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Research Article

Evaluation of DNA damage and repair in Radiographers and Dental Surgeons using X-ray machines in Dental Clinics.

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Abstract: Acute exposure to high dose of X-rays has potential to damage the DNA and chronic exposure to low dose radiation can also be deleterious because of the cumulative effects of X-ray. DNA damage is an indicator for various health problems including reproductive failure and cancer. Low dose X-ray radiation is used in medical diagnostics and dental clinics, occupational exposure to this may have harmful effects in the absence of necessary precautions. Some studies from other part of the world and a few from India have been carried out to identify the adverse effects of X-rays used for diagnostic purpose. However, the results from these studies are not consistent. Therefore, it is planned to study DNA damage in Dental Surgeons and Radiographers from Hyderabad, who use X-rays routinely in their clinics. Two sensitive techniques, Comet assay and Micronucleus test will be employed for evaluation. This study would help in providing guidelines for better health of Dental Surgeons and Radiographers

Keywords: DNA damage, DNA repair, Radiographers, Dental Surgeons.

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1. Introduction

X-rays are widely used in medicine not only for diagnosis but also for treatment of diseases. They also have application in industry and in research. If handled properly in therapy and diagnosis they are boon to man but their indiscriminate of improper use may lead to health hazards to patients, physicians and radiographers who are occupationally exposed to X-rays and used for diagnostic purposes are of extremely low doses, because large doses have harmful effects [1].

X-rays belong to a category of ionizing radiation and have the capacity to cause damage to the genetic material i.e., DNA. Large doses of acute exposure of X-rays produce chromosomal abnormalities. Similarly, small doses of chronic exposure of X-rays can also produce damage to the genetic material because small doses stretched over time have cumulative effects. Damage to the genetic material can lead to various diseases including Cancer.

Studies have been conducted on cytogenetic effects of radiation on the exposed workers in medical field. But studies conducted on exposed workers in Dental setup are few. Hagelstrum reported chromosomal aberrations (CA) in cultured peripheral blood lymphocytes from 10 subjects working in diagnostic X-ray and nuclear medicine areas in different hospitals of Buenos Aires, Argentina. A fourfold increase in CA was found in the exposed as compared to the control subjects. Maluf et al (2001) carried out micronucleus test and comet assay on 22 workers exposed to low levels of X-rays in a hospital in southern Brazil and 22 controls [2]. They observed a significant increase in dicentric bridges as well as DNA damage in the exposed individuals compared to controls. Age was significantly correlated with micronucleus frequency and DNA damage index in comet assay. Miyaji and Colus (2002) did not observe detectable differences between the frequencies of CA as well as the mitotic index in the dentists the controls of 28 dentists from Brazil who were exposed to diagnostic X-rays, for more then 10 years, and 24 controls [3]. Maddileti et al (2002) in 30 radiographers showed a significant increase in CA as compared

to the controls [4]. The frequency of dicentrices was 0.59 % in the exposed subjects whereas they were not seen in the controls. Moreover, the incidence of CA increased significantly with the duration of exposure. Ameerunisa carried out a study on 20 Dental clinicians and 20 Radiographers, working in Bangalore for 5-35 years, as well as 20 controls and showed an increase in CA in Dental personnel when compared with the unexposed individuals but the results were not statistically significant.

This Current research exploring a specific group of mediators known as indicate inconsistent results and small sample sizes, apart from most of the studies conducted Chromosomal Aberrations. Moreover, there are only a few studies from India. Therefore, it is planned to study the DNA damage in the Indian Dentists and Radiographers using two sensitive genotoxicity indicators i.e. Comet assay and Micronucleus test.

Considering the crucial role of the basal DNA damage as well as DNA repair using comet assay, and micronucleus test in Radiographers and Dental Surgeons using X-ray machine, as compared to age and sex matched controls.

Hence the aim of the study was to understand the consequence of the identify the harmful aspects of Radiation in Radiographers and the Dental Surgeons occupationally exposed to X-ray machines for diagnostic purposes in the context of the south Indian population.

