STUDIES ON GENOTOXICITY AND ANTIOXIDANT STATUS IN THE PERIPHERAL BLOOD LYMPHOCYTES OF INDIVIDUALS OCCUPATIONALLY EXPOSED TO IONIZING RADIATIONS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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MZU REGN NO: 118 0f 2015
Ph. D. REGN NO: MZU/Ph.D./1109 of 03.05.2018

DEPARTMENT OF ZOOLOGY
SCHOOL OF LIFE SCIENCES
APRIL 2022

STUDIES ON GENOTOXICITY AND ANTIOXIDANT STATUS IN THE PERIPHERAL BLOOD LYMPHOCYTES OF INDIVIDUALS OCCUPATIONALLY EXPOSED TO IONIZING RADIATIONS

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Submitted
In partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology of Mizoram University, Aizawl

CERTIFICATE

I certify that the thesis entitled **"Studies on genotoxicity and antioxidant status in the peripheral blood lymphocytes of individuals occupationally exposed to ionizing radiations"** submitted to the Mizoram University for the award of the degree of Doctor of Philosophy in Zoology by **Mary Zosangzuali** is a record of the research work carried out during the period 2017 - 2021 under my supervision and guidance and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other titles in this University or any other University or institution of higher learning.

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DECLARATION

I, **Mary Zosangzuali**, hereby declare that the subject matter of this thesis is the record of work done by me, that contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University or Institute.

This is being submitted to Mizoram University for the degree of **Doctor of Philosophy** in the Department of Zoology.

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ACKNOWLEDGEMENT

I was able to complete this doctoral thesis with the kind support I received from several individuals. I would like to express my sincere gratitude to them.

First and foremost, I express my heartfelt gratitude and appreciation to my supervisor **Dr. Zothansiama**, Assistant Professor, Department of Zoology, Mizoram University for his constant support, intellectual guidance and thorough scrutiny I received throughout my research work. His commitment, self-sacrifice, encouragement and scientific approach helped me in the better completion of this thesis.

I am grateful to the current and former heads of the Department of Zoology, **Prof. H.T. Lalremsanga** and **Prof. G. Gurusubramanian** for providing me with departmental facilities for the completion of my research work.

I am grateful to **Prof. Senthil Kumar**, Dean, School of Life Sciences, Department of Biotechnology, Mizoram University for the advice and support he extended for the fulfillment of this thesis.

I am highly obliqued to thank all the teachers of Zoology Department, Mizoram University for allowing me to use their laboratory equipment for my research work whenever needed. I would especially thank **Dr. Amit Kumar Trivedi**, Assistant Professor, Department of Zoology, Mizoram University for his profound kindness and support in the completion of my gene expression studies in this thesis. I would also like to thank **Dr. Vikas Kumar Roy** and **Dr. Esther Lalhmingliani** for their suggestions and scientific inputs.

I would further like to extend my gratitude to all the non-teaching staff of the Department of Zoology for their kind assistance whenever needed.

I am deeply grateful to **Dr. Kham Suan Pau**, Radiation Safety Officer, Mizoram State Cancer Institute (MSCI), Zemabawk, Aizawl, Mizoram for his endless support and cooperation with the collection of data. I would also like to thank the Head of Department and staff of the Department of Radio-diagnosis and Imaging, Civil

Hospital, Aizawl, Mizoram for allowing me to use their X-ray machine for my research work.

I also thanked **Dr. Lalchhandami Tochhawng**, Scientific Officer, Mizoram Science, Technology and Innovation Council, Aizawl, Mizoram, for all her advice and support throughout my research work.

I am grateful to have received Inspire fellowship from the Department of Science and Technology (DST), Government of India for financial support throughout my Ph. D program.

This thesis would not have been completed without the participation I received from the volunteers involved in the study, who willingly donate their blood samples as required. I would also like to thank **F. Lalsangpuii**, **Lalrinngheta** and **V.L. Malsawmdawngliani** for their kind assistance in blood sample collection.

I would like to extend my gratitude to all my lab-mates**, C. Lalmuansangi**, **Marina Lalremruati**, **F. Nghakliana**, **Lalfakawmi**, and **Esther Laltlanmawii**. Their considerate support and encouragement helped me in the better completion of my research work. I would further like to extend my gratitude to my fellow research scholars and all my seniors especially **Dr. Annie Lalrawngbawli**, **Dr. Jeremy Malsawmhriatzuala**, **Dr. Zothanmawii** and **Charles Lalnunfela** for their scientific input, inspirational advice, help and support.

I am thankful to many of my friends, both from academic and non-academic backgrounds who helped me and gave me moral support throughout my studies.

My eternal appreciation goes to my loving family whose prayers and support had been my strength to have gotten me this far in my work. This thesis is wholeheartedly dedicated to them.

Most importantly, I thank the Almighty God for His amazing love and grace throughout my life.

(MARY ZOSANGZUALI)

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CHAPTER I

GENERAL INTRODUCTION

Radiation is an energy that comes from a source and travels through space at the speed of light. It comes in different forms including heat, sound, radio waves, microwaves, visible light, X-rays, and nuclear radiation. There are four major types of radiation: alpha, beta, neutrons and electromagnetic waves. They differ in mass, energy and how deeply they can penetrate objects. Natural sources of radiation include ultraviolet (UV) rays from the sun, cosmic rays from outer space, and nuclear radiation emitted by naturally occurring radioactive elements. Medical, industrial and military activities have created additional sources of high-energy radiate on, mainly in the form of X-rays and radioactivity (Upton et al., 2021).

Radiation is often categorized as either ionizing or non-ionizing depending on the energy of the radiated particle. Ionizing radiation is a type of energy released by atoms in the form of electromagnetic waves or particles. Due to its high energy, ionizing radiations have the ability to remove electrons from molecules. Ionizing radiation has many beneficial applications, including uses in medicine, industry, agriculture and research. X-rays, gamma rays, and other forms of ionizing radiation are widely used to diagnose and treat some medical conditions. In medical field, ionizing radiation has become an inescapable tool used for the diagnosis and treatment of a variety of medical conditions. Medical exposures are the largest man-made source of exposure to ionizing radiation (Do 2016). When ionizing radiation is used in medicine for diagnostic and therapeutic purposes medical staff are inevitably exposed to some degree. According to UNSCEAR (2008), medical workers are the largest occupational group who are exposed to ionizing radiation at low doses (7.35 million workers around the world, accounting for 75% of workers exposed to man-made sources of radiation). As the use of ionizing radiation increases, so does the potential for health hazards if not properly used or contained.

Biological effects of ionizing radiation

Interaction of ionizing radiations with the living system induces a variety of biological effects depending on the quality of radiation as well as its dose and the dose rate at which radiation is delivered (Sutherland et al., 2000; Budworth et al., 2002). All components of a living cell or system are the target of radiation-induced damage. It includes the cell membrane, different cellular organelles, metabolites,

cellular microenvironment and the genetic material, DNA (Wang et al., 2018). Many lines of evidence support the view that DNA is the principal target for the biological effects of ionizing radiations (Bedford and Mitchell 1973; Broerse et al., 1968; Elkind et al., 1965; Howard and Pelc 1953). Even though all cellular components may be involved in the manifestation of the biological impact of the radiation-induced damage, it is widely accepted that the most critical target of radiation-induced damage is the genetic material (Reisz et al., 2014). The interaction of ionizing radiation with DNA has been described as resulting from a direct or an indirect interaction.

Direct action of ionizing radiation: Any form of radiation directly interacts with target structures to cause ionization, thus initiating the chain of events that leads to biological changes. Direct effects include the direct ionization of the DNA molecule, which may result in genetic damage that can disturb the cell's reproduction and survival (USNRC 2014). Depending on the quality and dose of radiation, damage such as nucleotide or base manifestations, including other oxidative alterations, mutation of different types, single-strand breaks (SSB), double-strand breaks (DSB), and complex clusters of damage form distinct lesions in DNA molecule (Blaisdell and Wallace 2001).

Indirect action of ionizing radiation: The indirect action of radiation is caused by electromagnetic radiations: X-rays and gamma rays, which do not produce biological changes themselves, but the energy absorbed may cause radiolysis of water molecules to produce free radicals, which can diffuse far enough to interact with the critical targets and cause damage. Exposure of biological materials to ionizing radiation triggers the formation of a rapid burst of reactive oxygen species (ROS) due to the ability of ionizing radiation to ionize water molecules and/or directly ionize target molecules (Riley 1994; Le Caer 2011; Kohanoff and Artacho 2017) which mediate radiation damage. Ionizing radiation produces these free radicals within microseconds, however, their effects persist long after their production due to the cascade of events triggered by these free radicals at the molecular and cellular levels. This finally leads to an increase in the oxidative stress (OS) and instability of the genome in the exposed cells (Einor et al., 2016). The free radicals formed by

radiolysis of water molecules has been reported to damage 60%-70% of the DNA molecules. Free radicals also attack proteins and lipids, resulting in protein oxidation and lipid peroxidation (Edwards et al., 1984).

