PHAGOCYTIC FUNCTIONS OF POLYMORPHONUCLEAR NEUTROPHILS IN DENTAL X-RAY TECHNICIANS

*SULTAN A. MEO, MBBS, M.Phil, Ph.D.

ABSTRACT

The dental professionals including dental X-ray technicians compass a variety of occupational risks including X-ray radiation. Therefore, the aim of the present study was to determine the effects of X-ray radiations on the phagocytic activity of Polymorphonuclear neutrophils (PMNs) in dental X-ray technicians.

The present pilot study was conducted in the Department of Physiology, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia during the year of 2003. A group of 8 apparently healthy dental X-ray technicians were recruited with age ranging from 22-49 years. They were matched with another group of 8 control healthy subjects in terms of age, sex and ethnic variation. Phagocytic activity of whole blood and PMNs was determined by measuring chemiluminescence (CL) response through a chemiluminescence luminometer.

The mean values for chemiluminescence (CL) response was not decreased in dental X-ray technicians compared to their controls.

A better protection and low dose exposure to X-ray radiation does not affects the physiological functions of PMNs by means of chemiluminescence response. However, a large size studies are further required to confirm the affects of Dental X-ray radiation on the phagocytic activity of Polymorphonuclear neutrophils (PMNs) in dental X-ray technicians.

Key words: Occupational hazards. X-ray radiation, Dental technicians, Phagocytosis, Polymorphonuclear leukocytes, Chemiluminescence response.

INTRODUCTION

Radiological investigations are most frequently requested significant diagnostic tool in the field of medical science. In spite of recent technological advances and other laboratory investigations, radiology remains the corner stone and has maintained a time-honored position in the diagnosis of different diseases. X-rays are part of the electromagnetic energy traveling waves, which includes radio waves, microwaves and visible light. They are the same as gamma rays except that gamma rays are emitted from the nucleus of an atom while X-rays originate from the atoms electron cloud. Analytical X-rays are produced by accelerated electrons from a cathode to an anode in an X-ray tube and can be detrimental. The higher the voltage of the generator the more will be the penetrating radiation. When ionizing radiation penetrates living tissues, it can destroy living cells or make them functionally abnormal.

Ionizing radiations such as alpha, beta and gamma rays and neutrons with sufficient energy to generate ion pairs such as electrons, can causes chemically...
active free radicals to be formed and damage the molecular structure, resulting in cell dysfunction (somatic effect) or mutations (genetic damage). Workers exposed to X-ray radiations are prone to develop life-threatening diseases related to different organs of human body including blood and/or immunity. Phagocytosis is one of the main defense mechanisms by which the immune system protects the body against the infection and is mediated by macrophage and polymorphonuclear leukocytes. It involves the ingestion and digestion of the microorganisms, insoluble particles, damaged or dead host cells, cell debris, activated clotting factors and other particulate material. The main steps implicated in phagocytosis are chemotaxis, adherence, pseudopodium, phagosome and phagolysosome formation and finally the bacterium is subsequently killed by the production of superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radicals on the surface of the phagosome. The production of reactive oxygen species by the NADPH oxidase complex is an important bactericidal weapon of phagocytes. Phagocytosis is affected directly or indirectly in different conditions such as quantitative and qualitative defects of neutrophils and monocytes, Leukocyte adhesion deficiency syndrome, Diabetes mellitus, Asplenia, Chronic granulomatous disease (CGD), Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Myeloperoxidase deficiency, Chediak Higashi Syndrome, Lazy Leucocyte Syndrome and in Jobs Syndrome. Keeping in view the available information, it is worthwhile to observe the effects of dental X-ray radiation on the phagocytic activity of leukocytes by measuring the Chemiluminescence (CL) response in whole blood and isolated polymorphonuclear neutrophils (PMNs) stimulated with opsonised zymosan (OPZ) in dental X-ray technicians compared with their matched controls.

SUBJECTS AND METHODS

Subjects: The present study was conducted in the Department of Physiology, College of Medicine, King Saud University Riyadh, Kingdom of Saudi Arabia during the year of 2003. This was a matched case control cross sectional pilot study. In this study all the participants were recruited through personal visit and two groups were formed 8 subjects each. In the first group 8 apparently healthy volunteered dental X-ray technicians (6 males and 2 females) with age 22-49 years were selected from the Radiology Department, College of Dentistry, King Saud University, Riyadh. They were matched with a second group of 8 healthy control subjects (6 males and 2 females) with the same age group. X-ray technicians worked in dental X-ray Department 8 hours daily. The mean radiation dose from March 15 2003 to June 15 2003 was 0.15 mSv. However, the duration of exposure in these X-ray technicians was 14.12 ± 2.15 years (mean ± S.E.M) range 1-25 years.

All subjects completed a questionnaire, which included anthropometric data and a consent form. Both groups met with exclusion criteria as per standard.

