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Effects of Low Dose Ionizing Radiation on the Hematological Parameters in Medical Imaging and Therapeutic Technologists Working in Selected Governmental Hospitals, Addis Ababa, Ethiopia

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Research Project Submission Form

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Abbreviations

APD	Avalanche Photodiode
BASO	Basophil
CBC	Complete Blood Count
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
EOS	Eosinophil
EQA	External Quality Assessment
FMoH	Federal Ministry of Health
Gy	Gray
Hgb/HGB	Hemoglobin
Hct/HCT	Hematocrit
IQC	Internal Quality Control
IR	Ionizing Radiation
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
mGy	mili Gray
MONO	Monocyte
MPV	Mean Platelet Volume
mSv	mili Sievert
NUET	Neutrophil
PCT	Platelet Concentrate (Plateletcrit)
PDW	Platelet Distribution Width
P-LCR	Platelet Large Cell Ratio

Plt/PLT	Platelet
RBC	Red Blood Cell
RD	Radiograph
RDW	Red Cell Distribution Width
RT	Radiotherapy
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
Sv	Sievert
TASH	Tikur Anbessa Specialized Hospital
TLD	Thermo luminescent Dosimeter
WBC	White Blood Cell

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Summary

Background: Exposure to ionizing radiations including x-rays and gamma rays leads to abnormal hematological findings, cancer (including leukaemia), birth defects in the future children of exposed parents, and cataracts. There is no published report in Ethiopia addressing the effect of low dose radiation on hematological parameters.

Objective: To compare the hematological profile such as (RBCs count, RBC indices, Hb, Hct levels, WBCs, platelets count and peripheral morphology) of medical imaging and therapeutic technologists and controls of selected Governmental Hospitals in Addis Ababa, Ethiopia

Method: A comparative cross-sectional study with 182 participants in the period October 2015 to June 2016 was carried out. Of them, 91 were radiation exposed and 91 were controls. Hematological parameters were analyzed using Sysmex XT-2000i. Peripheral blood morphology was done from a stained smear. Data was entered, cleaned and analyzed using SPSS version 21. Student t-test was used to compare the hematological parameter means between the two groups, the exposed and the control. Bivariate correlation statistics was used to draw association between the dependent and independent variables. P values ≤ 0.05 were considered statistically significant. Data was cleaned, entered and analysed using SPSS 21.

Result: Mean values of White Blood Cells, Mean Cell Hemoglobin, Mean Platelet Volume, Platelet Distribution Width, Platelet Large Cell Ratio, Lymphocytes, Monocytes and Basophils have shown significant difference from the control group. The mean MCH, PDW, P-LCR were higher while WBC, MPV, LYMPH, MONO, and BASO were lower in the exposed group. Atypical lymphocytes were significantly higher in the exposed group; 65/91 of the exposed and only 7 of the non-exposed group have such abnormal picture. There were larger effects on the lymphocyte of exposed workers with high number of atypical lymphocytes. A smaller but not negligible effect was noted on white blood cells and medium effects on mean cell haemoglobin, platelet distribution width, mean platelet volume, platelet large cell ratio, Basophil and monocytes.

Conclusion: It is not deniable that low dose ionizing radiation is imposing impact on the haematological as well as immunological system of medical imaging and therapeutic technologists as there are larger effects on the lymphocyte and basophil subsets of exposed workers.

Key words: *ionizing radiation, hematological parameters**,** atypical lymphocyte*

1. Introduction

1.1.Background

X-rays and gamma rays are forms of radiant energy, like light or radio waves. Unlike light, both x-rays and gamma rays can penetrate the body, which allows a radiologist to produce pictures of internal structures [1]. They can also be defined as an electromagnetic ionizing radiation with an extremely short wavelength and high frequency which tells that they are energetic. X-rays were first discovered by Wilhelm Röntgen in 1895 and were quickly applied to medical diagnostic use. Today, x-rays remain a valuable tool in diagnosis and treatment of many injuries and diseases. Quickly followed, in June 1896, by Becquerel who discovered natural radioactivity and in 1898, by Curie who isolated radium, there came fundamental discoveries that have paved the way for the main techniques of radiotherapy. Generally there are three medical practices involving exposure to ionizing radiation. These are diagnostic radiology (and image-guided interventional procedures), nuclear medicine and radiotherapy [2-5].

X-rays and gamma rays have also a wide application in the medicine, industry and other sciences. In the contrary the use of radiation is not without risk; it leads to exposure of the patient and the radiographer. Although the radiation dose is low in diagnostic examinations, exposure to ionizing radiation cannot be avoided in medical imaging facilities. Therefore, attention should be given in order to minimize unnecessary exposure for members of the public and occupational workers [6]. Computed Tomography (CT) examinations have considerably larger organ doses than those from the corresponding conventional radiograph [7]. The radiation doses received by occupationally exposed workers can be measured by using one of the various types of monitors: thermoluminescent dosemeter (TLD monitor), extremity (or Finger) TLD, the neutron monitor, and special TLD [8].

Radiation exposure can be expressed in certain units. The absorbed dose is measured in grays (Gy) or milligray (mGy) which is the amount of energy deposited in human tissue per unit of mass while the equivalent dose is often expressed in sieverts (Sv) or millisieverts (mSv)

which is the biological risk of exposure to radiation, absorbed dose multiplied by a converting factor based on the medical effects of the type of radiation. For x-rays and gamma rays (and beta particles), the equivalent dose in Sv is the same as the absorbed dose in Gy. Less common radiation dose units include rads, rems, and roentgens [9,10].

X-rays and gamma rays have dangerous biological effects as they can impose ionizing effect when penetrating the living tissue, destroy living cells, cause chromosomal aberrations and impose carcinogenic impact [11]. Damage can be caused to living cells, especially to Deoxyribonucleic acid (DNA) in the cell nucleus when there is exposure to ionizing radiation where the degree of this cellular damage depends on the amount of radiation administered [12]. Damage is also dependent on the radiosensitivity of the species whereas large individual differences have been greatly demonstrated in different studies [13,14].

Ionizing radiation exposure directly damages hematopoietic stem cells and alters the capacity of bone marrow stromal elements to support and/or maintain hematopoiesis **in vivo** and *in vitro*. Exposure to ionizing radiation (IR) induces dose-dependent declines in circulating hematopoietic cells not only through reduced bone marrow production, but also by redistribution and apoptosis of mature formed elements of the blood [15].

Generally, exposure to ionizing radiations including x-rays and gamma rays will lead to abnormal hematological findings, cancer (including leukaemia), birth defects in the future children of exposed parents, and cataracts.This risk associated with each imaging procedure is extremely low but, does slowly increase with the increasing number of exposure medical imaging technologists have [16].

1.2.Statement of the problem

Radiographers and dark room technicians are exposed to a variety of potential chemical hazards and ionizing radiation during their work [17]. A radiation injury of hematopoiesis can lead to hemorrhage, to endo- and exo-infections, and to anemia [18]. In mammalian organisms, the lymphohematopoietic system is the most radiosensitive tissue and radiation-induced suppression of hematopoiesis and immune function has been considered to be one of the most life-threatening consequences of radiation exposure [19]. The risk of cancer among radiologists and radiographers exposed to ionizing radiation in the workplace has been a subject of study since 1940s, when increased mortality from leukemia was reported among radiologists and radiographers compared to mortality among other medical specialists [20]. Epidemiological studies indicate that radiographers employed before 1950 were at increased risk of leukaemia and skin cancer, due to the fact that ionizing radiation used in a variety of imaging procedures can damage cells most likely due to the lack of use of radiation monitoring and shielding. The most consistent finding in this study was increased mortality due to leukaemia [21].

Occupational exposure to carcinogenic substances is an important cause of death and disability worldwide. There has been an estimated 7,000 deaths of leukemia due to exposure to occupational carcinogens. Ionization radiation is one of the responsible carcinogens for the disease outcome of leukemia other than benzene and ethylene oxide [22]. As referred by Sont WN *et al* significant positive excess relative risks have been reported for leukemia as well as for cancer of the rectum, pancreas, and lung. The association between exposure to ionizing radiation and leukemia has been well established and is one of the main outcomes of the studies on atomic bomb survivors [23].

On a multinational retrospective cohort study of cancer mortality, excess relative risk for leukemia excluding chronic lymphocytic leukemia was 1.96 per sievert (Sv) of radiation, which was higher than other causes of cancer [24].A cohort study of 308,297 radiation-monitored workers employed for at least 1 year provided strong evidence of positive associations between protracted low-dose radiation exposure and leukaemia [25].

In a follow up of 27,011 diagnostic X-ray workers in China, a 21% greater incidence of cancer than expected based on the experience of 25,782 physicians who did not routinely use X-rays (RR = 1.21; 95% CI: 1.08 to 1.35) was revealed. It was further suggested that patterns of risk associated with duration of work, and with age and calendar time of initial employment, and the excesses of leukemia and skin cancer and possibly cancers of the breast and thyroid, were due to occupational exposure to x-rays [26]. In addition to the above effects of radiation, there has been a report on diminished cellular and humoral immunity in occupationally exposed workers to low levels of ionizing radiation [27].

