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CD4⁺ T Cell and Total Lymphocyte Counts among Health Workers in Radiology Department of Some Hospitals Within Sokoto MetropolisAbdullahi, I.*¹, Muhammad, S.I.¹, Makusidi, M. A.², Yeldu, M.H.³, Hamid, K.M.¹, Mustapha, U.K.¹, Muhammed, O.M.⁴, Abdu, M.⁴

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Abstract

Exposure to ionizing radiation has lethal effects on helper T cells (CD4⁺ T cell), total lymphocyte count (TLC) and other immune cells. Most of the studies performed focused surprisingly not among Radiology workers. Indeed, evaluation of their immune status can provide useful information on the status and potential effect that the exposure can cause. Knowledge of the risks and effective strategies of protection are of paramount importance for them. The study aims at estimating the CD4⁺ T cells, Total lymphocyte counts and other immune cells among health workers in Radiology Department of some Hospitals within Sokoto metropolis. The CD4⁺ T cell was measured using Flow cytometer and the other immune cells using haematological analyzer. The result shows that there is decrease in the CD4⁺ T cell count of the Health workers as compare to the Healthy controls group. There is significant difference on CD4⁺ T cell count, TLC and CD4⁺/TLC ratio between the Health workers and Control group ($p < 0.05$). There is significant difference on monocytes and neutrophils counts between the Health workers and control ($p < 0.05$). The decreased number of circulating CD4⁺ T cell and granulocytes by radiations can lead to infections and long-term work on radiations can induce immune suppression. Routine checkup and proper use of effective protective equipment is recommended.

Keywords: CD4⁺ T cell, Total lymphocyte count, Health workers, Radiology

Introduction

Radiation is the emission or transmission of

energy in the form of waves or particles through space or through a material medium (Weinmann *et al.*, 2000). Radiation is often categorized as either ionizing or non-ionizing depending on the energy of the radiated particles. Non-ionizing radiations do not have as much energy and are not able to ionize cells. These include radio waves, microwaves, and light waves (Hardell and Sage, 2008). Whereas ionizing radiation such as alpha, beta, gamma rays, X rays, and neutrons have sufficient energy to generate ion pairs (electrons), which can generate chemically active free radicals (Mohammed *et al.*, 2014). These radicals in turn can damage the molecular structure resulting in cell dysfunction (somatic effect) or mutations (genetic damage) (Masumura *et al.*, 2002). These ionizing radiations penetrate the living tissues and can destroy living cells or make them functionally abnormal (Baker *et al.*, 1990). Workers over-exposed to radiations are prone to develop life-threatening diseases (Rozaj *et al.*, 1999). A study suggested that exposure to radiation such as X-ray causes atypical changes in lymphocyte morphology (Mohammed *et al.*, 2014).

Lymphocytes co-ordinate the immune system's response and play a central role in cell mediated immunity (Lee *et al.*, 2012; Ahmadi *et al.*, 2001). Lymphocyte subset may include; Helper T cells (CD4⁺ T-cells), Cytotoxic T cells (CTLs or CD8⁺ T-cells), Memory T cells and Regulatory T cells (Treg cells) (Bosire *et al.*, 2013). Upon encounter with antigens, CD4⁺ T cells become activated and proliferates rapidly secreting cytokines that sends signals and maintain active immune response (Arens and Schoenberger, 2010). On

the other hand, the CD8⁺ T-cells destroy virally infected cells and tumor cells, and remain inactivated when there is no foreign antigen (Bevan, 2011). CD4⁺ count provides a picture of immune system competence, with higher CD4⁺ counts typically signifying healthier immune systems (Ampofo *et al.*, 2006).