Deterministic and stochastic effects of ionizing radiation

Radiation exposure can produce biological effects as either a dose-dependent effect or a dose-dependent probability (Hamada and Fujimichi 2014). The health effects of ionizing radiation are usually classified into two categories: deterministic and stochastic effects. Deterministic effects are threshold health effects that are related directly to the absorbed radiation dose and the severity of the effect increases as the dose increases. Deterministic effects have a threshold below which no detectable clinical effects do occur. The threshold may be very and may vary from person to person. For doses between 0.25 Gy and 0.5 Gy, blood changes may be detected by medical evaluations and for doses between 0.5 Gy and 1.5 Gy blood changes will be noted and symptoms of nausea, fatigue and vomiting may occur (USNRC 2014). High doses can cause visually dramatic radiation burns or rapid fatality through acute radiation syndrome. Acute doses below 250 mGy are unlikely to have any observable effects. Acute doses of about 3 to 5 Gy have a 50% chance of killing a person some weeks after the exposure if a person receives no medical treatment. Deterministic effects can ultimately lead to a temporary nuisance or to a fatality (Herman and Thomas 2009). Stochastic effects of ionizing radiation occur by chance, generally occurring without a threshold level of dose. The probability of occurrence of stochastic effects is proportional to the dose but the severity of the effect is independent of the dose received (Connor 2019). The biological effects of radiation on people can be grouped into somatic and genetic effects. Genetic effects are abnormalities that may take place in the future generations of exposed individuals. These effects involve mutations of sperm or egg cells. Mutations of these reproductive cells can be passed to the next generation. Cancer risk is usually mentioned as the main stochastic effect of ionizing radiation, but also hereditary disorders are stochastic effects (Herman and Thomas 2009).

Radiation safety regulations for workers

International Commission on Radiological Protection (ICRP) provides systematic and useful standards for radiation protection including medical, occupational, environmental, and exposure controls against radiological accidents without unduly limiting the beneficial practices giving rise to radiation exposure (Dariusz 2014). The duration of radiation exposure, distance from the radiation source, and physical shielding are the key facets in reducing exposure. It was recommended for workers exposed to medical radiation sources to follow and apply all the requirements established in the International Basic Safety Standards (BSS) for protection against ionizing radiation and the safety of radiation sources. Dose limits are introduced to ensure that the occupational exposure of any worker is controlled and below a certain dose per period, as outlined in the International BSS. The radiation dose to workers is expressed in terms of effective dose and equivalent dose for extremities and eye lenses as stated by the International Commission on Radiological Protection (ICRP) report number 60. The personal dose equivalent H_p (10) is now the internationally recommended operational quantity in the field of radiation protection by individual monitoring. It is the dose received by tissue (effective dose) at a 10-mm depth from the skin surface and is considered to be the dose to the whole body. From the basic safety standards (BSS) recommendation, the equivalent doses limits should apply i) to the whole body, as represented by the operational quantity H_p (10); and ii) to the extremities, via the operational quantity H_p (0.07). The BSS defines the H_p (0.07) dose as the dose at a depth of 0.07 mm and is considered to be the dose received by the skin of the workers (Covens et al., 2007; Aleksandra et al., 2008; Kopec et al., 2011; Ho et al., 2002). The dose limit for workers proposed by the ICRP was established as an annual effective dose. According to ICRP 2007, an effective dose limit of 20 mSv/year averaged over 5 consecutive years; 50 mSv in any single year has been set for persons employed in radiation work. The cumulative effective dose over five years shall not exceed 100 mSv. Where equivalent dose in any calendar year to the lens of the eye shall not exceed 300 mSv and to the hands, skin and feet shall not exceed 500 mSv. In case of women workers of reproductive age, the dose limit to the surface of women's abdomen is 2 mSv for the remainder of the

pregnancy. Thermoluminescent dosimetry is the most suitable method to carry out measurements on personal dosimeters (Janssen et al., 1992; Niklason et al., 1994; Karppinen et al., 1995).

Radiation protection and work practices

To ensure the radiation safety of medical workers, physical radiation shielding is accomplished with different forms of personal protective equipment (PPE). Some fluoroscopy suites contain ceiling-suspended lead acrylic shields, which can reduce doses to the head and neck. Portable rolling shields, which do not require installation, can protect staff in operating rooms and interventional settings. These mobile shields have been shown to decrease the effective radiation dose to staff by more than 90% when used correctly (López et al., 2018). In cases where it is not feasible to shield oneself behind a physical barrier, all personnel should wear leaded aprons for protection. Leaded aprons, which are required in most states, commonly come in thicknesses of 0.25 mm, 0.35 mm, and 0.5 mm. Aprons that wrap circumferentially around the body are preferred to front aprons, given their increased surface area coverage. Transmission through leaded aprons is typically between 0.5% and 5%. Leaded aprons should always be companied by a thyroid shield. (Kaplan et al., 2016).

Background and scope of the study

High-dose radiation carcinogenesis is currently considered to be a generally accepted long-term radiation health effect. High doses are known to produce deleterious consequences in humans such as inducing cancer, cardiovascular diseases, cataracts, invading the hematopoietic system, and causing leukemia (UNSCEAR 2006; Baselet et al., 2016; Behjati et al., 2016; Grant et al., 2018; Hong et al., 2019; Richardson et al., 2019; Seo et al., 2018). However, the potential health risk arising from low-dose exposure is still debatable (Feinendegen et al., 2011; OECD 2016). Regarding human exposure to ionizing radiation, UNSCEAR (2008) report, ICRP (2007) and BEIR VII report defined that doses below 0.1 Gy are classified as “low doses”, while doses normally used in medical procedures, such as Radiation Therapy (RT) (2-3 Gy) are classified as high-doses (Leuraud et al., 2015; Ray et al., 2012). Whether low doses of ionizing radiation can cause cancer is the most critical and long-debated question

for radiation protection standards. In developed countries, the contribution of diagnostic X-rays to cancer ranged from 0.6-1.8 % of the cumulative risk of cancer up to the age of 75 years (Gonzalez and Darby 2004). Various studies have reported increased levels of chromosomal aberrations in lymphocytes of workers occupationally exposed to ionizing radiation as compared to unexposed controls (Evans et al., 1979; Lloyd et al., 1980; Jha and Sharma 1991; Andreassi et al., 2009; Zakeri and Hirobe 2010; Sakly et al., 2012; Vellingiri et al., 2014). Moreover, some epidemiological studies have also revealed that individuals who are occupationally exposed to ionizing radiation may have an increased risk of leukemia and other cancers (Smith and Doll 1981; Aoyama 1989; Muirhead et al., 1990; IARC 2000; Gilbert 2009; Wang et al., 2016). However, contradictory results have also been reported on the genotoxic effects of occupational exposure to low doses of ionizing radiation by various workers (Demirel et al., 1997; Cardoso et al., 2001; Maffei et al., 2002; Thierens et al., 2002; Joseph et al., 2004). Among the many biological parameters that have been studied in this connection, chromosomal damage in lymphocytes is most promising (Streffer et al., 1998). The blood-forming cells were one of the most sensitive cells due to their rapid regeneration rate (USNCR 2014).

Outcomes of health examinations belonging to the workers in low-dose ionizing radiation have been given considerable attention by the public since the second half of the twentieth century. Although the radiation exposure dose remains below the recommended levels, there is a high risk for workers handling X-ray machines due to their long-term exposure to low doses of ionizing radiation. Concerning the risk associated with medical X-rays, the study was carried out to investigate the genotoxicity and antioxidant status in the peripheral blood lymphocytes of individuals occupationally exposed to ionizing radiation, working in different hospitals in Aizawl, Mizoram. To the best of our knowledge, no studies had been performed or reported in the present study area in the past. The main objectives of the study are:

- To determine the effects of low-dose irradiation in the antioxidant status of medical workers exposed to ionizing radiation.

- To assess DNA damage among medical workers exposed to ionizing radiation using comet assay.
- To investigate chromosomal damage among the occupationally exposed group using micronucleus assay.
- To assess the alteration in the gene expression of the exposed group using qPCR technique.
- To study the effects of low-dose irradiation (X-ray) on chromosomal damage and antioxidant status in cultured human peripheral blood.

CHAPTER II

SELECTION OF SUBJECTS

1. SUBJECTS

The study was carried out in Aizawl city (23°43′37.58′′N and 92°43′3.49′′E), Mizoram, India, from 2017 to 2021. It is voluntary participation and all the individual from the study groups were contacted and explained about the study. The study was performed on 80 individuals who were divided into two groups – the exposed group and the control group.