Medical imaging can undoubtedly confer substantial benefits in the healthcare of patients, but not without exposing them to effective doses ranging from a few microsieverts to a few tens of millisieverts [28]."There appears to be no threshold below which exposure can be viewed as harmless," said Abrams, professor emeritus of radiology at Stanford and Harvard Universities [29]. As cited by Agrawala PK *et al* there is general scientific consensus that no matter how small, radiation exposure always increases the risk of cancer [30].

Diagnostic X-rays are the largest man-made sources of radiation exposure to the general population, contributing about 14% of the total annual exposure worldwide from all sources. Although diagnostic X-rays provide great benefits, their use involves some small risk of developing cancer is generally accepted [31]. Our aim is to assess the effect of low dose radiation on the hematological parameters of medical imaging and therapeutic technologists.

1.3.Rationale

Nowadays, there has been a rapid increase in the use of medical diagnostic and therapeutic tools like imaging tests of CT scan, x-ray, nuclear medicine tests and radiotherapy which may fuel concern about the long-term consequences of exposure to these ionizing radiations. There remains considerable uncertainty on how to extrapolate radiation risks to low doses and low dose rates, especially in a developing country like Ethiopia. Studies on occupational exposures can provide useful information in this regard. There is much uncertainty about the risks of hematological abnormalities or aberrations and leukemia after repeated or protracted low dose radiation exposure typical of occupational, environmental, and diagnostic medical settings. As the insurance of good health of workers and prevention of diseases is a main and first concern in any organization, specifically medical institutions, there needs to be a way to assess occupational risks and intervene gaps. The present study will quantify associations between protracted low-dose radiation exposures and hematological abnormalities.

2. Literature review

There have been different literatures focusing on determination of the effects of ionizing radiation in view of its impact on the hematopoietic system. One was in Italy in 2012 which has recruited 266 non-smokers, 133 radiation exposed workers and 133 controls. The mean values of total white blood cell were significantly decreased in the exposed workers of both sexes compared to controls. The average values of neutrophils were significantly low in female health workers compared to female controls. Thus, the researchers concluded that ionizing radiation can influence some lines of the hematopoietic system in the exposed workers [32].

Recently, a study evaluated haematological findings in healthy workers of Radiology Department of a hospital of Mashhad, Iran. The study was carried out in 2015 on 55 participants including 25 individuals working with x-ray machines and 30 healthy volunteers as controls. Blood samples had been analysed for the basic and routine cell counts which included hemoglobin content (Hb), hematocrit (Hct%), red blood cell (RBC) count, white blood cell (WBC) count and platelet (Plt) and other indices such as Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Mean platelet volume (MPV), RBC distribution width (RDW), Platelet distribution width (PDW), and P-LCR (platelet large cell ratio). Of all the hematologic parameters, PDW and P-LCR showed significant increase in the X-ray technicians than the control groups [33].The same kind of study was conducted in Iran in 2008, seven years back from the above study. 60 males occupationally exposed radiotherapeutic and diagnostic workers working for the last 14 years on an average with a group of 60 healthy control subjects in the same range of age, gender and ethnic origin were recruited. Radiation field workers had significantly decreased platelet and white blood cell count in comparison to controls [34].

A case-control study conducted in Iraq by 2011 has assessed the effects of radiation on the hematological parameters in 47 apparently healthy x ray technicians as compared with 20 apparently healthy controls. By performing laboratory tests of the hematological parameters and blood cell morphology the study observed no significant variation in the hematological

parameters while significantly high percentage of atypical lymphocytes was observed. Positive correlation was found between atypical lymphocyte percentage in the exposed group and duration of exposure to radiation in years [35].

In Iran, a study had incorporated 40 exposed and 40 non-exposed participants in 2013-2014. Radiation workers with at least 10 years work record showed lower Hg and MCV than the control group. Radiology workers showed decreased RBCs compared to other radiation workers. It has been concluded that monitoring of haematological parameters of radiation workers can be useful as biological dosimeter [36].

In a similar but genetic cross sectional study 30 occupationally exposed workers and 7 controls were selected in Iran in 2015. There was significant increased incidence of chromatid gap and chromatid break in nuclear medicine and CT scan workers blood cells [37].

A study done in the Kingdom of Saudi Arabia in the year 2002 had recruited 40 apparently healthy male x-ray technicians and another 40 apparently healthy matched controls to perform complete count of blood cells and determine observable changes. The study reported a significant decrease in the mean value of platelet counts in the exposed x-ray technicians. The study did not find a significant change in red cell and white cell counts, which additionally did not report a significant association with time of exposure [38].

Another recent study was conducted in 2014 by Saman Shahid and his colleagues in Pakistan. This study was comprised of 20 radiotherapy (RT) workers, 41 radiology (RD) workers, 31 nuclear medicine workers and 55 radiation unexposed workers. Hemoglobin, white blood cell, hematocrit, MCH, MCHC, neutrophil and platelet were low in most of the radiation exposed workers as compared to the non-exposed while RBC and lymphocytes were in the high range [39]. In the same year, effect of radiation on lymphocytes of 28 radiographers was determined by a Cross-sectional study in Mataram town, capital of the Indonesian province of West Nusa Tenggara and results indicated that characteristics of the radiologist Age ($p = 0.028$), radiation protection training ($p = 0.046$), use of Avalanche like use of Photodiode (APD) radiation detector ($p = 0.026$) and radiation dose ($p = 0.046$) [40] affect the lymphocytes.

Survey on low-dose medical radiation exposure in occupational workers was conducted in 2013 in Seoul, Republic of Korea. This was on 370 occupational workers and 335 controls. WBC counts were decreased in male and increased in female workers when the occupation period was longer. The RBC counts were lower in male workers while eosinophil counts in female workers were lower as compared to the control group. When the cumulative dose was large, the lymphocyte counts decreased in workers of both sexes. Platelet count and RBC count were lower in male and female workers than in the control group respectively. Abnormal distributions of some blood indices were observed in the occupational radiation workers compared with the controls [41].

The incidence of chromosomal aberrations were evaluated in the lymphocytes of peripheral blood of 40 persons working in different dental colleges and clinics in and around Bangalore occupationally exposed to X-rays. The investigators have observed that the radiographer showed a significant increase of chromosomal aberration in the lymphocytes of their peripheral blood. This might lead to the origin of atypical lymphocytes and future risk of cancer [42].

A study by Rozga and his colleagues in Zagreb Croatia, aimed to study whether chromosomal aberration and hematological alterations could be used as biomarkers for possible injury in workers exposed to ionizing radiation. The study consisted 483 participants where 76 were radiologists, 46 were pulmonologists, 201 x-ray technicians and 160 controls. Blood samples were taken for both chromosome analysis and blood cell count. Though the incidence of all types of chromosomal aberrations was higher in the exposed group, no significant changes in the hematological findings typically leukocyte, lymphocyte and thrombocyte counts were found. The study concluded that chromosomal aberrations are more sensitive biomarkers for radiation injury than hematological findings [43].

With a comparable objective with the above, there are studies which were aimed at determining the effect of radiation exposure on phagocytic activity of polymorphnuclear neutrophils, neutrophil adherence and spontaneous migration of leukocytes. In the study of Hrycek *et al,* 44 individuals operating x-ray equipment and controls were examined. In the persons employed in radiology departments statistically significant reduction of neutrophil adherence was shown, which especially in the subgroup of men was observed. Statistically

significant reduction of spontaneous migration area of leukocytes was revealed and it concerned both the subgroup of men and subgroup of women [44].

In a similar study in 2003 in Saudi Arabia a group of 8 x-ray technicians and 8 control groups were recruited for determination of polymorpho-nuclear neutrophil's phagocytic activity. This was determined by chemiluminescence response by luminometer. The study has concluded that a better protection and low dose exposure to X-ray radiation does not affect the physiological functions of polymorpho-nuclear neutrophils by means of chemiluminescence response. However, they have recommended the requirement of large size studies to confirm the effects of Dental X ray radiation on the phagocytic activity of Polymorpho-nuclear neutrophils (PMNs) in dental X-ray technicians [45].

By 2002 Hrycek A. *et al* conducted a research on the effects of radiation on lymphocytes and interleukins. The mean absolute number of peripheral blood lymphocytes in workers operating radiological equipment was slightly lower than that in the control group but the difference was not statistically significant. There were no statistically significant differences in the absolute number of peripheral blood lymphocytes in the subgroups selected with respect to sex and employment period. Nevertheless, the lowest absolute number of peripheral blood lymphocytes was revealed in women subgroup operating X-ray equipment [46].

A case-control study was carried out in Egypt in 2011 with participants of 20 nuclear medicine workers and 20 controls, from the administrative staff of Assiut University. Reports of bleeding tendency and recurrent infections were high in the workers than controls with subsequent lower count of lymphocytes in the workers group. It has been concluded that immunological status of health care providers is affected by radiation through its effect on lymphocytic subset [47].