Homeostasis of the T-cell immune system plays a key role in human health as well as in the development of various diseases (Lundstrom *et al.*, 2012; Moschovakis *et al.*, 2012), and this system is highly vulnerable to the effects of radiation exposure (Kusunoki and Hayashi 2008). Sub lethal total body irradiation (TBI) of mammals causes generalized immunosuppression, in part by induction of lymphocyte apoptosis attributable to dysfunction of immune regulation (McFarland *et al.*, 2012). Exposure to low doses of ionizing radiation (IR) is a fact of life in certain occupational settings. Radiation accidents, while unfortunate at the minimum and devastating in the worst cases, will no doubt continue to occur (Postow *et al.*, 2012). Fortunately, most radiation exposures involve low doses (<1 Gy) do not have immediate life-threatening effects (Golden *et al.*, 2013). However, long-term effects of low-dose exposures may be real and should be given serious consideration (Tucker, 2008). Long-term exposure to low doses of ionizing radiation may affect cells and tissues and may result in various adverse health effects (Sultan, 2004). Studies on risk of long-term exposure to low doses of ionizing radiation among health workers in radiation fields should be encourage.

Materials and Methods

Study Design

This is a case-control study which is set to compare the CD4⁺ T lymphocyte and Total lymphocyte count of health workers in Radiology Department of some Hospital within Sokoto metropolis and healthy controls. Blood sample was collected from both exposed and non-exposed individuals. The CD4⁺ T lymphocyte and Total lymphocyte counts were determined using flow cytometer and auto hematological analyzer respectively. A questionnaire was administered to obtain other necessary information of the participants.

Ethical approval was sought from Sokoto State ministry of health and the participants were consented before the commencement of the study.

Study Area

This study was carried out at Radiology Department of Sokoto Specialist Hospital, Maryam Abacha women and children Hospital, Veterinary Teaching Hospital, Medistop and Orthopedic Hospital Wamakko. The Hospitals are located within the Sokoto metropolis. Sokoto is the capital city of Sokoto State. The state is located in the extreme Northwest of Nigeria, near to the confluence of the Sokoto River and the Rima River. It has dry Sahel, surrounded by sandy savannah and isolated hills, with an annual average temperature of 28.3°C (82.9°F). The projected population of Sokoto State is around 4.9 million (NPC, 2006).

Study Population

The study population includes health workers in Radiology Department and healthy controls (age and gender- matched). The controls are individuals not working in Radiology Department.

Ethical Approval and Informed Consent

The ethical approval for this research was obtained from the Ethics and Research Committee of Sokoto State ministry of health in a letter with reference number SMH/1580/V. IV. The research was carried out in accordance with the declaration of Helsinki concerning the ethical principles for medical research involving human subjects. Informed consent for inclusion into the study was obtained from each participant using a standard informed consent form.

Sample Collection

About 3ml of whole blood was collected from the participants via venipuncture using BD vacutainer system into K₃ EDTA anticoagulated tube under strict aseptic condition. EDTA anticoagulated sample was used for the analysis of the CD4⁺ T cells and Total lymphocyte counts. The samples were processed at the Infectious Disease Laboratory of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.

Statistical Analysis

The data obtained from the intended study was presented in the form of tables and figures. The result obtained was entered into Microsoft Office Excel 2010 and analyzed using Statistical Package for Social Sciences (SPSS) version 21. The data obtained in the study was expressed as mean \pm SD. Pair wise comparison was made using *t*-tests and chi-square tests. P-value of \leq 0.05 was considered as statistically significant.

Result

The total number of subjects that participated in this study was 95. Of the 95 subjects, 50 (52.6%) were health workers in Radiology Department while 45 (47.4%) were recruited as healthy controls. The age range of both groups was between 24-52 years. The mean age \pm standard deviation (SD) of the Health workers is 32.96 ± 6.82 that of control is 33.69 ± 6.61 . The Mean \pm SD of CD4+ T count among the groups (Health workers; 328.180 ± 173.756 vs. control; $1371. \pm 204.266$), Mean \pm SD of total lymphocytes count among the groups (TLC) (Health workers; 2.118 ± 0.591 vs. control; 4.049 ± 0.596), Mean \pm SD of CD4+/TLC ratio among the groups (Health workers; 164.198 ± 54.345 vs. control; 347.0 ± 120.384), Mean \pm SD of eosinophils count among the groups (Health workers; 0.180 ± 0.1399 vs. control; 0.140 ± 0.1095), Mean \pm SD of Basophils among the groups (Health workers; 0.028 ± 0.0640 vs. control; 0.018 ± 0.0386), Mean \pm SD of neutrophils among the groups (Health workers; 3.310 ± 0.1470 vs. control; 5.647 ± 0.8969), and Mean \pm SD of monocytes

among the groups (Health workers; 0.406 ± 0.1766 vs. control; 0.518 ± 0.1419).