Exclusion criteria: Subjects with gross anemia, known history of diabetes mellitus, cardiopulmonary disease, autoimmune disease, malignancy, subjects with current or previous history of tobacco (smoked or chewed) addictions or who chewed betel nut were excluded from participating in the study. In addition, subjects who had working experience in any industry were also excluded from the study.

METHODS

Collection of blood sample: 8 ml of blood was collected from each subject by venipuncture and a disposable syringe, blood was heparinized (10 IU/ml) was used for measurement of phagocytic activity by measuring the Chemiluminescence (CL) response. Each specimen bottle was labeled with the subject identification code number.

Opsonization of zymosan: Zymosan (Sigma Chemical Co., St. Louis, MO, USA) was opsonized by suspending 50mg in 3ml human serum and 1ml PBS. The suspension was incubated for 30 minutes at 37°C and then centrifuged at 300 xg for 10 minutes. The supernatant was then removed and the pellet washed twice with 4ml buffer. After the last washing, the pellet was re suspended in phosphate buffer saline (PBS) at a concentration of 1.25mg/ml and stored in the freezer until use. The concentration of opsonized zymosan used was 2mg/ml.

Preparation of luminol: Luminol (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in DMSO to give a concentration of 10^-2 M and this stock solution was further diluted in PBS to 10^-4 M prior to use.
Polymorphonuclear leukocyte (PMN) separation:
PMN was separated by using neutrophil isolation medium (NIM) (Cardinal Associates Inc., PO Box 5220, Santa Fe, NM 87502). Five to seven milliliters of heparinized blood was layered over 4ml of NIM in a 15 ml tube and then centrifuged at 400xg for 30 min at room temperature. The leukocyte-rich plasma was carefully removed with a Pasteur pipet and transferred to a 15 ml conical centrifuge tube. Fill the tube with phosphate buffered saline (PBS) and centrifuged at 350xg for 10 min in a Heraeus centrifuge (Model GmbH, Osterode). Two milliliters of lysing buffer (E-Lyse) from the same company was added to lyse the residual erythrocytes, vortex to resuspend the pellets and centrifuged at 250xg for 10 min. The supernatant was discarded and the sediment suspended in 1 ml of 5% foetal calf serum (FCS). The cells was then counted and adjusted to the desired final concentration.

Chemiluminescence assay: Luminol-enhanced chemiluminescence A Berthold (AutoLumatPlus LB 953) luminometer with a constant temperature (37°C) controller (Bethold Technologies GmbH & Co. KG, Calmbacher StraBe 22, D-75323 Bad Wildbad-Germany) connected to a computer was used. The reaction mixture consisted of 100µl of whole blood or PMN suspension and 900µl medium containing 10⁻⁴ M luminol (5- amino-2,3-dihydro,1,4-phthalazinedione Sigma Chemical Co., St. Louis, MO, USA) and 2ng/ml (OPZ) Sigma Chemical Co., St. Louis, MO, USA. Light emission was recorded in millivolts (mV) and the readings were recorded at 1 minute intervals for 30 minutes. CL emission was quantified as the peak height in mV.

Statistical Analysis: The results of CL response were expressed as mean ± SEM. Comparison between groups was analyzed by using a Students paired t-test (two-tailed). The level of significance was taken as p value < 0.05.

RESULTS

Table 1 summarizes the comparison of the phagocytic activity in whole blood by measuring the CL response between dental X-ray technicians and their control group. The mean duration of exposure in X-ray technicians was 14.12 ± 2.51 years (mean ± S.E.M) range 1-25. The mean values of CL response in whole blood were not decreased in dental X-ray technicians compared to their matched control subject.

Table 2, demonstrates the phagocytic activity in isolated polymorphonuclear neutrophils (PMNs) by measuring the CL response. The mean values of CL response in isolated polymorphonuclear neutrophils were not decreased in dental X-ray technicians compared to their matched control subjects.

**DISCUSSION**

X-ray radiations have potentially harmful affects by removing an electron from water molecules in the cell converting them to free radicals. These in turn

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Un-exposed subjects (n=8)</th>
<th>Dental X-ray technicians (n=8)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL peak response</td>
<td>13.5±1.4 21.4±5.0</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Values are expressed as mean ± SEM; PMNs concentration=5x10⁶ cells/ml; OPZ concentration=1.25mg/ml; Luminol concentration=10⁻⁴M; NS= Non significant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Un-exposed subjects (n=8)</th>
<th>Dental X-ray technicians (n=8)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL peak response</td>
<td>493.2±52.5 626.4±102.9</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Values are expressed as mean ± SEM; PMNs concentration=5x10⁶ cells/ml; OPZ concentration=1.25mg/ml; Luminol concentration=10⁻⁴M; NS= Non significant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
break the DNA chain or scramble its coding. Recently National cancer institute (NCI) of USA considering to add medical X-rays to human carcinogenic list7.