In 2011, the effect of x-ray radiation on hematopoietic system of radiology technologists was studied on 95 male workers in Khartoum state hospital, Sudan. Samples from the participants were analysed for hematologic parameters with a final report of significantly decreased leukocyte, neutrophil and lymphocyte count compared to controls. Duration of exposure had also have a greater significance in reducing cell counts. However, there was no significant difference in the other parameters except the above three [48].

Taken together, most of the studies reviewed above revealed effect of low dose radiation on the hematologic parameters of exposed workers, though some of them did not establish clear association. However, there is no published report in Ethiopia investigating the effect.

3. Objectives

3.1.General objective

- To evaluate the effects of low dose ionizing radiation on the haematological parameters in medical imaging technologists of selected governmental hospitals, Addis Ababa, Ethiopia

3.2.Specific objectives

- To compare means of complete blood count parameters between the exposed and control groups
- To determine morphological abnormalities in the exposed and control groups
- To determine the association between sex and age of participants with morphological abnormalities
- To determine the association between work experience and morphological abnormalities
- To determine the association between practice of using personal protective equipment with morphological abnormalities

4. Hypothesis

- There is no statistically significant difference in the complete blood count parameters and cell morphology of medical imaging and therapeutic technologists and controls.
- There is no statistically significant association between morphology of cells and sex, age, use of personal protective equipment and work experience of participants.

5. Materials and methods

5.1.Study area

This study is conducted in selected governmental hospitals of Addis Ababa, Ethiopia namely; Tikur Anbessa Specialized Hospital, St Paul Millennium Medical College Hospital, Yekatit 12 Hospital, Zewditu Memorial Hospital, Ras Desta Damtew Memorial Hospital, Minelik Hospital, ALERT Hospital and Tirunesh Beijing Referral Hospital. Currently, Addis Ababa, the capital city of Ethiopia, has 12 state run and more than 40 private hospitals. Many of the later were built in the past 21 years. In sharp contrast however, all of the state run hospitals were built more than 30 years ago. For a city of an estimated four to five million population, state run hospitals are the best medical care alternative centers used mostly by the middle-to-low income inhabitants of the city. However, Tikur Anbessa Specialized Hospital is the largest referral hospital in the country where even the sick wealthy are referred to before flying out of the country. Out of the 12 hospitals, the Federal Ministry of Health (FMoH) administers four, two are under the Army and Police, five are under the city government of the Addis Ababa health bureau and one (Tikur Anbessa Specialized Hospital) is under the Addis Ababa University [49]. All the selected eight hospitals have high number of patient flow where high number of professionals or medical imaging and therapeutic technologists are expected to work in.

5.2.Study period

The study was conducted from October 2015 to June 2016 where the data collection took a month and half from April 2016 to May 2016.

5.3.Study design

A comparative cross-sectional study was conducted to assess effects in the hematological profile and blood cell morphology of medical imaging and therapeutic technologists in selected governmental hospitals, Addis Ababa, Ethiopia.

5.4.Population

5.4.1. Source population

All health professionals working in Tikur Anbessa Specialized Hospital, St Paul Millennium Medical College Hospital, Yekatit 12 Hospital, Zewditu Memorial Hospital, Ras Desta Damtew Memorial Hospital, Minelik Hospital, ALERT Hospital and Tirunesh Beijing Referral Hospital.

5.4.2. Study population

All medical imaging technologists and radiotherapy workers of the selected hospitals

5.4.3. Controls

Healthy controls, with the same range of age, sex, and area of residence in 1:1 ratio with the exposed workers were taken.

5.5.Inclusion and exclusion criteria

5.5.1. Inclusion criteria

All apparently healthy workers with work experience of one year (1year) and above were included.

5.5.2. Exclusion criteria

Participants, both exposed and unexposed, with gross anemia, pregnancy, known history of diabetes mellitus, cardiopulmonary disease, acute or chronic infection, autoimmune disease, malignancy, those who have taken radiotherapy or chemotherapy, those who are taking any drug during the study period, and those who have taken vaccines in the last 6 months were all excluded.

5.6.Variables

5.6.1. Dependent variable

- Hematological parameters

5.6.2. Independent variables

- Age
- Sex
- Place of work/Hospital

- Use of protective equipment
- Work experience

5.7.Measurement and Data collection

5.7.1. Sampling method

Convenient sampling method was used to collect data from the study sites. The participants were on job while collecting data.

5.7.2. Sample size determination

Sample size was determined by taking all the radiographers, nuclear medicine workers and radio therapeutic technologists in the eight hospitals available through the data collection period who are fulfilling the explained criteria and who are volunteers to participate by giving their informed consent. In this study 182 participants were recruited. A total of 91 apparently healthy occupational radiation exposed workers and a total of 91 apparently healthy and unexposed controls were included.

5.7.3. Data collection procedure

Details of the socio-demographic background, occupational and medical history regarding work-related exposure to mutagenic agents, safety measures taken, duration of exposure, use of therapeutic drugs, recent vaccination, smoking, and drinking was obtained from a questionnaire that was completed by each study participant. The information was used to include or exclude participants.

About 3ml of venous blood was collected from volunteer participants, who have fulfilled the criteria, into lavender Ethylenediaminetetraacetic acid (EDTA) tube for complete blood count and blood cell morphology tests. In this collection process a 20-21 gauge needle was used in order to avoid clotting or hemolysis. For proper mixture of blood and anticoagulant, collected specimen was mixed by inverting the tubes 8-10 times. Each specimen was checked for the presence of clots prior to labelling and analysis. Standard venous blood collection procedure was followed to ensure the quality of specimen. Complete Blood Count was performed within four hour of collection while smears for morphology were prepared as soon as blood was collected, as to prevent the anticoagulant in the EDTA tube from affecting the morphology of cells. The standard operating procedure for venous blood collection is annexed [50].

5.8. Hematological analysis

The collected blood samples were analyzed for all the hematological parameters aimed to be assessed in this study. Complete Blood Count (CBC) and morphological tests were performed by sysmex XT-2000i automated analyzer and manual smear review of wright stained blood film, respectively. Details of the procedures are annexed.

5.8.1. Principle of CBC by sysmex XT-2000i

Sysmex XT-2000i performs analysis based on the electrical resistance detecting method (hydro dynamic focusing method), flow cytometry method using semiconductor laser and SLS-hemoglobin method. The following are the principles of the analyzer:

Hydro dynamic focusing method

Inside the detector, the sample nozzle is positioned in front of the aperture and in line with the center. After diluted sample is forced from the sample nozzle into the conical chamber, it is surrounded by front sheath reagent and passes through the aperture center. By passing through the aperture center, the cells provide nice shape of cell signals. After passing through the aperture, the diluted sample is sent to the catcher tube.

Flow cytometry method using semiconductor laser

Cytometry is used to analyze physiological and chemical characteristics of cells and other biological practices as they flow through an extremely small pathway. A blood sample is aspirated, measured, diluted to the specified ratio, and stained. The sample is then fed into the flow cell. This sheath flow mechanism improves cell count accuracy and reproducibility. Since the blood cell particles pass in a line through the center of the flow cell, the generation of abnormal blood pulses is prevented and flow cell contamination is reduced. A semiconductor laser beam is emitted to the blood cells passing through the flow cell. The forward scattered light is received by the photodiode, and the lateral scattered light and lateral fluorescent light are received by the photo multiplier tube. This light is converted into electrical pulses, then making it possible to obtain blood cell information.

SLS-hemoglobin method

The SLS-hemoglobin method is an analysis method that makes use of the advantages of two methods, Cyanmethemoglobin and oxyhemoglobin. As with the oxyhemoglobin method, the hemoglobin conversion speed of the SLS-hemoglobin method is fast and the method does not use poisonous substances, making it a suitable method for automation. Similar to the cyanhemoglobin method, the SLS-hemoglobin method can also accurately measure blood, containing methemoglobin, such as control blood. In the SLS-hemoglobin method, surfactants lyse the red blood cell membrane releasing hemoglobin. The globin group of the hemoglobin molecule is altered by the hydrophilic alkyl group of sodium lauryl sulfate. This includes the conversions of hemoglobin from the ferrous (Fe^{+2}) to the ferric (Fe^{+3}) state forming methemoglobin, which combines sodium lauryl sulfate to become SLS-Hbhemichrome molecule.

The analyzer uses seven reagents, CELL PACK (EPK), STROMATOLYSER-4DL (FFD), STROMATOLYSER-4DS (FFS), SULFOLYSER (SLS), STROMATOLYSER-FB (FBA), RET-SEARCH (II) (dye solution), RET-SEARCH (II) (diluents) (RED) and CELL CLEAN, which are all in a closed system.