Table 2 shows the frequency distribution of educational qualification of the health workers. As depicted from the table, most of the health workers 26/50 (52%) have a National diploma while 3/50 (6%) have a secondary school certificate. Table 3 shows the frequency distribution of the health workers based on their Hospitals. Of the 50 health workers who participated in the study, majority 30 (60%) work at Specialist Hospital, Sokoto, the remaining health workers were distributed among Orthopedic Hospital Wamakko 10 (20%), Medistop diagnostic center 3(6%), Maryam Abacha women and children Hospital 3(6%) and Veterinary teaching Hospital, UDUS 4 (8%). Table 4 shows the frequency distribution of the health workers based on exposure history. Health workers with 1-4 years working experience are more common 23 (46%), while 7 (14%) have worked in the field for 9-12 years. Based on contact hours, 34 (68%) work between 4-6 hours daily, whereas 16 (32%) work for a duration of 7-9 hours daily. All the health workers are exposed to X-ray machine. Out of these health workers only 10 (20%) are not using protection while working.

Based on Medical history of the health workers, none of them have history of Autoimmune diseases, Immuno-deficiency diseases, Cancer, Viral Infection and history of using Immunosuppressive drugs.

Table 2: Frequency distributions of educational qualification of the health workers

Qualification	Frequency (N)	Percent (%)
National Diploma	26	52
Bachelor's degree	6	12
Certificate	12	24
Diploma	3	6
Secondary	3	6
Total	50	100

Table 3: Frequency distribution of the health workers based on their Hospitals

Hospitals	Frequency (N)	Percentage (%)
Orthopedic Hospital Wamakko	10	20
Medistop Diagnostic Center	3	6
Maryam Abacha Women and Children Hospital	3	6
Veterinary Teaching Hospital, UDUS	4	8
Specialist Hospital Sokoto	30	60
Total	50	100

Table 4: Frequency distributions of the health workers based on exposure history

Characteristic	Frequency	Percent (%)
Working experience		
1-4	23	46
5-8	20	40
9-12	7	14
Total	50	100
Contact hours		
4-6	34	68
7-9	16	32
Total	50	100
Protection		
Yes	40	80
No	10	40
Total	50	100

Table 5 shows the relationship between contact hours and CD4+ T cells counts of the health workers. Most of the health workers have low CD4+ count, workers with contact hours of 4-6 hours are 28/43 (65.1%) and workers with contact hours of 7-9 hours are 15/43 (34.9%). Whereas those with normal CD4+ from 4-6 contact hours is 6/7(85.7%) and for 7-9 is 1/7(14.3%). However, a statistical analysis by Fisher's exact test Shows that there is no significant relationship between contact hours and CD4+ T cells count among the health workers (Fisher's exact test value = 0.418, p-value = 0.406).

Table 6 shows the relationship between working experience and CD4+ T cells counts of the health workers. Of the 43 health workers with low CD4+ count, those with 1-4 years as working experience have the highest frequency 19(44.2%) while the least is 9-12 years 7(16.3). Among those with normal CD4+ count, 1-4 years have higher frequency 4(57.1%). However, fisher's exact test statistical analysis shows that there is no significant relationship between working experience and CD4+ counts among the health workers (Fisher's exact test value = 0.977, p-value = 0.741).