The mononuclear phagocyte system (MPS) and the polymorphonuclear neutrophils (PMNs) provide the human body with powerful defense against various microorganisms. The ability of phagocytes to destroy target pathogens depends in part on their capacity to generate toxic oxygen products. Soluble agents or particular stimuli can stimulate the phagocytic cells. The particulate activators, however, are exemplified by opsonized zymosan (serum-treated zymosan particles), or opsonized glucan8.

The ability of phagocytes to produce massive amount of superoxide anion (O2•−), peroxide (H2O2) and hydroxyl radicals (OH•) in response to the above stimuli has attracted considerable attention in recent years as a paradigm of trans-membrane signaling. Superoxide and peroxide may subsequently interact to produce hydroxyl radicals (OH•) and singlet oxygen (O2). In addition, H2O2 and Cl− combine in the presence of myeloperoxide released from the phagosome to produce hypochlorite (HOCl) which is also bactericidal. The products of oxygen reduction (O2•−, H2O2, OH•) constitutes key components of the oxygen-dependent anti-microbial capacity of the cells. Oxygen metabolites are toxic and kill the bacteria, fungi and parasites9-11. However, failure to produce these metabolites results in decreased immunity and ultimately prone to develop an infection. The present study shows that the mean values of OPZ are not decreased in dental X-ray technicians compared to their matched control subject. Damagh et al., (2003)12 conducted a study on the phagocytic activity of Polymorphonuclear neutrophils in Chest X-ray technicians and reported that the Chemiluminescence (CL) was significantly decreased in chest X ray technicians compared to their controls. The probable reason for this contradiction was a dose response between chest and dental X-ray radiation. Jha and Sharma (1999)13 estimate the level of radiation exposure of personnel handling diagnostic X-ray machines and chromosomal aberrations was analyzed in peripheral blood lymphocyte cultures. These occupationally exposed individuals showed higher frequencies of dicentrics as well as eccentrics than normal controls.

Rozgaj et al., (1999)14 reported that long-term exposure to low doses of ionizing radiation may affect the cells and tissues and result in various adverse health effects. However, they did not show time dependent recovery.

Szymielski et al. (1975)15 studied the action of EMF on the granulopoietic reaction in rabbits and observed consistent reduction in bone-marrow reserve pool and a depressed lysozyme activity. In addition, Szymielski (1975)18 also demonstrates increased number of dead cells and rise in the liberation of lysosomal enzymes after exposure to EMF in rabbit cells.

Takeuchi, (1992)16 suggested that the antibody-producing ability, delayed type hypersensitivity reaction and mitogenic activity were sensitive to soft X-ray irradiation.

Shandala et al., (1979)17 reported a significant disturbance in the immunological system of rats exposed intermittently to 500 uW/cm2 for 30 days and observed that blast cells in peripheral blood and the rosette-forming cells in the spleen and thymus were both altered.

Sokolova, (1973)18 observed the phagocytic capability in animals exposed to high-frequency EMFs and reported that neutrophil phagocytic activity and blood-plasma bacteriocidal activity were decreased.

Maltsev et al., (1979)19 conducted experiments on rats, dogs, and monkeys, irradiated with X rays or gamma rays and observed decreased in the indices of phagocytosis only 3-4 weeks after irradiation.

Minoru et al., (1997)20 found that X-ray radiation has a destructive action on immune system cells and organs and depresses their functional activity.

Hrycek et al., (1995)21 reported that workers handling X-ray equipment in X-ray laboratories have disturbances of peripheral blood neutrophil metabolism assessed by NBT test. In addition, they also observed that neutrophil phagocytic activity was weakened in persons working over five years with X-ray equipment.

Hrycek, et al., (1999)22 showed that subjects operating X-ray equipment in radiology departments have significant reduction in neutrophil adherence as well as spontaneous migration area of leukocytes.

The present pilot study suggests that dental X ray radiations are low dose radiations and does not adversely affects the phagocytic activity in dental X ray
technicians. However, these findings are of importance in that they demonstrate the need for further large sized studies to confirm the results of the present study. Even this pilot study failed to observe an association between the impairment of phagocytic activity of (PMNs) in dental X-ray technicians; it does not reflect that dental X-ray radiations are safe. It is advisable, that further large sized studies are required to confirm the hazards of dental X-ray radiations on the phagocytic activity of (PMNs). It is also suggested that the X-rays department workers, their employers and health officials should work together to adopt technical preventive measures such as wearing appropriate protective equipment, like Lead apparel, Lead goggles, thyroid shield etc. These measures will help to prevent the hazards of X-ray radiations.

ACKNOWLEDGMENT

We are thankful to Dr Prof. Ali S Al Tuwaijri, Professor and chairperson, Department of Physiology, Dr Erum Chohan, College of Dentistry, KSU and Dr Mohammad Salim, Registrar, Department of Nuclear medicine for their valuable suggestions. We also extend our thanks to Mr. James and Bayboy for their technical support and typing the manuscript.

REFERENCES

18. Sokolova, I.P. The effects of combined exposure to microwaves and soft X-rays on immunobiological reactivity of animals. JPRS. 1973; 633: 21, 139.