The hematological parameters generated by the automated analyser and included in this study were WBC (White Blood Cell count), RBC (Red Blood Cell count), Hgb (Hemoglobin), Hct (Hematocrit), MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin), MCHC (Mean Cell Hemoglobin Concentration), Plt (Platelet) , RDW (Red Cell Distribution Width) , PDW (Platelet Distribution Width), MPV (Mean Platelet Volume), P-LCR (Platelet Large Cell Ratio), PCT (Plateletcrit), NEUT (absolute Neutrophil count), LYMPH (absolute Lymphocyte count), MONO (absolute Monocyte count), EO (absolute Eosinophil count) , and BASO (absolute Basophil count).

5.8.2. Principle of Wright stain

Wright stain is an example of alcohol containing Romanowsky stains. These stains contain eosin Y which is an acidic anionic dye and azure B and other thiazine dyes (derived from the oxidation, or polychroming, of methylene blue) which are basic cationic dyes. When diluted in buffered water, ionization occurs. Eosin stains the basic components of blood cells, e.g. hemoglobin stains pink-red, and the granules of eosinophils stain orange-red. Azure B and other methylene blue derived dyes, stain the acidic components of cells. Nucleic acids and

nucleoprotein, stain various shades of mauve-purple and violet, the granules of basophils stain dark blue-violet, and the cytoplasm of monocytes and lymphocytes stains blue or blue-grey. The staining reactions of Romanowsky stains are pH dependent which is why the stains are diluted in buffered water of specific pH. The standard operating procedure is annexed. (50)

5.9.Data quality control

Specimens were analysed in a laboratory that the essential elements of a quality program, specifically internal quality control (IQC) and external quality assurance (EQA), are being applied to each laboratory assay performed in order to ensure test result accuracy and precision. Samples were properly collected, transported and stored. Analysis was performed by following standard operating procedure (SOP) for both CBC and peripheral smear tests. Three level hematology controls (High, Medium, Low) were run daily. Smears were microscopically examined by three laboratory technologists, including the principal investigator, and commonly agreed on results were taken as final results where atypical lymphocytes were confirmed by a pathologist.

5.10. Data analysis and Interpretation

Analysed results of the hematological tests were entered into Statistical Package for Social Sciences (SPSS) software version 21 for statistical analysis. Cross-tabulation was used to explain socio demographic characteristics, age and sex distribution of participants. Independent t-test or student t-test was used to compare the hematological parameter means between the two groups, the exposed and the control. Statistical values for $p < 0.05$ were considered significant. Cohen's d was manually calculated to measure the magnitude of the effect size. Cohen's d values less than or equal to 0.2 were considered as small effects, Cohen's d values less than or equal to 0.5 were considered as medium effects and Cohen's d values greater than 0.5 were considered as large effects. Tables, bar charts and figures are used to display results. Bivariate correlation statistics was used to draw association between the dependent and independent variables.

5.11. Ethical consideration

The study was commenced after getting ethical approval from the Ethical Review Committee of the Department of Medical Laboratory Sciences. A letter asking approval of this research study have been sent to Tikur Anbessa Specialized Hospital, St Paul Millennium Medical College Hospital, Yekatit 12 Hospital, Zewditu Memorial Hospital and Ras Desta Damtew

Memorial Hospital, Minelik Hospital, ALERT Hospital and Tirunesh Beijing Referral Hospital from Addis Ababa University, Graduate School of Medical Laboratory Sciences. Consent was obtained from the research participants. Before communicating the results to participants, we are consulting with hematologist and pathologist for further possible management and decision.

5.12. Dissemination of result

The result of this study will be communicated to the hospitals for appropriate action. The thesis will be publicly defended and submitted to the Graduate School of Medical Laboratory Sciences. Information will also be presented to the Medical and Scientific community at different conferences. Manuscript will be submitted to peer reviewed journals for possible publication.

6. Results

Background information of the study participants

Among 182 participants 91 were radiation exposed workers and the other 91 participants were from radiation unexposed workers. Radiation unexposed workers were from miscellaneous profession but from a same hospital compound from which the exposed workers were sampled, for example, laboratory technologists, nurses, and physicians. All radiation exposed workers and radiation unexposed workers included were from the eight selected hospitals. From the exposed workers 20 (21.98%) were from Tikur Anbessa Specialized Hospital, 12 (13.19%) from Yekatit 12 Hospital, another 12 (13.19%) from St Paul Millennium Medical College Hospital, 18 (19.78%), from Zewditu Memorial Hospital, 5 (5.49%) from Ras Desta Damtew Memorial Hospital, 9 (9.89%) from Minelik Hospital, 5 (5.49%) from ALERT Hospital and 10 (10.99%) from Tirunesh Beijing Hospital. (Figure 1)

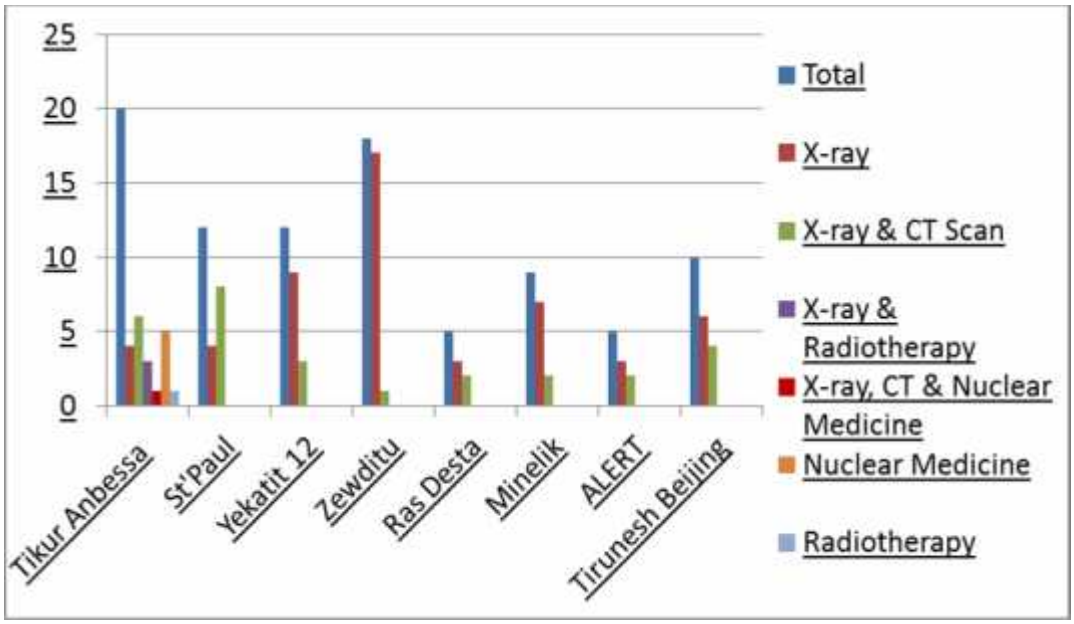

Figure 1. Distribution of Occupational Radiation Exposed Workers in Eight Hospitals, Addis Ababa, April-May, 2016 , (n=91)

Above half of the study participants, both the exposed and the controls, were in the age group 21-30, where 58 (63.74%) were in the exposed group and 71 (78.02%) were in the control group. Fourteen (15.38%) and 13 (14.29%) of the exposed group and the control group were in the 31-40 age group, respectively. In the age group 41-50, 11 (12.09%) were in the exposed workers and 5 (5.49%) were in the unexposed (control) group. Equal number of females and males were recruited in the two groups, which were 23 (25.27%) females and 68 (74.73%) males. As shown in the table, the majority did not use personal protective equipment (PPE) in the past one year (Table 1); all except those working in radiotherapy unit of Tikur Anbessa Specialized Hospital have no PPE.

Table 1. Age and Sex Distribution of exposed and non-exposed participants and usage of lead apron in Eight hospitals, Addis Ababa, April-May, 2016

Parameter	Occupational Radiation Exposed Workers (n=91) No (%)	Occupational Radiation Unexposed Workers (n=91) No (%)	Total
Age Group			
21-30	58 (63.74)	71 (78.02)	129 (70.88)
31-40	14 (15.38)	13(14.29)	27 (14.84)
41-50	11 (12.09)	5 (5.49)	16 (8.79)
51-60	8 (8.79)	2 (2.20)	10 (5.49)
Sex			
Female	23 (25.27)	23 (25.27)	46 (25.27)
Male	68 (74.73)	68 (74.73)	136 (74.73)
Use of PPE			
Yes	13 (14.28)		
No	78 (85.72)		
Total	91	91	182

As depicted in Figure 2, working experience of the occupational radiation exposed workers ranges from 1 year to 37 years, where 46 (50.55%) worked for 1-5 years, 27 (29.67%) worked for 6-10 years, 5 (5.49%) worked for 11-20 years, 10 (10.99%) worked for 21-30 years and 3 (3.3%) worked for 31-40 years.