Table 5: Relationship between contact hours and CD4+ T cells counts of the health workers

Contact Hours (hours)	CD4+T Cells Count (Cells/ μ l)	
	Low count N (%)	Normal count N (%)
4-6	28(65.1)	6(85.7)
7-9	15(34.9)	1(14.3)
Total	43(100)	7(100)

Fisher's exact test value = 0.418, p-value = 0.406, significant p-value < 0.05

Table 6: Relationship between working years and CD4+ T cells counts of the health workers

Working experience (years)	CD4+ T counts (cells/ μ l)	
	Low count N (%)	Normal count N (%)
1-4	19(44.2)	4(57.1)
5-8	17(39.5)	3(42.9)
9-12	7(16.3)	0(0.0)
Total	43(100)	7(100)

Fisher's exact test value = 0.977, p-value = 0.741, significant p-value < 0.05

As depicted from table 7, out of the 50 health workers 29 were males and 21 were females in the 45 healthy controls 22 were males and 23 were females. Males have higher frequency in health workers compared to control group (Health workers; 29(58%), Control; 22(48.9%), whereas females have higher frequency in controls group compared to health workers group (health workers; 21(42%), control; 23(51.1%). A statistical analysis by Chi square shows that there is no significance difference with gender between the groups ($\chi^2 = 0.791$, p-value = 0.415).

Table 8 shows the Comparison of CD4+ T cells range count between Health workers and control group. Among the participants with low CD4+ count (<500), the health workers have higher number compared with control group (Health workers: 43(86.0%) vs control: 0(0.0). Among those with normal CD4+ count (500-1500) the health workers have lower number compared with control group (Health workers: 7(14.0%) vs. control: 35(77.8%). None from health workers have high (>1500) CD4+ count (Health workers: 0(0.0% vs. control:

10(22.2%). A statistical analysis by Fisher exact test Shows that there is significant difference between health workers and control groups with CD4+ T cells count range (Fisher exact test 86.060, p-value < 0.001).

Table 9 Shows the comparison of Total Lymphocytes range count between health workers and control groups. Among those with normal TLC (1-5), health workers have higher number compared with control group (Health workers: 50(100%) vs. control: 44(97.8%). None among the health workers had high TLC (>5) (Health workers: 0(0.0%) vs. control: 1(2.2%). A statistical analysis by Fisher's exact test shows that there is no significant difference between health workers and control groups with Total lymphocyte count range (Fisher exact test 0.123, p-value 0.474).

Table 10 shows the comparison of eosinophils range counts between health workers and control groups. None among the health workers have low eosinophils count (<0.0) (Health workers: 0(0.0%) vs. control: 6(13.3%). Among those with normal eosinophils count

(0.0-0.4), health workers have higher number compared with control group (Health workers: 49(98.0%) vs. control: 38(84.4%). Both groups have equal number of those with high eosinophils count (>0.4). A statistical analysis by Fisher exact test shows that there is significant difference between health workers and control groups with Eosinophil count range (Fisher exact test 7.440, p-value <0.05).

Table 11 shows comparison of basophils range counts between health workers and control groups. Among those with normal basophils count (0.0-0.2), health workers have higher number compared with control group (Health workers: 41(82.0%) vs. control: 36(80.0%). Both groups have shared equal number of those with high basophils count (>0.2). A statistical analysis by Chi square test shows that there is no significant difference between health workers and control groups with Basophil count range (Chi square test 0.062, p-value >0.05).

As depicted from Table 12, a Mann-Whitney U Test indicates that there is significant difference in the CD4+ T cells count of Health workers (Md= 280, N=50) and Control group (Md=1325, N=45), U<0.001, p<0.0001. Similarly a Mann-Whitney U Test indicates that there is significant difference in TLC, CD4+ / TLC ratio, Neutrophils, Monocytes of health workers (Md=1.900, N=50), (Md=130.450, N=50), (Md=2.9000, N=50), (Md=0.300, N=50) and control group (Md=4.100, N=45), U= 46.500, p<0.0001, (Md= 342.300, N=45), U=179.000, p<0.0001, (Md=5.7000, N=45), U= 156.500, p<0.0001, (Md=0.500, N=45), U= 615.500, p<0.0001 respectively. However, there is no significance difference in Eosinophils and Basophils of Health workers (Md=0.1000, N=50), (Md=0.0001, N=50) and control group (Md=0.1000, N=45), U= 930.500, p= 0.103, (Md= 0.0001, N=45), U= 1102.500, p= 0.801 respectively.