The results of this study are expected to throw light on the occupational hazards faced by

Radiographers and the Dentists, while using X-ray machine for diagnostic purposes. The emphasis of the present study is on the need of further consideration of radiation protection principles, especially in the field of quality control and quality assurance programs in Dental clinics.

2. Materials and Methods

Study population:

The present study consists of a total of 200 (100 Dental Surgeons and 100 Radiographers) subjects that include 100 healthy controls and an equal number of patients whose diagnosis was confirmed by the clinicians based on the symptoms. During a patient visit at the clinic, demographic details on age, gender, tobacco usage (smoking and chewing), along with their clinical history were collected from all the patients in a detailed proforma via personal interview. Patients were recruited from Osmania General Hospital (Afzalgunj, Hyderabad, Telangana, India), and Panineeya Institute of Dental Sciences and Research Centre (Hyderabad, Telangana, India). The study was approved by the institutional ethical committee (25/IEC/IOG/OU/18). Informed consent was obtained from each subject before included in the study. Initially, patients were diagnosed with angiographically confirmed unstable angina cases; Non-ST-Elevated Myocardial Infarction (NSTEMI) and ST-Elevated Myocardial Infarction (STEMI), percutaneous coronary intervention, coronary artery bypass grafting patients with periodontal history confirmed by X-ray cases were recruited in the study. Patients who were having blood pressure >140/90mmHg, blood glucose levels ≥130 mg/dL and triglycerides (TRI) ≥150 mg/dL (hyperlepedimea patients) high-density lipoprotein (HDL) cholesterol <40 mg/dL and low-density lipoprotein (LDL) cholesterol 100mg/dL very low density lipoprotein (VLDL) 2 to 30 mg/dL and obesity patients with body mass index (BMI) ≥25 kg/m2 were considered for the study. TRI were estimated by Glycerol Phosphate Oxidase (GPO) method, and HDL cholesterol was estimated by Phosphotungstic acid method. Subjects with mild symptoms of CAD and other systemic diseases such as cancer, kidney diseases, liver disease, congestive heart failure, malignancy, diabetes mellitus (DM), and AIDS were excluded from the study. Patients who have Dental Surgeons and Radiographers DNA damages were been developed diabetes later in life were included, whereas, those patients who had diabetes before the damage were excluded from the study. A total of 100 age, gender, tobacco usage, and ethnicity-matched individuals who do not have any clinical family history of cases and controls from the same demographic area were included as healthy controls. Our sample size of (100 cases + 100 controls) is large enough and exceeds the estimated number of samples (~69 cases and controls) required to obtain a 90% statistical power.

Sampling and Methodology

From each subject 5 ml of peripheral blood, along with all the relevant clinical, details will be collected.

1. **COMET ASSAY/Single cell gel electrophoresis (SCGE) assay**

will be carried out by the modified method of Ahuja [5]. After collecting the blood sample from each subject it will be aliquoted to study the DNA damage as well as DNA repair and will be processed immediately for comet assay.

1.1 Preparation of slides

Onto a clean dry plain slide about 100ul of NMA(Normal Melting Agarose) will be dropped and smeared in one direction with the help of another plain slide inclined at about 45^0 C. This ensures uniformly thin layerd of agarose and provides better attachment for subsequent layers. These precoated slides will be dried at 37^0 C and stored in dust free boxes until use. For second layer, 30ul of Peripheral Blood sample, mixed with 110ul of LMA (Low Melting Agarose) will be layered on the slide and covered with a cover slip and will be kept at 4^0 C for 10 mints to allow the gel to solidify. The cover slip will be then removed and the third layer consisting of only LMA will be prepared in a manner similar to the second layer. Once the third layer is solidified, the cover slip will be gently removed and the slides will be incubated in cold lysis buffer at 4 0 C overnight.

1.2. Alkali Treatment and Electrophoresis of slides

After incubation in cold lysing solution overnight the slide will be remove from the lysing solution and placed side by side in a horizontal electrophoretic unit as close together as possible. The eletrophoretic reservoir will be filled with freshly prepared alkaline (PH>13) eletrophoretic buffer so as to immerse the slides completely. The slides will be incubated in the eletrophoresis buffer for 30 min to facilitate the DNA unwinding and expression of alkali labile sites. After alkali treatment the power supply will be turned on. Electrophoresis will be carried out for 30 mins at 30 mA @0.67V/cm and the current will be adjusted by raising or lowering the level of buffer.