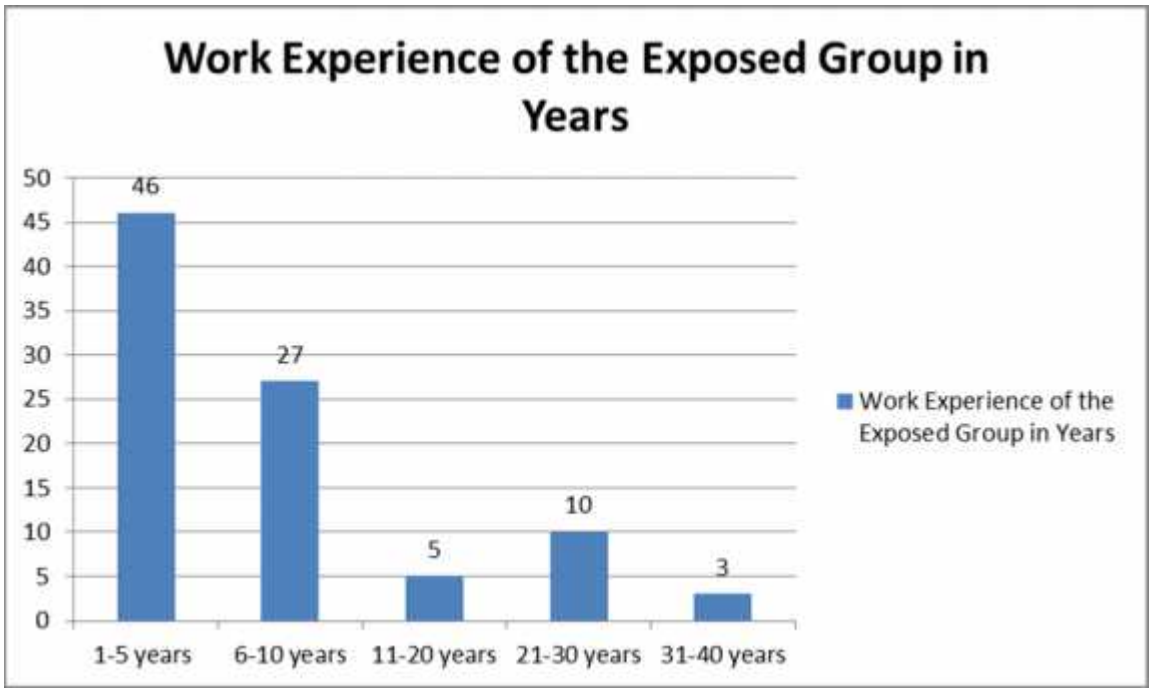

Figure 2. Working experience of the occupational radiation exposed workers in the Eight Hospitals, Addis Ababa, April-May, 2016 , (n=91)

Complete Blood Count

As displayed in Table 2a, the mean CBC values of the exposed group were lower than the control group for almost half of the CBC parameters analyzed, except for hemoglobin, hematocrit, MCV, MCH, MCHC, PCT (platelet concentrate), PDW, MPV, P-LCR and eosinophil. The level of significance of the differences for those with statistically significant differences (WBC, MCH, MPV, PDW, P-LCR, LYMPH, MONO, BASO) is independently tested and displayed in Table 2b.

Table 2a. Mean Complete Blood Count (CBC) values of radiation exposed and non-exposed group in the Eight Hospitals, Addis Ababa, April-May, 2016

	Exposed (n=91)	**Non-exposed (n=91)**
WBC (10^3/u L)	6.1266 ± 1.859	6.9499 ± 2.182
RBC (10^6/uL)	5.1860 ± 0.451	5.1949 ± 0.473
HGB (g/dL)	15.427 ± 1.300	15.206 ± 1.509
HCT (%)	44.789 ± 3.605	44.357 ± 3.637
MCV (fL)	86.176 ± 5.277	85.562 ± 3.977
MCH (pg)	29.8055 ± 1.493	29.2846 ± 1.524
MCHC (g/dL)	34.457 ± 1.234	34.240 ± 1.093
PLT (10^3/uL)	253.621 ± 72.056	267.341 ± 48.729
RDW (%)	13.911± 0.772	14.009 ± 0.885
PDW (fL)	13.1378 ± 2.099	12.2979 ± 1.819
MPV (fL)	10.6611 ± 1.463	10.1211 ± 0.794
P-LCR (%)	28.8200 ± 5.744	25.9978 ± 5.536
PCT (%)	0.2677 ± 0.061	0.266 ± 0.046
NEUT (10^3/u L)	3.1511 ± 1.357	3.5619 ± 1.681
LYMPH (10^3/u L)	2.020 ± 0.594	2.357 ± 0.606
MONO (10^3/u L)	0.4846 ± 0.175	0.569 ± 0.200
EOS (10^3/u L)	0.444 ± 0.377	0.426 ± 0.395
BASO (10^3/u L)	0.0208 ± 0.127	0.0261 ± 0.173

Table 2b. T test analysis of significantly different mean CBC values between radiation exposed and unexposed workers in the Eight Hospitals, Addis Ababa, April-May, 2016

Parameters	t-test for equality of means			
			95% confidence interval of the difference	
	T	p-value	Lower	Upper
WBC	-2.739	**0.007**	-1.41635	-0.23025
MCH	2.329	**0.02**	0.07950	0.96226
PDW	2.868	**0.005**	0.26205	1.41773
MPV	3.077	**0.002**	0.19365	0.88635
P-LCR	3.356	**0.001**	1.16267	4.48177
LYMPH	-3.777	**0.000**	-0.51345	-0.16106
MONO	-3.027	**0.003**	0.14023	0.02956
BASO	-2.347	**0.020**	-0.00982	-0.00085

Peripheral Blood Morphology of cells

Blood cell morphology of all the participants have shown no abnormality in the red blood cells while white blood cell lines specifically the lymphocyte morphology has shown a significant number of atypical lymphocytes in the radiation exposed workers than the control groups. Image from a participant is displayed in Figure 3. Atypical lymphocytes were seen in blood of 65 radiation exposed workers and only in 7 individuals' blood from the control group (Table 3).

Table 3. Frequency of atypical lymphocytes in radiation exposed and control group in the Eight Hospitals, Addis Ababa, April-May, 2016

	Atypical lymphocytes No (%)	Normal cells	Total	t-test	p-value	95% Confidence interval	
Exposed	65 (71.43%)	26 (28.57%)	91			Lower	Upper
Unexposed	7 (7.69%)	84 (92.31%)	91	11.528	**0.000**	0.528	0.746

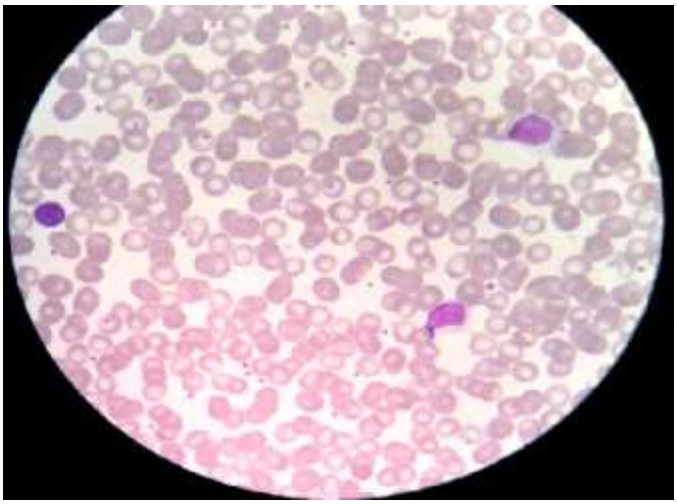

Figure 3. Blood film of radiation exposed workers showing atypical lymphocytes, high power field X1000

Effect size determination using Cohen's d value for hematological parameters showing statistically significant differences

After obtaining significant results of some parameters from the independent t-test, Cohen's d was calculated and interpreted to determine the effect of radiation exposure on hematological parameters (Table 4). Smaller effect on the white blood cell count, medium effect on MCH, PDW, MPV, P-LCR, Basophil and Monocyte, and larger effect on absolute Lymphocyte count and atypical lymphocyte has been recorded.

Table 4. Effect size of observed significant differences

Parameters	Cohen's d value	Effect size
WBC	0.2	Small effect
MCH	0.345	Medium effect
PDW	0.22	Medium effect
MPV	0.458	Medium effect
P-LCR	0.5	Medium effect
LYMPH	0.562	Large effect
MONO	0.45	Medium effect
BASO	0.351	Medium effect
Atypical lymphocytosis	1.69	Large effect

The study also tried to analyse if there are any association between abnormal blood cell morphology and characteristics of the study participants like sex, age, work experience, use of protective equipment and place of work. Accordingly, all the independent variables have no statistically significant association with the blood cell morphology of the participants (Table 5).

Table 5. Association of the independent variables with cell morphology

	Abnormal Morphology	
Variables	Pearson Chi-Square	p-value
Sex	0.005	0.945
Age in group	2.892	0.409
Work Experience in group	7.922	0.094
Use of Protective equipment	4.867	0.182
Hospital	4.052	0.774

7. Discussion

Exposure of cells to ionizing radiation induces damage in various cellular compartments and results in complex biological responses [51]. It has been described that blood cell counts immediately drop soon after irradiation with high doses of ionizing radiation like in radiotherapy [52]. Some studies have demonstrated the negative effect of low dose radiation on haematological parameters while others detect the change at genetic analysis level only. No published study is available from our country. Thus, this study has recruited x-ray technicians, radiotherapists and nuclear medicine workers as they are continuously exposed to occupational ionizing radiation typically x-ray and gamma ray. In view of the importance of having biological dosimeters in addition to the physical ones this study has analysed blood samples of the exposed workers for complete blood count and cell morphology and compared it with controls.