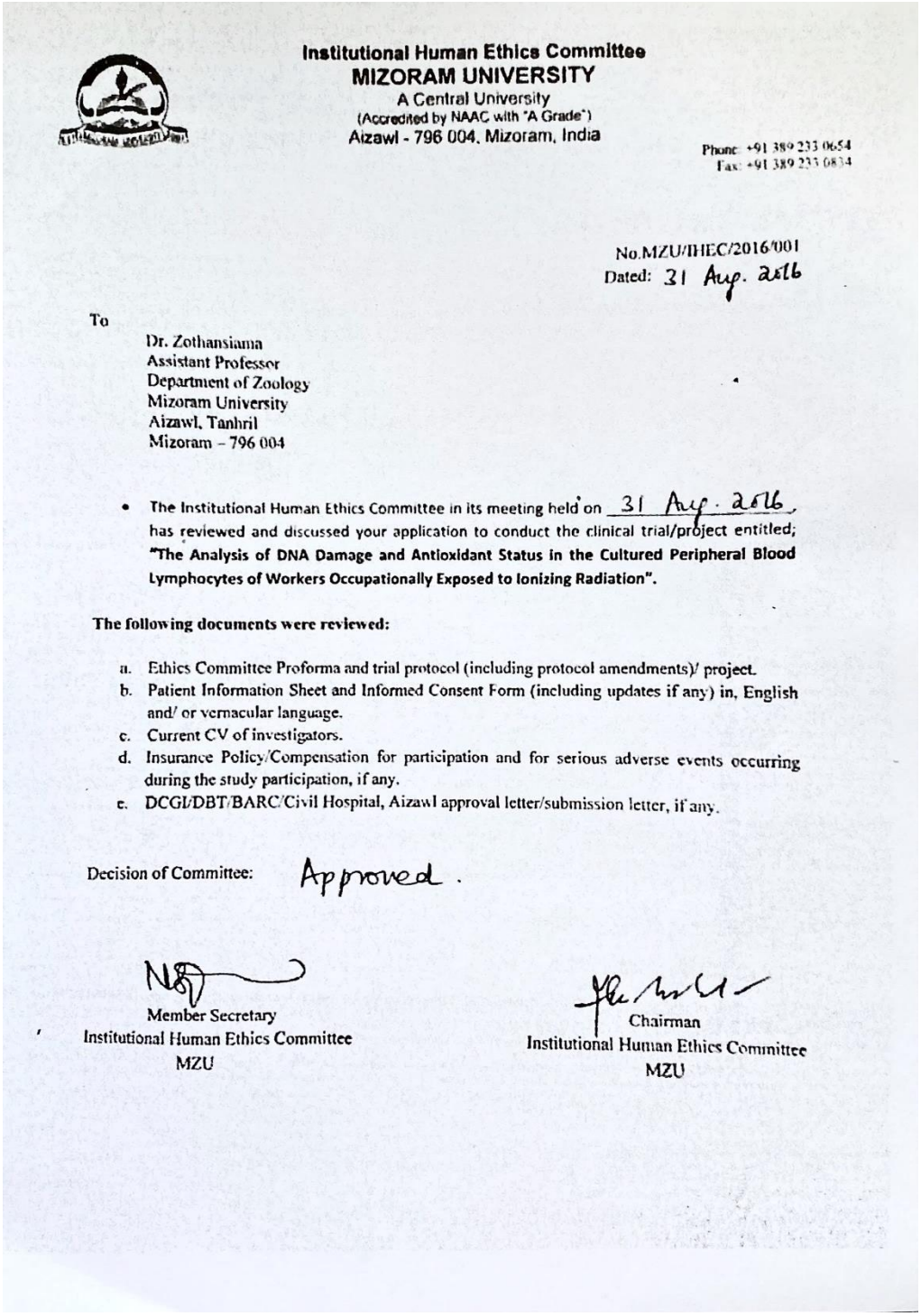
1.1. Exposed group: Exposed group consists of 40 healthy radiology technicians working in different hospitals in Aizawl, India, who fulfilled the inclusion criteria of being above 18 years of age and have been occupationally exposed to X-rays for medical diagnostic purposes. The occupational exposure to ionizing radiation is monitored by a personal thermoluminescence dosimeter (TLD badges) every 3 months.

1.2. Control group: Control group comprised of 40 healthy volunteers matched for age and gender, residing in the same urban area, and did not have any work-related exposure to ionizing radiation or to any other known genotoxic agents. The individuals of either group had no known history of recent infection and did not receive irradiation for diagnosis or treatment purposes that could influence the results of the present study.

None of the participants lived close to (at least 500 m) mobile phone base stations, radio and television transmitters, electric transformer/s and high-tension electric power lines to eliminate the effect on account of high residential exposure to non-ionizing radiation including radiofrequency and extremely low-frequency electromagnetic radiation. There is no report of medical treatment from the participants before blood sampling.

2. ETHICS APPROVAL

The study was approved by the Human Ethics Committee, Mizoram University, Aizawl, India vide approval No. MZU/IHEC/2016/001.



Institutional Human Ethics Committee
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No.MZU/IHEC/2016/001
Dated: 31 Aug. 2016

To

Dr. Zothansiama
Assistant Professor
Department of Zoology
Mizoram University
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Mizoram – 796 004

- The Institutional Human Ethics Committee in its meeting held on 31 Aug. 2016 has reviewed and discussed your application to conduct the clinical trial/project entitled; **"The Analysis of DNA Damage and Antioxidant Status in the Cultured Peripheral Blood Lymphocytes of Workers Occupationally Exposed to Ionizing Radiation".**

The following documents were reviewed:

a. Ethics Committee Proforma and trial protocol (including protocol amendments)/ project.
b. Patient Information Sheet and Informed Consent Form (including updates if any) in, English and/ or vernacular language.
c. Current CV of investigators.
d. Insurance Policy/Compensation for participation and for serious adverse events occurring during the study participation, if any.
e. DCGI/DBT/BARC/Civil Hospital, Aizawl approval letter/submission letter, if any.

Decision of Committee: Approved.

Member Secretary
Institutional Human Ethics Committee
MZU

Chairman
Institutional Human Ethics Committee
MZU

3. CONSENT FORM AND QUESTIONNAIRE

The individuals from each group were asked to sign a consent form stating the volunteer's willingness to participate in the study. A set of questions relevant to this study was prepared and all the participants were asked these questions individually. A questionnaire was prepared to collect information on demographic data such as a family history of cancer, lifestyle including betel nut chewing, tobacco chewing, smoking habit (15–25 cigarettes in a day), alcohol consumption (wine intake range of 1–3 liters/week) and dietary pattern (vegetarian or non-vegetarian). The information related to previous exposure to ionizing radiation for diagnosis or treatment purposes of both groups was also collected using a questionnaire. In addition, the exposed group was also requested to fill up occupation-related information such as duration of employment and the average number of patients handled per day. The consent form and questionnaire used during the study are given below:

E.3

Consent Form

I Lalbialfima fully agree to provide my samples for research purpose on biochemical and molecular aspects. I fully understand that the researchers in MZU will use my samples for DNA sequencing, testing the biochemical profiles and cell culture assays. I also understand that the results of these studies will reflect any changes in the blood parameters due to radiation effect or any other possible effects. I also ascertain that the result of this study will not affect me in anyway and that will not attract any legal or social issues.

Signature of the Investigator

Signature of the Volunteer

Date: 12/8/21

Volunteer Questionnaire for study on

GENOTOXIC EFFECTS OF RADIATION ON PERIPHERAL LYMPHOCYTES

Cases/ controls

Serial number: _____ Date: _____/_____/_____

1. Hming/ Name: _____
2. Kum/ Age: _____
3. Pian ni leh kum/ Date of birth: _____/___/_____
4. Sex: Mipa/hmeichhia (Male/ Female)_____
5. Permanent Address:

_____

_____

6. Nupui/pasal I nei tawh em? (Marital status):
   (a) Married [ ]
   (b) Unmarried [ ]
7. Kum engzat I nih in nge nupui/pasal I neih? (Age at marriage): _____
8. Fa engzat nge I neih? Kum engzat te nge an nih? (Number of children with their age):

   _____

   _____

   _____

9. Phone number: _____

I. Radiation laboratory a hnathawh chungchang (Information about working in radiation laboratory):

1. Radiation laboratory a I hnathawh hming (Designation of particular work in radiation laboratory)?

_____

2. Kum engzat nge radiation laboratory ah I thawh tawh (Time since working in radiation laboratory)?
   (a) Kum 1 aia tlem (Less than 1 year) [ ]
   (b) Kum 1-5 inkar (Between 1-5 years) [ ]
   (c) Kum 5-10 inkar (Between 5-10 years) [ ]
   (d) Kum 10 aia rei (More than 10 years) [ ]

3. Nikhat ah patient engzat nge I neih thin (Number of patients handled per day)?
   (a) 1-5 inkar (Between 1-5) [ ]
   (b) 5-10 inkar (Between 5-10) [ ]
   (c) 10-15 inkar (Between 10-15) [ ]
   (d) 15 aia tam (More than 15) [ ]

4. I awmna atanga hnai tak ah electric transformer/electric power line sang tak/radio or television transmitter a awm em (Presence or not of an electric transformer at less than 10m/very high tension electric power line at less than 100m/radio and television transmitter at less than 4km)?
   (a) Awm (Present) [ ]
   (b) Awmlo (Absent) [ ]

5. Radiation laboratory ah hian I pi/pu/nu/pa thawk an awm em? Khawngaihin han sawi ciang teh (Any biological relatives working in radiation laboratory. If any, please specify)?
   (a) Awm (Present) [ ]

   _____

   (b) Awmlo (Absent) [ ]

6. Engtik nge hri danna I lak hnuhnun ber (Time since last vaccination was taken)?
   (a) Kum 1 aia tlem (Less than 1 year) [ ]
   (b) Kum 1-3 inkar (Between 1-3 years) [ ]
   (c) Kum 3-5 inkar (Between 3-5 years) [ ]
   (d) Kum 5 aia rei (More than 5 years) [ ]

7. Damdawi ei lai I nei em (Any medication taken)?
   (a) Nei (Yes) [ ]
   (b) Neilo (No) [ ]

8. Hriselna chungchang ah in sawiselna I nei em (Any health complaints)?
   (a) Lu na (Headache) [ ]
   (b) Ngaihmawhawm (Irritability) [ ]
   (c) Awm nuamlo (Discomfort) [ ]
   (d) Luak chhuak (Nausea) [ ]
   (e) Mu tha theilo (Sleep disruptions) [ ]
   (f) Chau (Fatigue) [ ]
   (g) Beng kiu (Tinnitus) [ ]

9. I thisen zawmpui la dam/boral tawh ah cancer natna nei an awm em? Khawngaihin han sawi chiang teh (Any family history of cancer. Please specify).