Table 7: Comparison between Health workers and controls based on gender

Gender	Health workers N (%)	Controls N (%)
Males	29(58)	22(48.9)
Females	21(42)	23(51.1)
Total	50(100)	45(100)

χ^2 test value = 0.791, p-value = 0.415, significant p-value <0.05

Table 8: Comparison of CD4+ T cells range count between Health workers and control groups

CD4+ T cells range count (cells/μl)	Health Workers N (%)	Control N (%)
Low (<500)	43(86.0)	0(0.0)
Normal (500-1500)	7(14.0)	35(77.8)
High (>1500)	0(0.0)	10(22.2)
Total	50 (100)	45 (100)

Fisher's exact test value 86.060, p-value <0.001, significant p-value <0.05

Table 9: Comparison of Total Lymphocytes range count between health workers and control groups

TLC range (cells/ μ l)	Health Workers N (%)	Control N (%)
Normal (1-5)	50(100%)	44(97.8%)
High (>5)	0(0.0%)	1(2.2%)
Total	50(100%)	45(100%)

Fisher's exact test value 0.123 p-value 0.474, significant p-value <0.05, TLC=total lymphocytes count

Table 10: Comparison of Eosinophils range counts between health workers and control groups

Eosinophils range count (cells/ μ l)	Health Workers N (%)	Control N (%)
Low (<0.0)	0(0.0%)	6(13.3%)
Normal (0.0-0.4)	49(98.0%)	38(84.4%)
High (>0.4)	1(2.0%)	1(2.2%)
Total	50(100%)	45(100%)

Fisher's exact test value 7.440, p-value 0.018, significant p-value <0.05

Table 11: Comparison of Basophils range counts between health workers and control groups

Basophils range count (cells/ μ l)	Health Workers N (%)	Control N (%)
Normal (0.0-0.2)	41(82.0%)	36(80.0%)
High (>0.2)	9(18.0%)	9(20.0%)
Total	50(100%)	45(100%)

Chi square test value 0.062, p-value >0.05, significant p-value <0.05

Table 12: Comparison of CD4+T cells count, TLC, CD4+/TLC ratio, Monocytes and Granulocytes counts between health workers and control groups

Variables	Health workers (N=50) Median	Controls (N=45) Median	Mann Whitney U - value	P-value
CD4+ T cells count	280.0	1325.0	<0.001	<0.0001
Total lymphocytes	1.900	4.100	46.500	<0.0001
CD4+ / TLC ratio	130.450	342.300	179.000	<0.0001
Neutrophils	2.9000	5.7000	156.500	<0.0001
Eosinophils	0.1000	0.1000	930.500	0.103
Basophils	0.0001	0.0001	1102.500	0.801
Monocytes	0.300	0.500	615.500	<0.0001

Significant p-value <0.05

Discussion

It is well known that dividing cells of the haematopoietic system are highly radiosensitive (Smirnova, 2010). Role of the functional cells of the lymphopoiesis and granulocytopoiesis system is very important for immune protection against infections. However, these alterations are dependent on an effective radiation dose range and exposure time (Ismail and Jaafar, 2011). It is found in this study that radiation exposed worker's immune parameters had been more altered as compared to radiation unexposed controls. Homeostasis of the T-cell immune system plays a key role in human health as well as in the development of various diseases (Lundstrom *et al.*, 2012; Moschovakis *et al.*, 2012) and this system is highly vulnerable to the effects of radiation exposure (Kusunoki and Hayashi, 2008). Workers over-exposed to radiations are prone to develop life-threatening diseases (Rozaj *et al.*, 1999). A study suggested that exposure to radiation such as X-ray causes atypical changes in lymphocyte morphology (Mohammed *et al.*, 2014).