Our study revealed that a number of the CBC parameters are affected in the radiation exposed workers as compared to workers who were not exposed to radiation. For example, the mean white blood cell count of radiation workers was significantly lower than controls. This is similar with Italian study in 2012 [32], with Iranian study in 2008 [34], Pakistani study in 2014 [39], and with Sudanese study in 2011 [48] which all have reported lower leukocyte count in radiation exposed technologists than the respective controls. Lowering in the count of white blood cells in ionizing radiation exposed workers might imply the effect of radiation on the disease or infection prevention ability of workers i.e. the immunity of radiation workers might be deteriorating.

In the present study the mean cell haemoglobin was one of the complete blood count parameters which showed a statistically significant difference between the exposed and the non-exposed groups. MCH was higher in the exposed group than the control. This finding is comparable only with the Pakistani study conducted in 2014 whereas all other studies had not found a significant effect on MCH. Here in our study MCH is higher in the exposed group which is the opposite of the Pakistani study finding [39].

From our findings PDW, P-LCR and MPV are the other higher values in the radiation exposed group in comparison with the control. Similarly higher value in PDW and P-LCR has been reported by a study in Iran by 2015 but MPV was not significant in their study [33].

This Iranian study is the only study that has incorporated hematologic parameters like MPV, P-LCR and PDW where we can make comparison with. Increment in platelet large cell ratio (P-LCR) is associated with thrombocytopenia. P-LCR has a direct relation with PDW and MPV while it is indirectly related to platelet count [53] as there was no statistically significant effect on platelet count in our study.

As the immune system is so vulnerable to the exposure of ionizing radiation and specifically lymphocytes being the most radiosensitive cells [27] our study with other different and vast studies have found a very significant effect on the lymphocytes of exposed personnel. The absolute lymphocyte count as well as morphology of lymphocytes of exposed workers was significantly different from the control group. The mean value of lymphocyte count was significantly lower in the radiation technologists than the controls. From 91 of exposed workers 65 of them showed atypical lymphocytes in their blood smear which is very significant. Egyptian and Sudanese studies had also revealed lowering of lymphocytes in exposed workers [46, 47]. In line with our finding on lymphocyte morphology, significant atypical lymphocytosis was appreciated by researchers in Iran by 2011 [35].

In the current study, there was no significant association between occurrence of atypical lymphocytosis in the peripheral blood smear and sex, age, work experience or exposure time and use of protective equipment of the participants. This finding differs from others who demonstrated high rate of atypical lymphocytosis. The aforementioned Iranian study [35] as well as a study by Indonesian researchers has got association between lymphocyte abnormality and different characteristics of radiologists like age, exposure period and others [39]. So, based on our finding we cannot identify factors that could be aggravating risk factors associated with low dose of ionizing radiation exposure. Cells respond to variable environments by changing gene expression and gene interactions [54]. This might be the responsible cause for the abundance of atypical lymphocytes in the exposed workers as their lymphocytes are changing their character in response to ionizing radiation. Though atypical lymphocytosis can be witnessed in the presence of viral infections, chronic bacterial infections and drug interactions [55], our study have purposefully excluded participants with such complications and medication statuses in order to particularly and exclusively see the impact of ionizing radiation on the hematological parameters.

Mean absolute monocyte and basophil counts have also been statistically significant differences in our study. Both values were lower in the personnel occupationally exposed to

radiation than the unexposed group. No other study has revealed this. Many of the other parameters like; RBC, HGB, MCV, MCHC, RDW, PLT, PCT, NEUT, and EOS have not been significantly different from that of controls though there had been a lower or a higher value of those parameters in between the groups.

In general, some studies show consistent findings [35, 39] and others documented controversial findings [38] in the effort of explaining effects of ionizing radiation on hematological parameters, which can imply that individual differences in sensitivity and responsiveness to stimulus of ionizing radiation are playing a great role. Individuals who are exposed to ionizing radiation for a longer period might have the same or lesser response when compared to responses by individuals exposed to a shorter period. The vice-versa also works. That is individuals who are exposed for a shorter period might aggressively respond to radiation stimulus.

Taken together, the data reported herein revealed some hematological abnormalities in the low dose radiation exposed group. The finding of atypical lymphocyte in a remarkable proportion of participants is of concern and needs further investigation. For example, data on annual average radiation exposure of workers were not obtained because thermoluminescent dosimetery (TLD) badges have not been worn by the workers for about a year and readings were not available. Almost 78 of the 91 exposed workers do not wear lead apron while working for the purpose of protecting themselves. Most have claimed that they use the principle of distance to be protected. Thus, it is possible that participants might experience effect of the radiation resulting in the abnormalities documented in this study.

8. Strength and Limitation of the study

8.1.Strength

- This study is the first to be done in Ethiopia that it can provide information and alert concerning bodies to fill gaps.
- It also paves a way to further researches on occupational radiation issues.
- Unlike most of the reviewed literatures this study incorporated 18 complete blood count parameters along with cell morphology.
- This study can be very representative as most of the governmental hospitals in Addis Ababa are incorporated with a very satisfactory response rate of participants.

8.2.Limitation

- Information about participants' current medical status was only made by taking histories without further medical diagnosis.
- The future fate of atypical lymphocytosis in the exposed group is not studied.

9. Conclusion and Recommendation

9.1. Conclusion

- Eight (WBC, MCH, MPV, PDW, P-LCR, LYMPH, MONO, BASO) of the 18 CBC parameters studied show statistically significant difference between exposed and non-exposed groups. That is, the mean MCH, PDW, P-LCR were higher while WBC, MPV, LYMPH, MONO, and BASO were lower in the exposed group
- Atypical lymphocytosis was recorded in 65/91 of the exposed and 7/91 of the non-exposed participants
- There are larger effects on the lymphocyte and basophil subsets of exposed workers with high number of atypical lymphocytes.
- A smaller but not negligible effect on white blood cells and medium effects on mean cell haemoglobin, platelet distribution width, mean platelet volume, platelet large cell ratio, and monocytes are the major reports of this study.
- Nonetheless, there is no established threshold for initiation of biologic changes as a consequence of exposure to low levels of irradiation. Therefore, despite technologic advances in diagnostic equipment and implementation of protective measures, professionals remain at risk of the low-dose radiation to which they are exposed. It is not deniable that low dose ionizing radiation is imposing impact on the hematological as well as immunological system of medical imaging and therapeutic technologists.

9.2. Recommendation

- We recommend that there should be a more standard system of radiation protection for radiation workers.
- It is advisable to have a regular check-up of the hematologic parameters of radiation exposed workers for a better monitor of their immune status.
- There should be Thermolumunescent Dosimeter (TLD) badge and regular record of readings to monitor the annual average exposure
- Cohort type researches are recommended in order to have a clearer image of the effects of ionizing radiation.

- Genetic studies might also add values to the knowledge of the effects of ionizing radiation, particularly for Ethiopian setting.

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Annexes

Annex I. Procedure for Venous Blood Collection and blood sample transportation

1. Select a sterile, dry, preferably plastic syringe of the capacity required, e.g. 2.5 ml, 5 ml, or 10 ml. Attach to it a 19 or 20 SWG needle (preferably a disposable one). If the patient is a child or adult with small veins, use a 23 SWG needle.

   Note: When not using a disposable syringe or needle, check the syringe for good suction and the needle for any blockage, directing the syringe and needle safely away from the patient. Ensure all air is expelled from the syringe. Whenever possible use a disposable needle and syringe.

2. Apply a soft tubing tourniquet or velcro fastening arm band to the upper arm of the patient to enable the veins to be seen *and felt*. Do not apply the tourniquet too tightly or for longer than 2 minutes. Ask the patient to make a tight fist which will make the veins more prominent.

3. Using the index finger, feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt. If a vein cannot be felt, apply a pressure cuff above the elbow and raise the pressure to 80 mm (deflate the cuff once the needle is in the vein).

4. Cleanse the puncture site with 70% ethanol and allow to dry. Do not re-touch the cleansed area.

5. With the thumb of the left hand holding down the skin below the puncture site, make the venepuncture with the bevel of the needle directed upwards in the line of the vein. Steadily withdraw the plunger of the syringe at the speed it is taking the vein to fill. Avoid moving the needle in the vein.

If the plunger is withdrawn too quickly this can cause haemolysis of the blood and the collapse of a small vein.

6. When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a piece of dry cotton wool.

7. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped. Remove the needle from the syringe and carefully fill the container(s) with the required volume of blood. Discard the needle safely. Do not attempt to re-sheath it because this can result in needle-stick injury.