   (a) Yes [ ] (b) No [ ]

II. Zuk leh hmuam chungchang (Life style and habits):

1. Kuhva (Betel chewing habit):
   (a) Ei ngailo (Never) [ ]
   (b) Nghei tawh (Past chewer) [ ]
   (c) La ei mek (Current chewer) [ ]

2. Sahdah (Tobacco):
   (a) Hmuam ngailo (Never) [ ]
   (b) Nghei tawh (Past chewer) [ ]
   (c) La hmuam mek (Current chewer) [ ]

3. Meizial zuk dan (Smoking habit):
   (a) Zu ngailo (Never) [ ]
   (b) Nghei tawh (Past smoker) [ ]
   (c) La zu mek (Current smoker) [ ]

4. Zu in dan (Alcohol drinking habit):
   (a) In ngailo (Never) [ ]
   (b) Nghei tawh (Past drinker) [ ]
   (c) La in mek (Current user) [ ]

5. Ei leh in chungchang (Food habit): Sa I ei ngai em?
   Vegetarian/ Non-vegetarian:_____

4. DEMOGRAPHIC DATA OF EXPOSED AND CONTROL GROUPS

Student "t" test and Chi-square tests were used for comparison of demographic variables of the exposed and control groups. The results are expressed as mean ± standard error of the mean (SEM). Statistical analyses was conducted using SPSS Ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA). A p-value of less than 0.05 was considered statistically significant**.**

The demographic characteristics of both exposed and control groups are depicted in Table 1. The exposed and control groups were matched for all the demographic characteristics such as age, gender, smoking habit and alcohol consumption, betel nut and tobacco chewing (Table 1). Dietary pattern was not included in the analysis as all the participants were non-vegetarian and none of the subjects had a peculiar dietary habit.

The average age for the exposed group was 37.40 ± 2.19 years and the control group was 37.89 ± 1.22 years. Among the occupational exposure group, the cumulative effective dose ranged from 3.14 to 144.5 mSv (40.88 ± 39.86 mSv) depending on the duration of employment, which ranged from 3-29 years (10.27 ± 1.06 years) (Table 1).

Table 1: Demographic parameters of exposed and control groups.

Characteristics	Category	Exposed group		Control group		t/χ2 value	p value (t/χ2)
		N%	Mean ± SEM	N%	Mean ± SEM		
Gender	Male	25 (62.5)		24(60)		-/0.008	0.928
	Female	15 (37.5)		16 (40)			
Age (years)	20-40	24 (60)	37.40 ± 2.19	27 (67.5)	37.89 ± 1.22	0.198/-	0.884
	41-60	16 (40)		13 (32.5)			
Smoking habit	Smokers	13 (32.5)		16 (40)		-/1.168	0.28
	Non-smokers	27 (67.5)		24 (60)			
Betelnut chewing	Chewers	18 (45)		20 (50)		-/1.290	0.256
	Non-chewers	22 (55)		20 (50)			
Tobacco chewing	Chewers	15 (37.5)		11(27.5)		-/1.100	0.294
	Non-chewers	25 (62.5)		29 (72.5)			
Alcohol consumption	Alcoholic	10 (25)		12 (30)		-/0.231	0.63
	Non-alcoholic	30 (75)		28 (70)			
Family history of cancer	Yes	17 (42.5)		14 (35)		-/0.516	0.431
	No	23 (57.5)		26 (65)			
Cumulative dose (mSv)	≤50	21 (52.5)	40.88 ± 39.86				
	>50	19 (47.5)					
Duration of employment (years)	≤10	18 (45)	10.27 ± 1.06				
	>10	22 (55)					
Patients handled per day	≤15	17 (42.5)	15.54 ± 0.81				
	>15	23 (57.5)					

CHAPTER III

EFFECTS OF LOW-DOSE IRRADIATION IN THE ANTIOXIDANT STATUS OF MEDICAL WORKERS EXPOSED TO IONIZING RADIATION

ABSTRACT

Regular low dose occupational exposure to ionizing radiation may induce deleterious health effects. Although the radiation exposure dose remains below the recommended levels, there is a high risk for workers handling X-ray machines due to their long-term exposure to low doses of ionizing radiation. The potential risk for deleterious effects associated with low doses in hospital workers still remains debatable. Therefore, the present study was conducted to analyse the alteration in the antioxidant status by measuring the levels and activities of various antioxidants in the peripheral blood of hospital workers occupationally exposed to low doses of X-rays. The occupationally exposed group showed a significant decline in the activities of glutathione-s-transferase (GST) and catalase (CAT) when compared to the control group, with a significant rise in lipid peroxidation (LPO). However, no significant changes were observed in the level of glutathione (GSH) and superoxide dismutase (SOD) activity between exposed and control groups. We have observed that protracted low dose exposure to ionizing radiation is an inevitable occupational hazard leading to the persistence of oxidative stress in the radiological technicians depending on the time spent with X-rays, cumulative dose received and the number of patients handled daily raising the potential risk of cancer development.

1. INTRODUCTION

Ionizing radiations (IR) are like a double-edged sword, that can be either useful in medicine for the treatment and diagnosis of human ailments or a predominant contributor to the occurrence of various diseases. High doses of ionizing radiation are clearly known to produce deleterious consequences in humans such as cardiovascular diseases and cataracts (UNSCEAR 2006; Baselet et al., 2016), the potential health risk arising from low-dose exposure is still debatable (Feinendegen et al., 2011; OECD 2016). By triggering the formation of various types of damage in the DNA structure (such as single-strand and double-strand DNA breaks, DNA base alterations, and DNA–DNA cross-links), ionizing radiation can cause genomic instability and mutations thereby leading to cancer induction in the exposed individuals (Ward 1998; Mavragani et al., 2017).

Exposure of biological materials to ionizing radiation have also been reported to trigger rapid bursting of reactive oxygen species (ROS) due to their ability to ionize water molecules and/or directly ionize other target molecules (Riley 1994; Le Caer 2011; Kohanoff and Artacho 2017). Ionizing radiation produces these free radicals within microseconds, however, their effects persist long after their production due to the cascade of events triggered by these free radicals at the molecular and cellular level. This finally leads to an increase in the oxidative stress (OS) and instability of the genome in the exposed cells (Einor et al., 2016). Although cells are equipped with an impressive repertoire of antioxidants, both enzymatic as well as non-enzymatic small molecule antioxidants to counterbalance oxidative stress by neutralizing ROS, the excess generation of ROS may overwhelm the endogenous antioxidants and cause oxidative cellular damage (Kono and Fridovich 1982; Jagetia and Shetty 2016). ROS, due to their extreme reactivity are not amenable to direct measurement in biological material. Therefore, altered levels of antioxidant enzymes such as catalase (CAT); superoxide dismutase (SOD); glutathione-s-transferase (GST); glutathione peroxidase (GPx); and small-molecule antioxidants such as glutathione (GSH) are routinely used to assess oxidative stress. Exposure of human lymphocytes with 3 Gy γ-rays significantly reduced the GST, CAT and SOD activities when compared to control (Bravard et al., 1999). Irradiation of mice resulted in a dose-dependent decline in the GSH concentration and activities of GPx;

SOD and CAT with a concomitant increase in the lipid peroxidation in the liver, intestine and skin indicating oxidative cellular damage (Jagetia and Reddy 2005; Jagetia and Rajanikant 2015; Jagetia and Shetty 2016). Stable by-products of free radical reactions are also routinely measured to quantify oxidative stress. ROS-induced oxidative damage to DNA produces stable by-products such as 8-hydroxyguanosine (8-OHG) DNA adducts whereas lipid peroxidation leads to well-studied cytotoxic products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) which have been used extensively to assess oxidative cellular damage. MDA appears to be the most mutagenic product of lipid peroxidation that arises when ROS reacts with lipids. The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicity and diseases (Gutteridge 1995; Yin et al., 2011; Gaschler and Stockwell 2017).

Hospital workers are the occupational exposure group that are consistently exposed to low doses of ionizing radiation (Mettler et al., 2008). In many hospital radiology units, although the radiation exposure dose remains below the recommended levels, there is a high risk for workers handling X-ray machines due to their long-term exposure to low doses of ionizing radiation. Therefore, the present study was conducted to analyze the cytogenetic damage associated with exposure to ionizing radiation by measuring the alterations in the antioxidant status in the blood plasma of hospital workers who are occupationally exposed to low doses of ionizing radiation and comparing the results with controls living in the same urban area.

2. MATERIALS AND METHODS

2.1. Chemicals

Glutathione reduced (GSH), nicotinamide adenosine dinucleotide (NADH), nitroblue tetrazolium (NBT) and n-butanol were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Methanol, acetic acid, Folin-Ciocalteu reagent, potassium tartrate, hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA), hydrochloric acid (HCl) and potassium chloride (KCl) were purchased from Merck India (Mumbai, India). Thiobarbituric acid (TBA) and phenazine methosulphate (PMS), 1-chloro-2, 4-nitrobenzene (CDNB) were purchased from Sigma Aldrich Chemical Co. (Bangalore, India) and 5, 5′ dithio 2- nitrobenzoic acid (DTNB) was procured from Tokyo Chemical Industry (Tokyo, Japan).

2.2. Selection of subjects

The volunteers were divided into two groups as described in Chapter 2.

***2.2.1. Exposed group*:** The group comprised of 40 healthy individuals that are occupationally exposed to X-rays for medical diagnostic purposes, working in different hospitals in Aizawl, Mizoram.

2.2.2. Control group: The group comprised of 40 healthy individuals matched for various demographic data, residing in the same urban area. The volunteers did not have any work-related exposure to ionizing radiation or any known genotoxic agent. After identifying suitable volunteers for the study, each individual was asked to sign a consent form and questionnaire as described in Chapter 2.

2.3. Blood sample collection

Blood samples were collected by venepuncture from each individual. The blood was allowed to sediment and the buffy coat was collected. The antioxidant status of the study groups was estimated in the blood plasma of each individual using standard protocols.

2.4. Biochemical estimations

Total protein contents were measured by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

2.4.1. Glutathione

Glutathione (GSH) contents were measured using the method given by Moron et al. (1979). Briefly, 80 µl of plasma was mixed with 900 µl of 0.02 M sodium phosphate buffer and 20 µl of 10 mM DTNB and incubated for 2 min at room temperature. The absorbance of the sample was read against blank at 412 nm in a UV-Visible spectrophotometer (SW 3.5.1.0. Biospectrometer, Eppendorf India Ltd., Chennai), and GSH concentration was calculated from the standard curve and expressed in µmol/mg protein.