This study involved 50 radiation exposed health workers and 45 unexposed controls. Table 8-11 indicate the percentage of those individuals which were having either lower, normal or higher values of the respective immune parameters compared to the normal control range. Overall, there were four affected immune parameters. The most affected immune parameter was only CD4+ T cell, which was found low in majority of health workers (86%), the second most affected parameter was basophil which was found high in majority of health workers (18%). Our study indicated that overall, health workers were more affected for immune parameters such as CD4+ T cell and basophil probably because of the extensive use of x-rays and energetic particles for therapeutic purposes for patients. These personnel can be accidentally exposed to the radiations from X-ray machines. This may happen if the worker receives primary beam radiations or this could be the secondary beam from patient scatter-radiations.

There is no statistically significant difference in TLC and Basophil count between the health workers and control group. Our observation is

consistent with a previous report (Billings *et al.*, 2014) which indicated that the dose response relationships for the numbers of peripheral blood cells of various types following the exposure of mice to gamma radiation were approximately the same, with the dose response curves being nearly identical. But Sanzari (2013) reported that the dose of radiation was the most significant contributing factor in the observed radiation-induced cell loss. The dose dependent decrease in peripheral WBC counts is consistent with previous findings in the mouse model after whole-body gamma irradiation (Sanzari *et al.*, 2013a) the difference in this finding may be because of the use of mouse in place of human.

Mann-Whitney U Test revealed a significant difference in the levels of CD4+, TLC, CD4+/TLC ratio, Neutrophil and Monocyte between the Health workers and control group ($p < 0.05$). This finding is supported by previous reports (Ware *et al.*, 2010; Wambi *et al.*, 2009) which indicated that the dose of radiation was the most significant contributing factor in the observed radiation-induced cell loss. The dose dependent decrease in peripheral WBC counts is consistent with previous findings in the mouse model after whole-body gamma irradiation ((Ware *et al.*, 2010; Wambi *et al.*, 2009). In addition, Hrycek *et al.* (2011) investigated the effects of low-dose ionizing radiation on peripheral blood lymphocyte subsets in eight radiologists exposed to low-dose radiation over a period of 6–27 years. They found no significant difference in T-helper/T-suppressor ratio between exposed radiologists and non-exposed (control). This disagrees with the findings of Graessle (2002), who reported that reductions in peripheral blood cell concentrations occurs at a dose-rate of more than 3 mGy/day. Mann-Whitney U Test revealed no significant difference in the levels of Eosinophil and Basophil count between the health and control group. Many other variables such as educational qualification, the hospital where subject was localized, contact hours and CD4+ T cell count of the health workers, working experience and CD4+ T cell count of health workers and gender of the health workers were evaluated with highest percentage among national diploma (52%), Specialist Hospital Sokoto (60%), 4-6 hour (65.1%), 1-4 years

(57.1%) and male (58%) respectively.

Conclusion

Haematopoietic system is found to be sensitive for radiation workers because mostly immune parameters were observed suppressed. Changes in immune parameters have been noted. Although these workers were working under low radiation doses, long-term use of radiations can show harmful effects on immune parameters. Significant differences were found with the CD4⁺ T cell parameters. Furthermore, the long-term impacts of low ionizing radiation doses on the immune functions in relation to human health should be evaluated. At the end of this study the following conclusions were made

1. There is decrease in the CD4⁺ T cell count of the radiation-exposed Health workers compared to the Healthy controls group.
2. There is significant difference in the CD4⁺ T cell count, TLC and CD4⁺/TLC ratio between the radiation- exposed Health workers and the non-exposed controls.
3. There is significant difference on some cells such as Monocytes and Neutrophils between the Health workers and control group.
4. The decreased number of circulating CD4⁺ T cell and granulocytes among radiation-exposed health workers can lead to infections and immune suppression.

Recommendations

At the end of this study the following recommendations were made

1. The corresponding safety measures should be kept in mind for such workers saving the workers from any accidental exposure.
2. Regular checkups of radiation workers and effective monitoring of all immune parameters should be undertaken in order to diagnose an early change in their entire health.

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