After electrophoresis, the slides will be carefully lifted from the buffer and placed on a staining tray. The slide will now be flooded gently with neutralizing buffer. The process will be repeated two more times to completely wash off any traces of alkali. The slides will be washed with distilled water thrice and air dried.

1.3 Silver Staining

The air dried slides will be immersed in the fixing solution for 10 mins and washed gently with double distilled water several times. The washed slides will be allowed to air dry with about 32ml of staining solution A (25g of sodium carbonate will be dissolved in 500ml double distilled water and stirred vigorously for 20-30 mins) and poured over the dried slides so as to cover the slides uniformly. This step will be repeated until with a fresh mixture of staining solution a grayish color develops on the slides. To stop staining, the slides will be immersed in stopping solution for 5 min. The slide will be washed with double distilled water and air dried.

1.4. Evaluation of DNA Damage

For visualization of DNA damage, a bright field transmission light microscope will be used. Comet tail length will be measured in each case using an ocular micrometer fitted in the eyepiece in 150 randomly select cells from each subject at 400X magnification. Mean tail length (um) will be calculated for each sample.

DNA repair

Three aliquots of one ml each from the remaining blood, after the first aliquot has been taken for DNA damage.will be incubated at 37 0 C for 1,2 and 3 hrs. These 3 aliquots will be processed the same way, as was done for the first aliquot, by Comet assay. The difference between the mean Comet tail lengths from the DNA damage and these 3 aliquots would give an estimate of DNA repair in a stepwise manner. The data of DNA damage and repair generated this way would also give information of DNA repair efficiency
at the individual level.

II. Micronucleus Test

Micronucleus is a chromatin structure in the cytoplasm surrounded by a membrane without any detectable link to the cell nucleus. Cytocalcin-B prevents the cells from completing cytokinesis resulting in the formation of nucleate cell, which enables the detection of Micronuclei [6].

The remaining blood from DNA damage and DNA repair studies will be used for micronucleus test. Whole blood cultures will be set up in duplicate in RPMI 1640 supplemented with fetal calf serum andantibiotics to culture for 72 hrs at 37 0 C. Cytocalacin-B solution (6 ug/ml) will be added 44 hrs. After
initiation of culture and incubated further for another 28 hrs. at 37 0 C. To harvest, the cultures will be treated with hypotonic solution (0.56% KCL) for 18 min at 37 0 C. After centrifugation the supernatant will be discarded and the pellet fixed in 3:1 methanol: acetic acid. The slides will be prepared in triplicates, will be flame dried and stained with 2% Geimsa solution for 10 min, then rinsed in water and air dried. The cells will be analyzed under a magnification of 400X using light microscope, and 1000 cells will be scored per subject for micronuclei.

2.4. Work Plan

It is planned to include 100 Dental Surgeons and 100 Radiographers from Hyderabad in the study occupational exposed to X-rays for more than three years. For comparison, 100 age and sex matched controls, who are not exposed to radiation, will be included. A Proforma has been designed to document age, sex, life style, and duration of exposure, medical history and a family history of the subjects.

2.5. Statistical analysis

All continuous data were expressed as mean ± SD. Open Epi software (version 3.01, http://www.openepi.com, Dean et al., 2014) was applied to perform the difference in demographic variables and to test the statistical significance by using Yates corrected $\chi 2$ test, Fisher exact test and Person values for both the groups. Hardy-Weinberg equilibrium was tested for damage in DNA. The risk of an individual were been calculated by considering the head and tail part of the damaged DNA The level of significance $p<0.05$ was considered for statistical significance.

Significance

The results of this study are expected to throw light on the safety aspects of the Radiographers and the Dental Surgeons during the use of X-ray machines for diagnostic purposes. The emphasis of present study is to evaluate genotoxic effects of radiation during the course of occupational exposure. This will be especially useful in the field of quality control and quality assurance programs in dental clinics.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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