   Do not fill a container with the needle attached to the syringe. Forcing the blood through the needle can cause haemolysis.
8. Mix immediately the blood in an EDTA or citrate anti-coagulated container. When required, make a thick blood film from the blood remaining in the syringe. Immediately label carefully all the blood samples.
9. Check that bleeding from the veinepuncture site has stopped. Cover the area with a small dressing.

Safe box system

WARNING: Do not close the Safe box lid until all the contents are inside the package as packaging cannot be reopened.

1. Samples must be in a 4.7mls EDTA tube. If there is a circumstance where you need to send more than one sample of blood in the same box, be please aware that no more than 3 samples (6 EDTA tubes) can be sent per Safe box.
2. Label the tubes clearly center with patient number, and date and time of sample collection.
3. Place the tubes in the absorbent white material, place in the plastic bag, seal the bag and then place in the clear plastic compartment
4. In the adjacent compartment within the safe box, place the blood taking & patient documentation form. Ensure the correct forms are placed with the matched blood samples.

5. Please ensure that all contents are inside the package before closing. Once the package has been closed it cannot be reopened without destroying it.
6. Remove the cardboard separator and place the lid over the top of the container and firmly press shut.
7. Peel the outer backing from the label and wrap around the Safe box.
8. Please ensure the outside of the SAFEBOX is clearly labeled with the name and address of the person responsible at site for sending the samples with a contact telephone number

Annex II. Procedure for Wright Stain

1. Cover the blood film (preferably methanol prefixed) with undiluted stain but do not flood the slide. If using a dropper bottle count the number of drops required to cover the film.

   Note: The undiluted stain not only acts as a fixative but also partially stains the smear. This stage is required to obtain the best possible staining results.

2. Add the same volume of pH 6.8 buffered water (i.e. equal the number of drops as stain). The diluted stain should not overflow. Ensure the water is well mixed with the stain by blowing on the diluted stain or mixing the stain and water using a plastic bulb pipette. Allow to stain for 5 minutes.

   Note: Diluting the stain in buffered water brings about full staining of the blood cells. The exact staining time to use should be decided when a new batch of stain is prepared.

3. Wash off the stain with tap water (filtered if not clean). Do *not* tip off the stain, because this will leave a fine deposit covering the film. Wipe the back of the slide clean and stand it in a draining rack for the smear to dry. The blood film should appear neither too pink nor too blue (check results microscopically).

   Tap water: *If the tap water is highly acidic, resulting in too pink a blood film or highly alkaline, resulting in too blue a blood film, try using boiled cooled water or filtered rain water. If neither of these is suitable, wash the film with pH 6.8 buffered water.*

Annex III. Procedure of sysmex 2000i and its reagents

Procedure of sysmex 2000i

Sampler (Auto) Mode

Standard precautions should be followed when handling specimens and performing all laboratory testing.

1. 1mL of sample required.
2. Place specimens in a rack with barcodes facing the front of the rack. Ensure that labels are securely adhered to tube with no loose edges.
3. Load up to 5 racks at one time (50 samples). A new rack may be added to the right rack pool at any time.
4. On the computer, click on the “Sampler” icon or press [F3] on the keyboard. The “Sample Number” dialog box displays.
5. Click [SAMPLER START] and [OK].
6. The specimen will be automatically mixed 10 times, aspirates, and analyzes the sample according to the tests ordered for specified barcode.
7. Results will print if specimen meets criteria that require further action by the technologist (ie. smear reviews, manual differentials, repeat of critical results).

Note: If Barcodes are not used, the sample number will increase by 1 as each sample is analyzed. The discrete test to be performed must be selected in the Sampler dialog box.

Manual Mode

1. 85 uL of sample required (short draw or pediatric capillary collection).
2. Click the “Manual” icon or press [F2] on the keyboard.
3. Enter the specimen number using the keyboard or the handheld barcode wand.
4. Discrete tests for manual mode are defaulted to C/D/R (CBC/Diff/Retic) unless changed by the operator.
5. Click [OK].
6. Mix the patient sample. Uncap the tube.

7. Place sample under the aspiration pipette so that the tip of the pipette is at the bottom of the sample tube.
8. After sample aspiration a part of the whole blood sample is diluted in 1:50 with lysing reagent stromatolyse4DL and then stromatolyer 4ds dye is added.
9. After a pre defined response time the stained sample is introduced into the detector, where forward light scatter and side fluorescent emission are measured. From this four leucocyte populations are computed: neut count (neu #), lymph count (lymp#), mono count (mono#) and eos count (eos#) as well as neutr percentage (neu %), lymp %, mono%, eos%.

Reagents of sysmex 2000i

EPK is ready to use diluents for impedance and photoelectrical analysis of whole blood.

FFB are ready-to-use diluents which are used for impedance and photoelectrical analysis of whole blood, for lysing reagent to analyse leukocytes and the basophilic granulocytes of a whole blood sample by resistance measurement and photometric measurement and for analyzing blood by resistance measurements and photometric measurement respectively.

FFS is used to stain the leukocytes in diluted and lysed blood samples. It serves for the determination of 4-part differential count (lymph, Mono, Eo, NetrBaso) with selected sysmex hematology analyzers.

FFD is a ready to use diluent for analyzing blood by resistance and photometric measurement.

SLS is cyanide-free reagent used for the determination of hemoglobin. It lyses the RBC and acts upon globin of hemoglobin to from a stable hemochrome.

RED is intended to dilute the sample while simultaneously staining the reticulocyte to assay the reticulocyte concentration in blood.

CELL CLEAN is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics system, transuducer, sample rotor valve, whole blood aspiration tube and the HGB flow cell.

Annex IV. Participant information sheet

Addis Ababa University College of Health Sciences School of Allied Health Science Department of Medical Laboratory Sciences

Title: Effects of Low Dose Ionizing Radiation on the Hematological Parameters in Medical Imaging Technologists of Selected Governmental Hospitals Addis Ababa, Ethiopia

Introduction

This information sheet and consent form is prepared by the principal investigator to clarify the study that you are asked to take part in. If there is any unclarity before you decide to participate or not you can ask freely.

Purpose

We have planned to conduct a study with objective of evaluating the haematological profile such as (RBCs count, RBC indices, Hb, Hct levels, WBCs, platelets count) and cell morphology in medical imaging technologists (Radiography, nuclear medicine and radiotherapy workers) of Tikur Anbessa, St'Paul Millennium Medical College, Zewditu Memorial, Yekatit 12 and Ras Desta Damtew, Minilik, ALERT and Tirunesh Beijing Referal Hospitals Addis Ababa, Ethiopia

Confidentiality

Any information that we collect about you during this research will be kept confidential. Information about your identity will be put away after recording your file; and kept in a secured place. Only the principal investigators will be able to link your identity with the code number.

Risk

There will be a slight pain or discomfort while we collect your blood from the puncture site on your arm but this pain will not persist long and will not cause you a permanent damage.

Benefit

Any abnormal finding will be communicated with the participant for proper management. Findings from this study will help us in setting prevention programmes and developing treatment protocols. You are not going to be paid for participating on this study and you are not going to be asked to pay for the participation.

Participation and Right to refuse

We are asking you and others to voluntarily participate in this study. Since participation in this study is entirely voluntary. You can refuse to participate in this research at any time. Your refusal to participate in this study will not affect any of the benefits you are supposed to get from the center.

Person to contact

Please direct any questions or problems you may encounter during this study to the principal investigator:

Eden Giragn,

Addis Ababa University College of Health Sciences School of Allied Health Science
Department of Medical Laboratory Sciences

Tel: +251-913781172 **email:**egiragn@yahoo.com

Department of Medical Laboratory Science Research Ethics committee +251 11 2755170

Amharic version Participant information sheet

አዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየህክምናላብራቶሪት/ክፍል

ርዕስ; በመጠንአነስተኛየሆነጨረርበደምምርመራውጤቶችላይያለውተጽዕኖ ፤ በተመረጡየመንግስትሆስፒታሎችውስጥበሚሰሩየጨረርሰራተኞችላይአዲስአበባኢትዮጲያ

መግቢያ

ይሕለተሳታፊዎችመረጃመስጫወረቀትእናየፍቃደኝነትማረጋገጫቅጽበዋናተመራማሪዋየተዘጋጀ ሲሆንአላማውምየሚሳተፉበትንጥናትማብራራትነው፡፡

የጥናቱአላማ

የደምህዋሳትምርመራለማካሄድእናየጨረርተጋላጭነትበምርመራውላይየሚኖረውንተጽዕኖለማ ጥናትአስበናል፡፡
በዚህምየነጭናየቀይየደምህዋሳትቆጠራ፣ቅርጽናመጠንምልከታ፤እንዲሁምሌሎችደምእንዲረጋየ ሚያደርጉየደምህዋሳትቆጠራእናምልከታየሚከናወንሲሆንተሳታፊዎችምለአነስተኛመጠንጨረር የተጋለጡየተመረጡየመንግስትሆስፒታልየጨረርሰራተኞችይሆናሉ፡፡ እነዚህምየጥቁርአንበሳ፣ የቅዱስጳውሎስ፣የየካቲት 12፣የዘውዲቱእናየራስደስታዳምጠውሆስፒታልሰራተኞችይሆናሉ፡፡

