiratory burst activity is not reduced in subjects with leukaemia in remission. It was also shown that within each group of leukaemia subtypes, there was no significant difference, except in subjects with lymphocytic leukaemia, which indicated a significant decrease. The result indicates that increased leucocytes count and percentage of immature cells have no influence on the generation of superoxide by lymphocytes.

In conclusion, the observations made in this study of respiratory burst function of PMNs in subjects with different leukaemia subtypes demonstrated that respiratory burst function in different leukaemia subtypes is varied; suggestive that subjects with myelogenous leukaemia might have an effective bactericidal function, while lymphoblastic leukaemia might have impaired bactericidal capacity.

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Conflict of interest
None

REFERENCES

1. Mehrzad, J., Duchateau, L., and Burvenich, C. (2009) Phagocytic and bactericidal activity of blood and milk-resident neutrophils against *Staphylococcus aureus* in primiparous and multiparous cows during early lactation, *Veterinary Microbiology,* Elsevier, 134 (1-2):106.
2. Chen, Y., and Junger, W. G. (2014) Measurement of the oxidative burst in neutrophils, *Methods in Molecular Biology;* 844:115 – 124
3. John-Paul, T., John, F. F., Peter, W., and Yoke, L. F. (2009) Respiratory burst function of ovine neutrophils, *Biomedical Central Immunology;* 10:25:1471 – 2172
4. Nussler A. K., Wittel, U. A., Nussler, N. C., and Beger, H. G. (1999) Leukocytes, the Janus cells in inflammatory disease, *Langenbecks Archives of Surgery;* 384:222 – 232
5. Akiko, T., Charles, G., and Nabeel, R. Y. (2006) NUP98-HOXA9 Induces Long-term Proliferation and Blocks

Differentiation of Primary Human CD34+ Hematopoietic Cells, *Cancer Research;* 66(13):6628 – 6637

6. Galeev, R., and Larsson, J. (2018) Cohesin in haematopoiesis and leukaemia, *Current Opinion in Hematology;* 25(4):259 – 265

7. www.nytimes.com, (2016) Leukemia In-Depth Report, retrieved from https://nytimes.com/Leukemia%20-%20In-Depth%20Report%20-%20NY%20Times%20Health.htm Accessed on 16th January 2016

8. Tayebeh, A. S., Sabariah, M. N., Patimah, I., and Fauziah, O. (2014) The Effects of Herbs and Fruits on Leukaemia, pp 1 - 8, Hindawi Publishing Corporation retrieved from http://dx.doi.org/10.1155/2014/494136 Accessed on 17th January 2016

9. Pokharel, M. (2012) Leukemia: A Review Article, *International Journal of Advanced Research in Pharmaceutical and Biosciences;* **2 (3):**397 – 407

10. Park, B. H., Fikrig, S. M., and Smithwick, E. M., (1968) Infection and nitroblue-tetrazolium reduction by neutrophils, *Lancet;* 2:532 – 534

11. Esfandiari N, Sharma RK, Saleh RA, Thomas AJ Jr., Agarwal A. (2013) Utility of the Nitroblue Tetrazolium Reduction Test for Assessment of Reactive Oxygen Species Production by Seminal Leukocytes and Spermatozoa *Journal of Andrology*; 24(6):862-870

12. Afzal M, Khan QM and Sessitsch A.(2014). Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. *Chemosphere*;117:232-42. doi: 10.1016/j.chemosphere.2014.06.078

13. Michele, A., Norma, A., Catherine, B., Trung, P. H. and Jacques, H. (1991) Effect of a Factor Released by K562 Malignant Cells in Culture on Human Neutrophil Bactericidal Activity, *Infection and Immunity*; 59(8):2673 – 2676

14. Tie-Shan, T., Ai-ling, J., Xin-Ying, J., and Yan-Zhang, L. (2017) Neutrophils and Immunity: From Bactericidal Action to Being Conquered, *Journal of Immunology Research*:1 - 14

15. Mashael, A. A., and Rasha, A. B. (2011) Chemotherapy effect on Neutrophil Function in Leukaemic Patients, *Journal of King Abdulaziz University of Medical Sciences*; 18(1):3 – 13

16. Ajdary, S., Hamidpoor, L., and Zandieh, T. (2002) Function of neutrophils in different phases of chronic myelogenous leukaemia, *Iran Biochemistry Journal;* 6(2 and 3): 83-88.

17. Abeer, A. A. (2013). Neutrophils phagocytic function in chronic myelogenous leukaemia after imatinib mesylate therapy, *Iran Journal of Cancer, Medicine and Genetics;* 6(2): 65 – 71