2.4.2. Glutathione-s-transferase

Glutathione-s-transferase (GST) activity was measured using the method given by Beutler (1984). Briefly, 850 µl of phosphate buffer was mixed with 50 µl of 20 mM CDNB, incubated for 10 min at 37˚C followed by the addition of 50 µl each of 20 mM GSH and plasma. The blank consisted of all the reagents and distilled water instead of plasma. The absorbance of blank and test was measured at 360 nm and the enzyme activity was expressed in Unit/mg protein.

2.4.3. Superoxide dismutase

Superoxide dismutase (SOD) activity was measured by the method of Fried (1975). Briefly, 100 µl each of plasma and 186 µM PMS were mixed with 300 µl of 3 mM NBT and 200 µl of 780 µM NADH. The mixture was incubated for 90 sec at 30°C and 1 ml of acetic acid and 4 ml of n-butanol were added to stop the reaction. The blank consisted of all the reagents and distilled H_2O was added instead of plasma. The absorbance of test and blank was measured at 560 nm using a UV-VIS spectrophotometer, and the enzyme activity was expressed in Unit/mg protein (1Unit = 50% inhibition of NBT reduction).

$$\% \text{ inhibition} = (\text{OD of blank OD of test=OD of blank}) \times 100$$

$$\text{SOD unit} = 1/50 \times \% \text{ inhibition.}$$

2.4.4. Catalase

Catalase (CAT) activity was determined using the modified protocol of Aebi (1984). Briefly, 200 µl of 3% H_2O_2 was mixed with 50 µl of plasma and 150 µl of 50mM phosphate buffer (pH 7.0). The absorbance was recorded at 240 nm in a UV-VIS spectrophotometer. The decomposition of H_2O_2 was followed directly by the decrease in absorbance. The enzyme activity was expressed in Unit/mg protein. The

catalytic activity of CAT at a time interval of 15 sec was calculated by the following formula:

$$K = 0.153 (\log A_0 / A_1)$$

where A_0 is the absorbance at 0 sec and A_1 is the absorbance at 15 sec.

2.4.5. Lipid peroxidation

Lipid peroxidation **(**LPO) was estimated by the method of Beuege and Aust (1978). Briefly, plasma was added to a mixture containing 10% TCA, 0.8% TBA and 0.02 N HCl in a 1:2 ratio. The mixture was boiled for 10 min in a boiling water bath, cooled immediately at room temperature and centrifuged at 1000 rpm for 10 min. The supernatant was collected and its absorbance was read at 535 nm against the blank. The blank contained all the reagents minus the plasma, which was substituted with distilled water. The MDA concentration of the sample was calculated using the extinction coefficient of $1.56 \times 10^{-6} M^{-1} cm^{-1}$.

2.5. Statistical analyses

The results are expressed as mean ± standard error of the mean (SEM). Student "t" test was performed to determine the significant differences between the exposed and control group for various antioxidant assays. Multiple linear regression analyses was carried out for the prediction of antioxidant status from demographic characteristics. Statistical analyses was conducted using Graph Pad Prism ver. 6.0 and SPSS Ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

Levels and activities of antioxidants such as glutathione (GSH), glutathione-s-transferase (GST), superoxide dismutase (SOD), catalase (CAT) along with the level of lipid peroxidation (LPO) in the serum of both exposed and control groups were measured using standard protocols. The occupationally exposed group showed a significant decline in the activities of GST and catalase when compared to the control group, with a significant rise in LPO irrespective of their demographic characteristics (Figure 1.1 B, D & E). However, no significant changes were observed in the level of GSH and SOD activity between exposed and control groups (Figure 1.1 A & C).

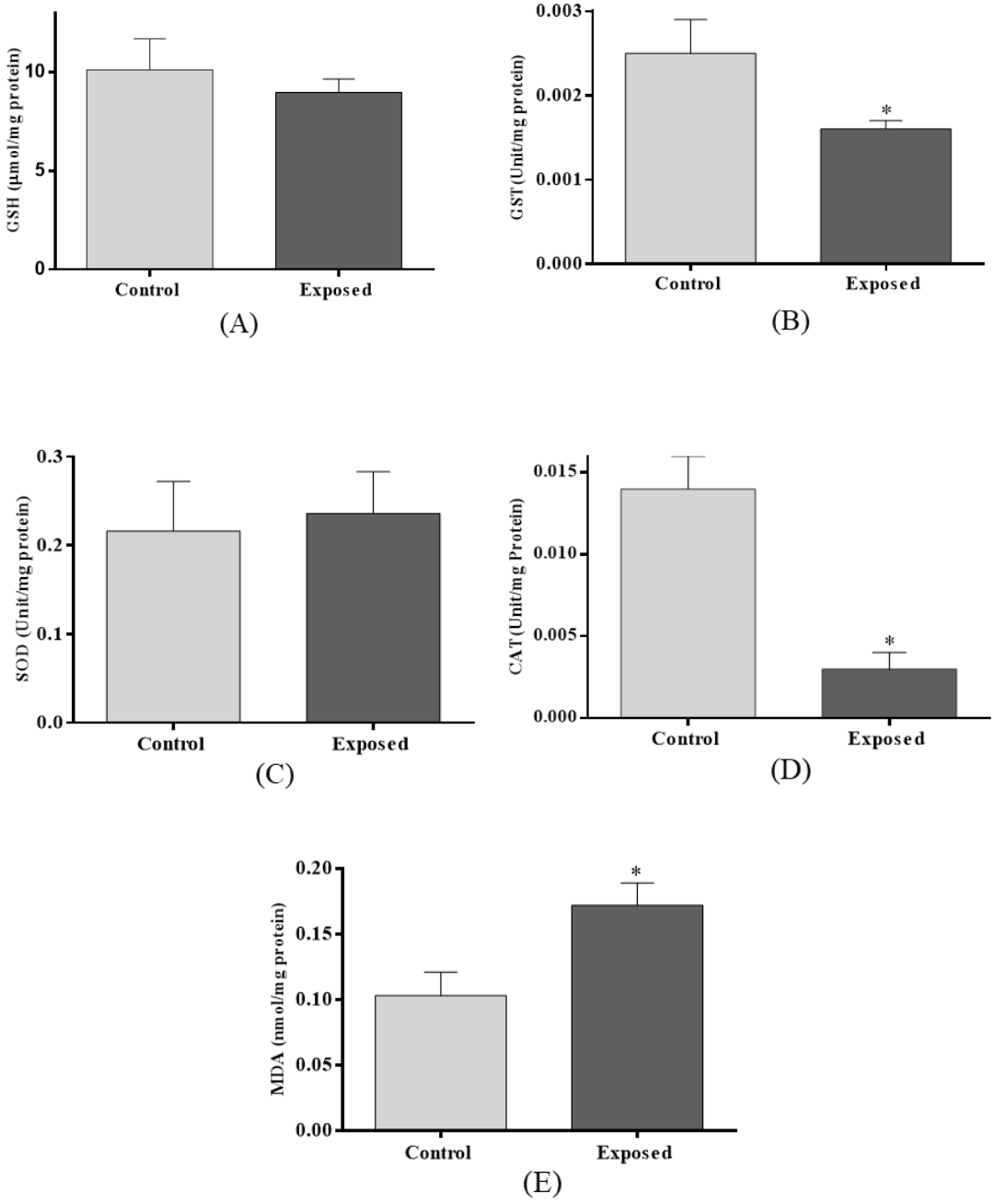
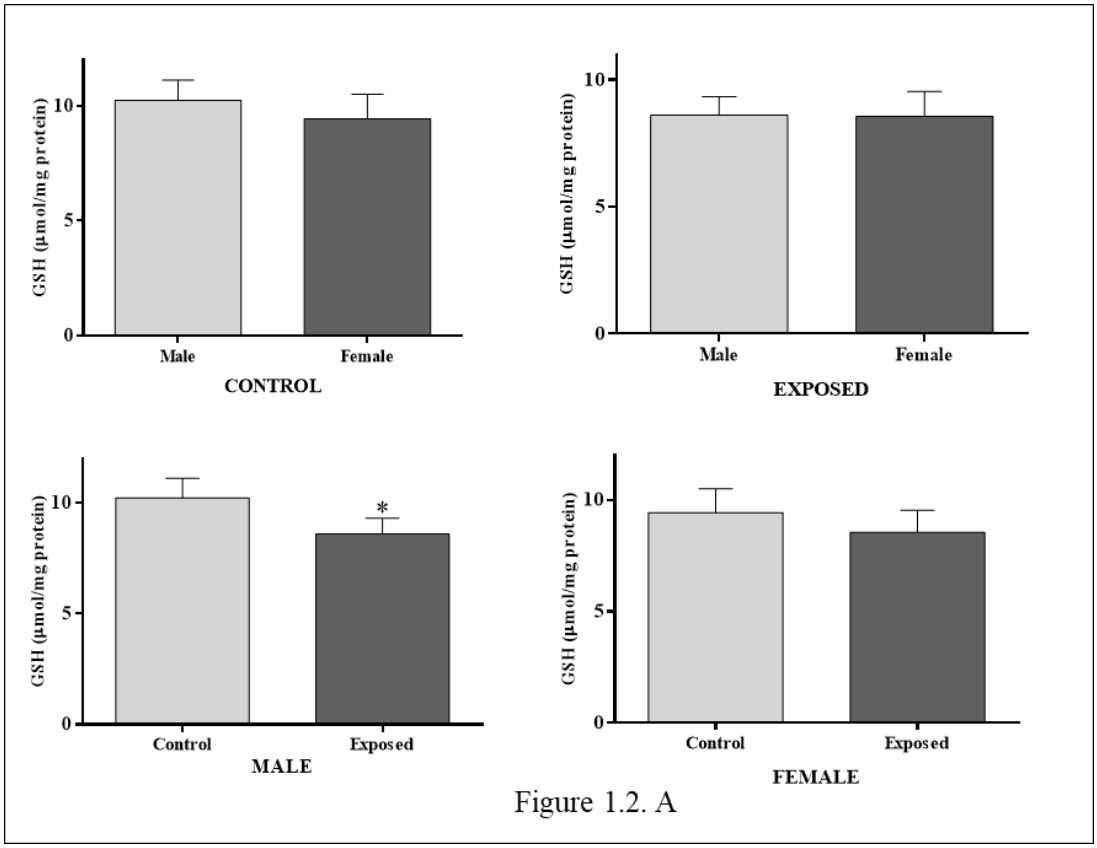