ምስጢራዊነት

ከእርስዎየምንወስደውማንኛውምዐይነትመረጃበምስጢርየሚጠበቅሲሆንማንነትዎንየሚገልጽማ ንኛውምመረጃየጥናቱመዝገብላይከሰፈረ
በኋላበተገቢውመልኩየሚወገድይሆናል፡፡የእርስዎንማንነትናየመለያቁጥርመለየትየምትችለውዋ ናዋተመራማሪብቻትሆናለች፡፡

አደጋ

የደምናሙናከክንድዎበምንወስድበትሰዓትመጠነኛየሆነህመምሊሰማዎይችላልሆኖምግንምንምአ ይነትየከፋጉዳትምሆነየረጅምጊዜአደጋአያደርስብዎትም፡፡

ጥቅም

በዚህየጥናትውጤትመሰረትየማንኛውምተሳታፊየምርመራውጤትአስጊሆኖቢገኝለተገቢወይምአ ስፈላጊየህክምናአገልግሎትሲባልተመራማሪዋለባለቤቱበግልጽታሳውቃለች፡፡ በተጨማሪምየዚህጥናትውጤትከጨረርመጋለጥጋርለሚክስቱአደጋዎችወይምየበሽታአይነቶችበ ቀጣይየመከላከያእናየመቆጣጠሪያእንዲሁምየህክምናዘዴዎችንለመቀየስይረዳል፡፡በዚህጥናትላይ ለመሳተፍወይምስለተሳተፉየሚጠየቁትወይምየሚከፈልዎትገንዘብአይኖርም፡፡

የመሳተፍእናያለመሳተፍመብት

እርስዎንእንዲሁምሌሎችንበዚህጥናትላይእንዲሳተፉስንጠይቅየእርስዎንመሉፈቃደኝነትመሰረ ትአድርገንነው፡፡በጥናቱላይያለመሳተፍመብትዎመሉበመሉየተጠበቀነው፡፡ አለመሳተፍዎየሚያመጣብዎትምንምአይነትጉዳትየለም፡፡

ለበለጠመረጃተመራማሪዋንማነጋገርይችላሉ

ኤደንግራኝ

አዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየህክምናላብራቶሪ ት/ክፍል

ስልክ; 251 913 78 11 72**ኢሜይል**; egiragn@yahoo.com

የ ህክምና ላቦራቶሪ ትምህርት ክፍል የኤቲክስ ኮሚቴ 251 ıı 275 5150

ANNEX V. Consent Form

Consent form

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind. I give consent to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand.

Name ----------
Signature: ----------

Amharic version of consent form

የተሳታፊነትማረጋገጫ

እኔስሜከታችየተገለጸውየጥናቱተሳታፊለመሆንስወስንየጥናቱአላማ፤ አሰራሮችአናቅድመሁኔታ ዎችንበግልጽበመረዳትእናለጥናቱተሳታፊነትፍቃደኝነቴንበማንኛውምደረጃየማንሳትመብቴንበማ ረጋገጥነው፡፡
በመሆኑምበጥናቱተሳታፊለመሆንስወስንበጥናቱሳቢያለኪሰቱየሚችሉአደጋዎችንበሚገባየተረዳ ሁእናከጥናቱበማንኛውምደረጃእራሴንለመሰረዝብወስንተገቢየሆኑእገዛዎችሁሉእንደማይነፈጉኝ በማመንነው፡፡

እነዚህንመረጃዎችሁሉበሚገባበምረዳውቋንቋየተገለጸልኝመሆኑንበፊርማዬአረጋግጣለሁ፡፡

ሙሉስም ----------ፊርማ----------

ANNEX VI. Questionnaire

Serial Number	List of questions	Choices to be circled or answers to be written according to the question	Skip	Coding column
001	Name of the organization	1. TikurAnbessa 2. St' Paul 3. Zewditu 4. Yekatit 12 5. RasDesta 6. Menelik 7. Tirunesh Beijing 8. ALERT		
002	Sex	1. Male 2. Female		
003	Age	Yea ☐		
004	Type of service you give in this organization	1. X-ray imaging 2. CT scan 3. Radiotherapy 4. Nuclear medicine imaging		
005	For how long have you been on this job?	Yea ☐		
006	Do you usually and properly use protective equipment while doing your job?	1. Yes 2. No		
007	Have you been exposed to mutagenic agents previous to your current job?	1. Yes 2. No		
008	Are you taking therapeutic drugs?	1. Yes 2. No		
009	Have you recently been vaccinated?	1. Yes 2. No		
010	Do you smoke?	1. Yes 2. No		
011	Do you drink alcohol?	1. Yes		

		2. No		
012	Are you pregnant? (Females only)	1. Yes 2. No		
013	Are you anemic?	1. Yes 2. No		
014	Are you diabetic?	1. Yes 2. No		
015	Do you have any cardiopulmonary disease?	1. Yes 2. No		
016	Do you have acute or chronic infection?	1. Yes 2. No		
017	Do you have autoimmune disease?	1. Yes 2. No		
018	Do you have any malignancy?	1. Yes 2. No		
019	Have you been treated with radiotherapy?	1. Yes 2. No		
020	Have you been treated with chemotherapy?	1. Yes 2. No		
021	Do you wear TLD badge?	1. Yes 2. No		
022	If yes for the above question, TLD reading			

Thank you so much for your kind participation!

Amharic version of the questionnaire

ተራ ቁጥር	የጥያቄዎችዝርዝር	አማራጮች	ዝለል	የምስ ጢርቁ ጥር
001	የሚሰሩበትመስሪያቤትስም	1. ጥቁርአንበሳ 2. ቅዱስጳውሎስ 3. የካቲት 12 4. ዘውዲቱ 5. ራስደስታ 6. ሚኒሊክ 7. ጥሩነሽ ቤጂንግ 8. አለርት		
002	ጾታ	1. ሴት 2. ወንድ		
003	እድሜ	በአመት ☐		
004	በመስሪያቤቱየሚሰጡትአገልግሎ ትአይነት	1. የራጅ 2. የ'ሲቲስካን' 3. የጨረርህክምና 4. የ'ኒውክላርሜድስንኢ ሜጂንግ'		
005	በዚህስራላይለምንያህልጊዜአገለገሉ ?	በአመት ☐		
006	በስራዎላይአስፈላጊየሆኑየመከላከ ያመሳሪያዎችንበአግባቡይጠቀማሉ ?	1. አዎ 2. አይደለም		
007	አሁንከሚሰሩትስራበፊትየዘረመል ንባህሪለሚለውጡነገሮችተጋልጠ ውያውቃሉ?	1. አዎ 2. አይደለም		
008	በአሁኑጊዜለማንኛውምበሽታየሚ ሆንመድሃኒትእየወሰዱነው?	1. አዎ 2. አይደለም		

009	**በቅርቡማንኛውንምአይነትክትባት ተከትበዋል?**	1. **አዎ** 2. **አይደለም**		
010	**ሲጋራያጨሳሉ?**	1. **አዎ** 2. **አይደለም**		
011	**የአልኮልመጠጥይጠጣሉ?**	1. **አዎ** 2. **አይደለም**		
012	**ነፍሰጡርናት?(ለሴቶችብቻ)**	1. **አዎ** 2. **አይደለም**		
013	**የደምማነስአለብዎት?**	1. **አዎ** 2. **አይደለም**		
014	**የስኩዋርህመምአለብዎት?**	1. **አዎ** 2. **አይደለም**		
015	**የልብእናየሳንባህመምአለብዎት?**	1. **አዎ** 2. **አይደለም**		
016	**አዲስወይምልማደኛየሆነ‹ኢንፌክሽን›አለብዎት?**	1. **አዎ** 2. **አይደለም**		
017	**የ‹አውቶኢሚውን›ህመምአለብዎት?**	1. **አዎ** 2. **አይደለም**		
018	**የካንሰርህመምአለብዎት?**	1. **አዎ** 2. **አይደለም**		
019	**የጨረርህክምናወስደውያውቃሉ?**	1. **አዎ** 2. **አይደለም**		
020	**የ‹ኬሞቴራፒ› ህክምናወስደውያውቃሉ?**	1. **አዎ** 2. **አይደለም**		
021	**የ‹ቲኤልዲባጅ› ያደርጋሉ?**	1. **አዎ** 2. **አይደለም**		
022	**የላይኛውጥያቄመልስአዎከሆነየ‹ቲኤልዲባጅ› ንባብ**			
ለቅንተሳትፎዎከልብእናመሰግናለን!!!				

Annex VII. Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name:

Signature _____

Place _____

Date of submission _____

This thesis has been submitted with my approval as University advisor.

Name _____

Signature _____

Place _____

Date of submission _____

Name _____

Signature _____

Place _____

Date of submission _____