Figure 1.1: Levels and activities of antioxidant enzymes and lipid peroxidation in the exposed and control groups. (A) Glutathione (GSH) level (µmol/mg protein); (B) Glutathione-s-transferase (GST) activity (Unit/mg protein); (C) Superoxide dismutase (SOD) activity (Unit/mg protein); (D) Catalase (CAT) activity (Unit/mg protein); (E) level of lipid peroxidation (LPO) (nmol/mg protein). * *indicates significant variations between the group ($p<0.05$).*

3.1. Effects of demographic characteristics on the level of glutathione among the exposed and control groups:

Males of the exposed group showed a significant decline in the level of GSH when compared with males of the control group. However, a significant change in the GSH level was not observed among the females of both groups (Figure 1.2 A). The younger members (20-40 years) and elder members (41-60 years) of the exposed group showed a significant decline in the levels of GSH when compared with their respective age groups in the control group. Also, elder members of the exposed group showed a significant decline in GSH levels when compared with younger members of the exposed group (Figure 1.2 B). Similarly, betelnut chewers among the exposed group showed a significant decline in the level of GSH when compared with the control group (Figure 1.2 D). Smoking, tobacco chewing, alcohol consumption and family history of cancer did not significantly alter the levels of GSH in both the exposed and control groups (Figure 1.2 C, E-G). Multiple linear regression analyses revealed a significant association of the reduced level of GSH with gender and age (Table 2).

Among the occupationally exposed group, technicians who have a cumulative effective dose of >50 mSv showed a significant decline in the levels of GSH when compared with workers having a cumulative effective dose of ≤50 mSv. Duration of employment and number of patients handled per day did not show a significant effect on the levels of GSH (Figure 1.3). Multiple linear regression analyses revealed a significant association between the reduced level of GSH and cumulative dose of occupationally exposed group (Table 2).



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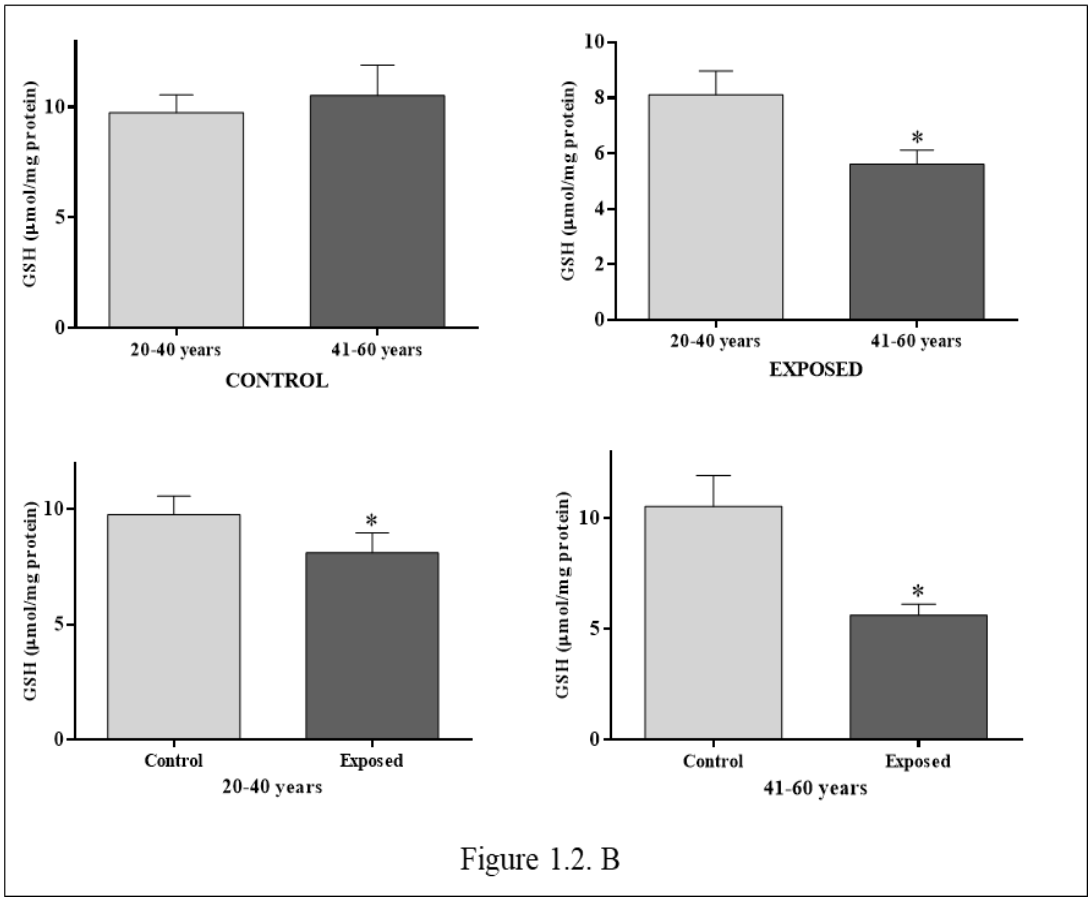


Figure 1.2. B

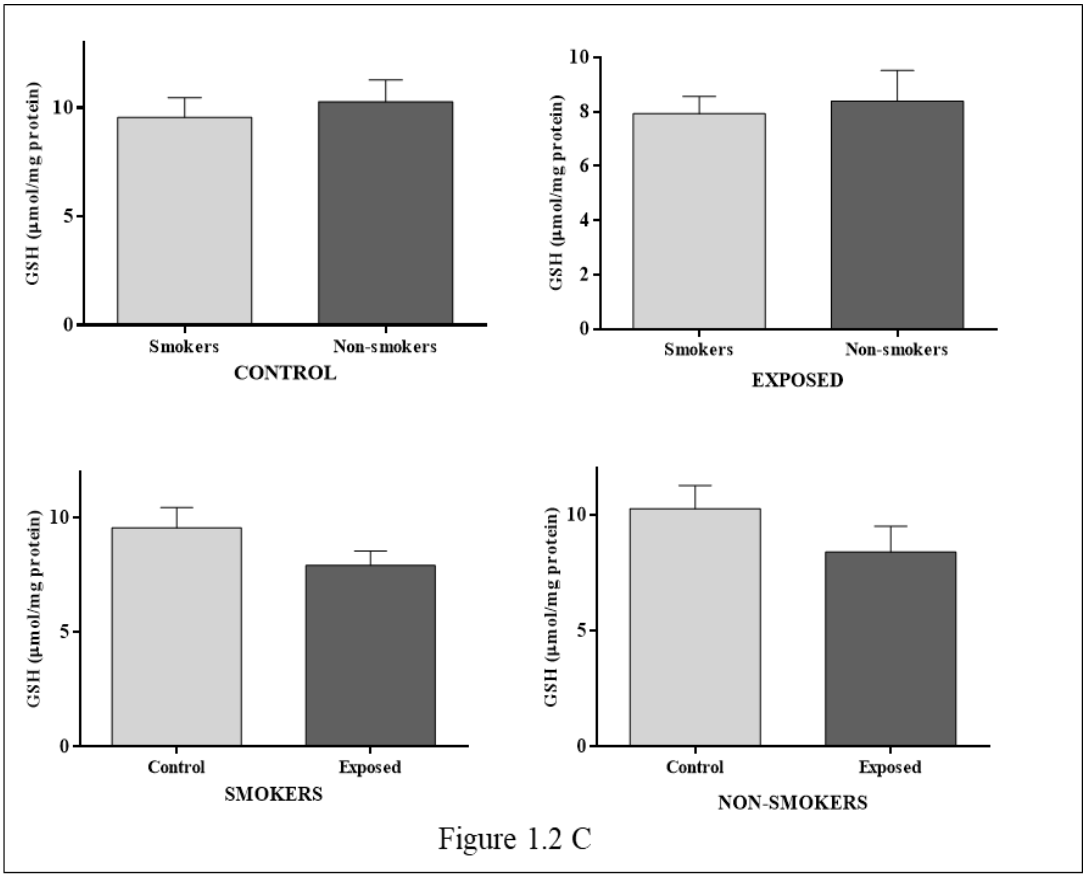
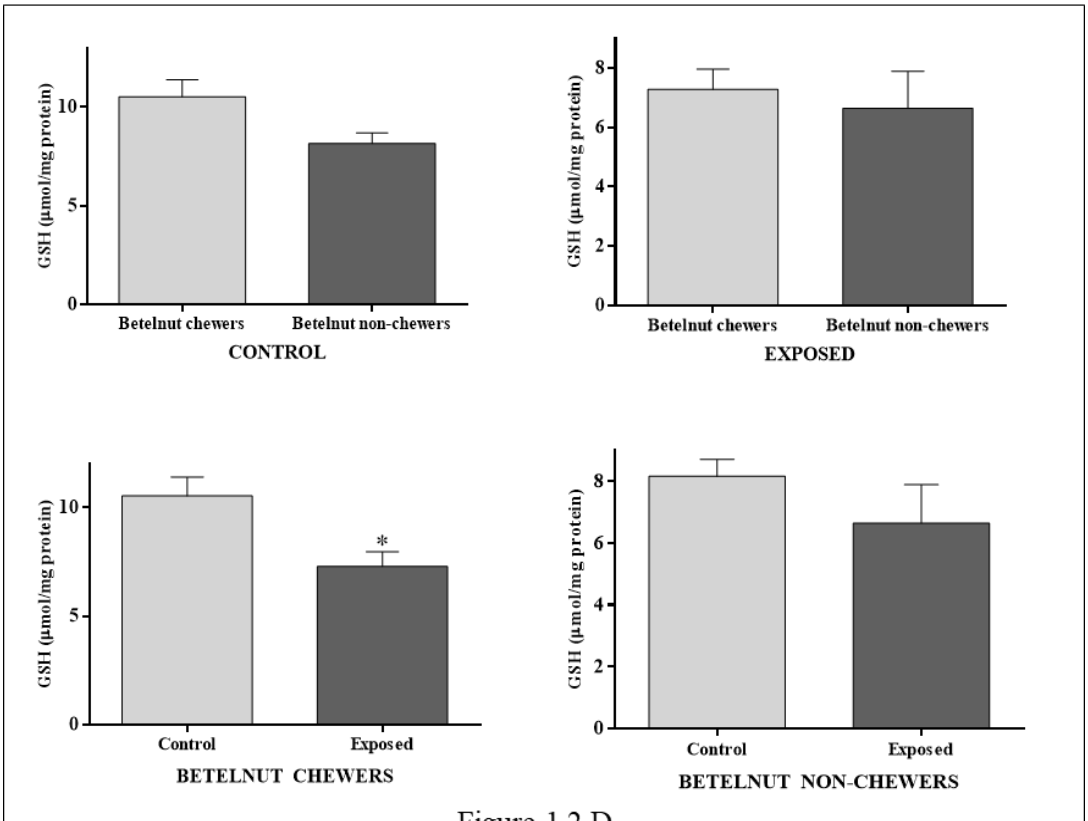


Figure 1.2 C



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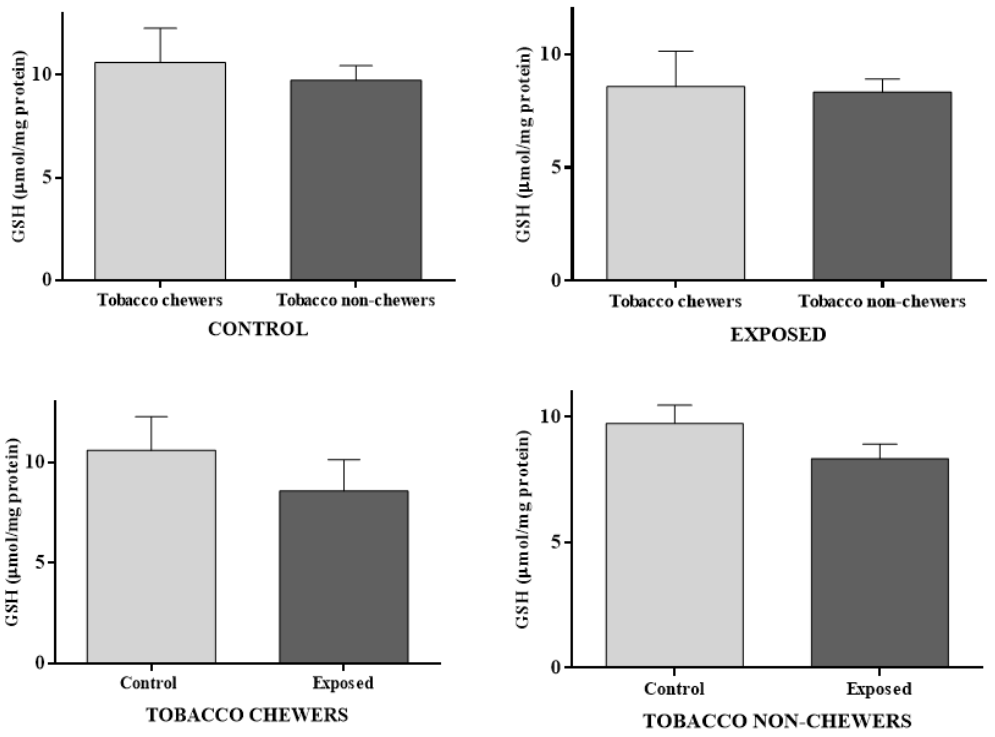

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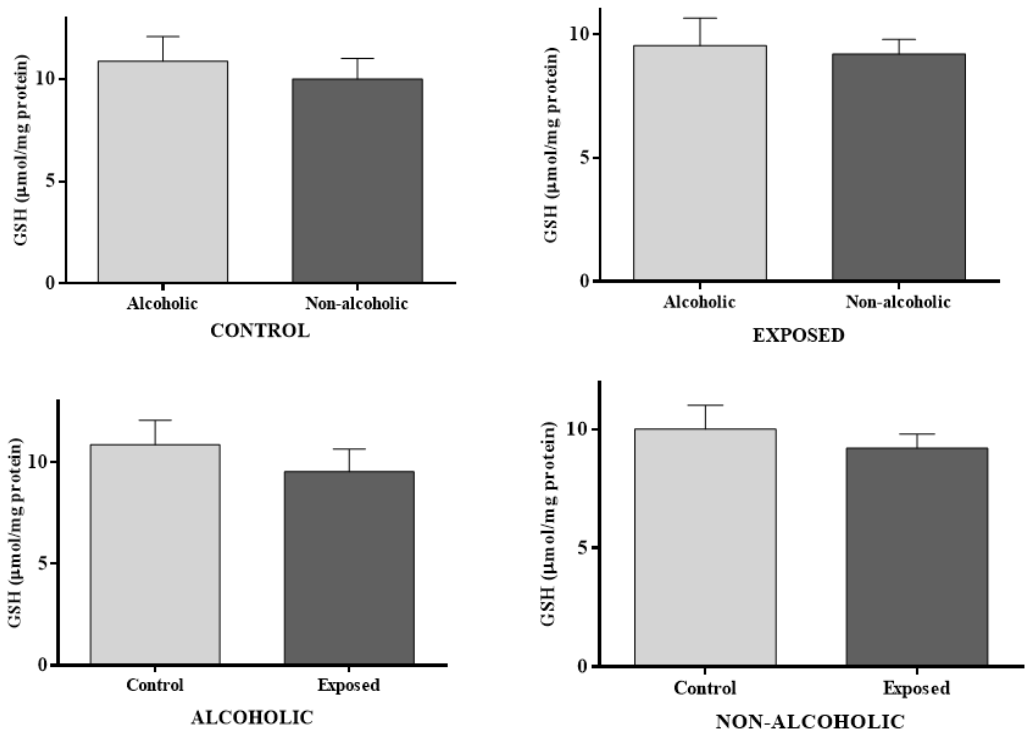

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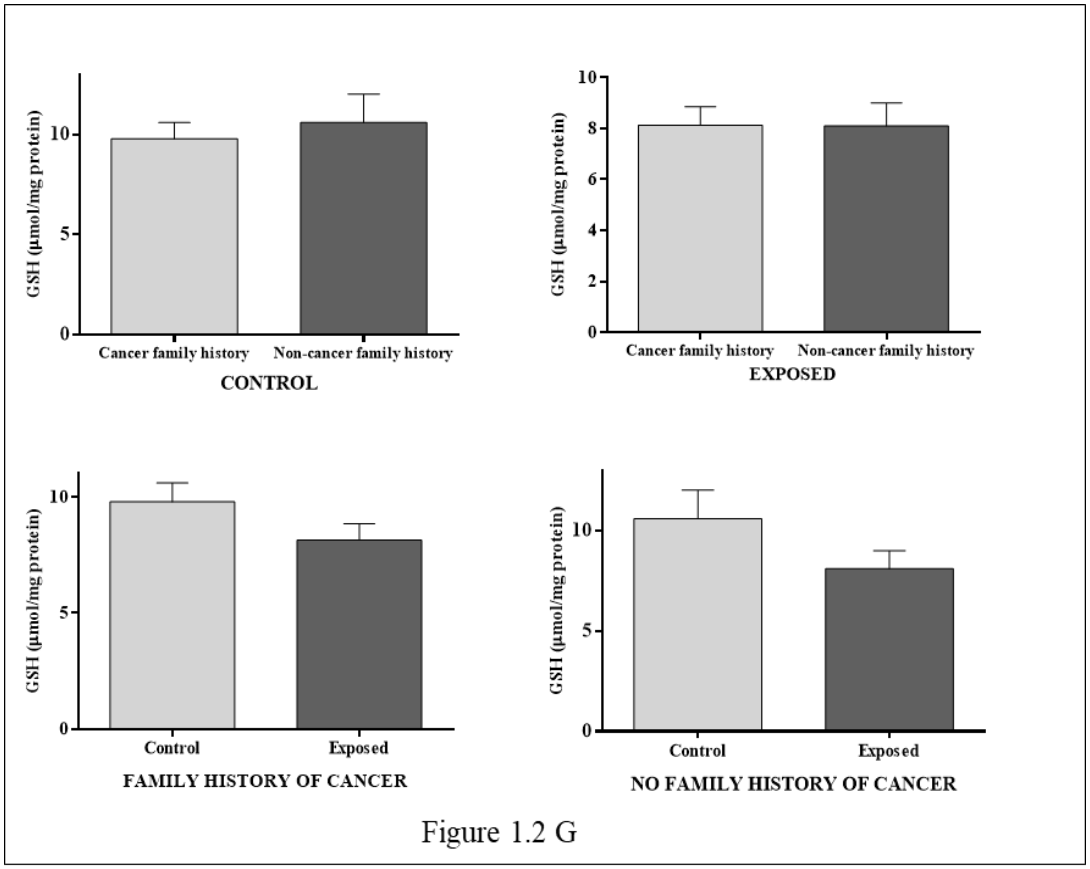


Figure 1.2: Functions of the demographic characteristics on glutathione (GSH) level (µmol/mg protein) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

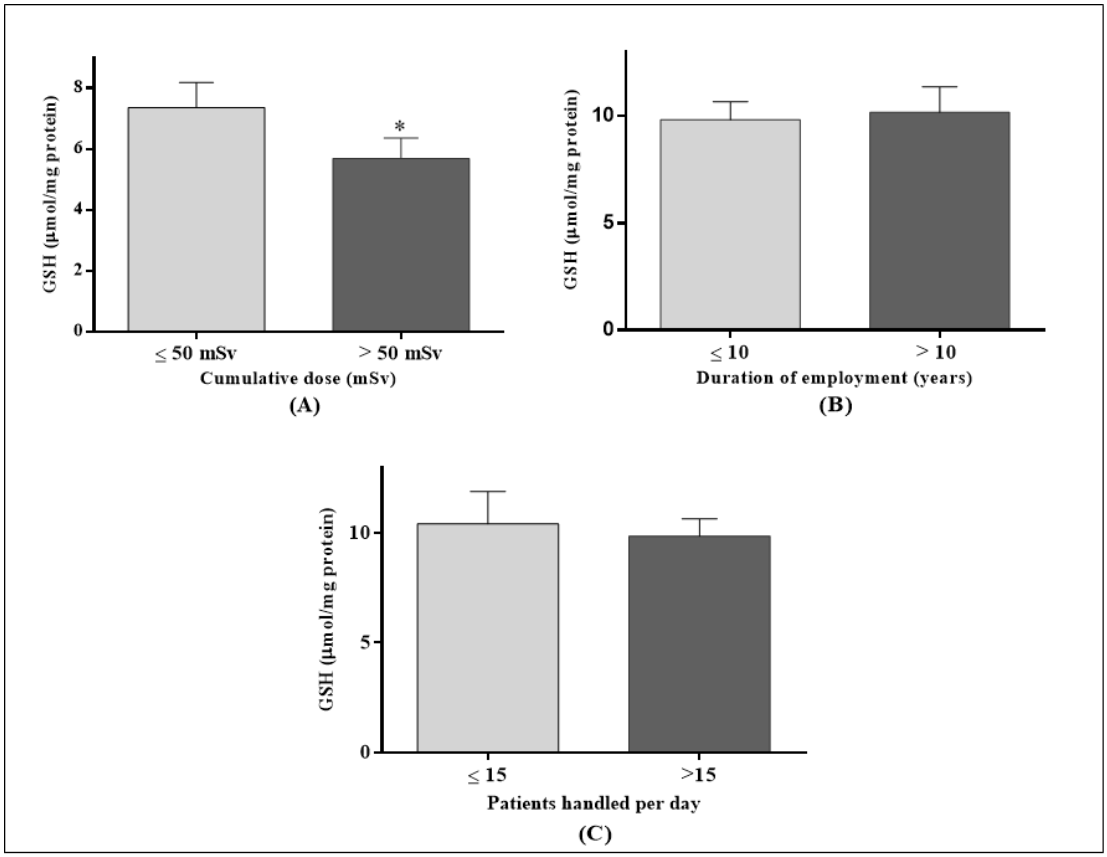


Figure 1.3: Functions of the demographic characteristics on glutathione (GSH) level (µmol/mg protein) of the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

3.2. Effects of demographic characteristics on the activity of glutathione-s transferase among the exposed and control groups:

Males of the exposed group showed comparatively lower GST activity when compared with males of the control group. A significant change was not observed in females of both exposed and control groups (Figure 1.4 A). The activity of GST was significantly lower in younger members (20-40 years) of the exposed group when compared with their respective age group of the control group. However, GST activity of elder members (41-60 years) of the exposed group was not significantly different from the control group (Figure 1.4 B). Smokers, betelnut chewers and alcohol users of the exposed group showed significantly lower GST activity when compared with those belonging to the control group (Figure 1.4 C, D, E & F). Family

history of cancer among the occupationally exposed group did not show significant variation in GST activity (Figure 1.4 G).

Cumulative dose (mSv), duration of employment (years) and number of patients handled per day did not show a significant effect on the activity of GST on individuals working with X-rays (Figure 1.5).

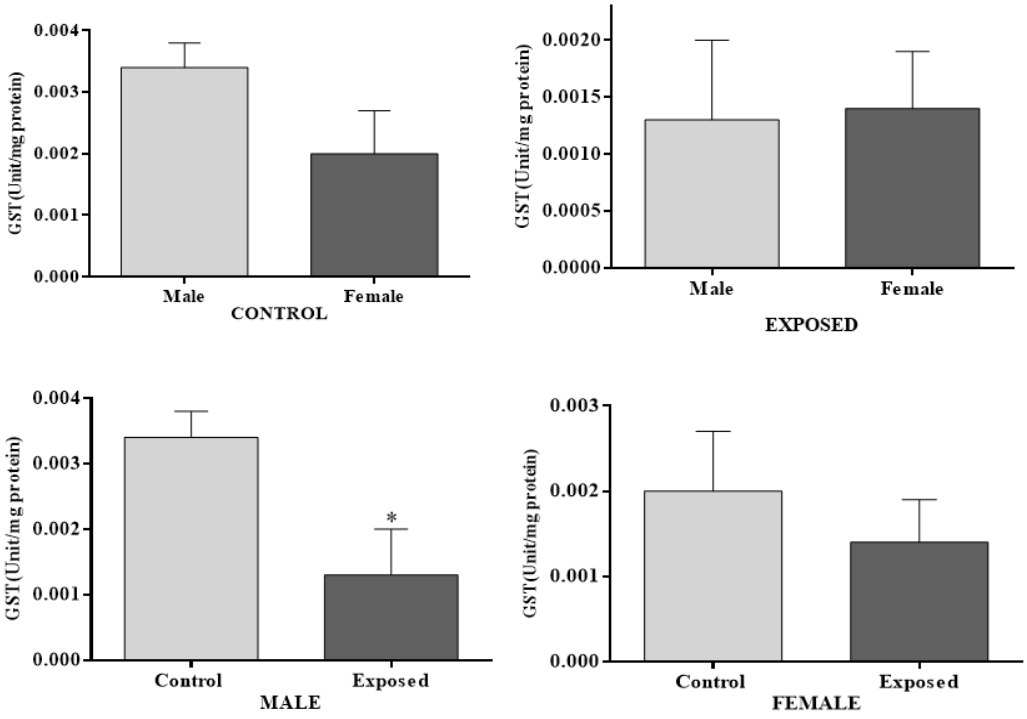


Figure 1.4 A

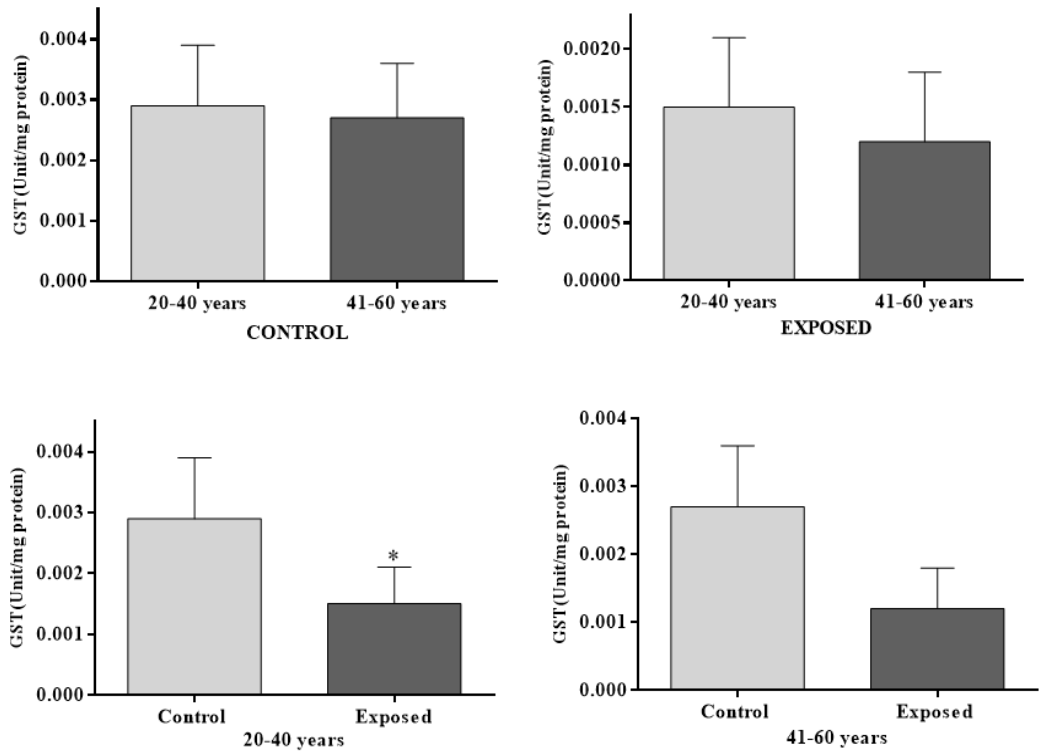


Figure 1.4 B

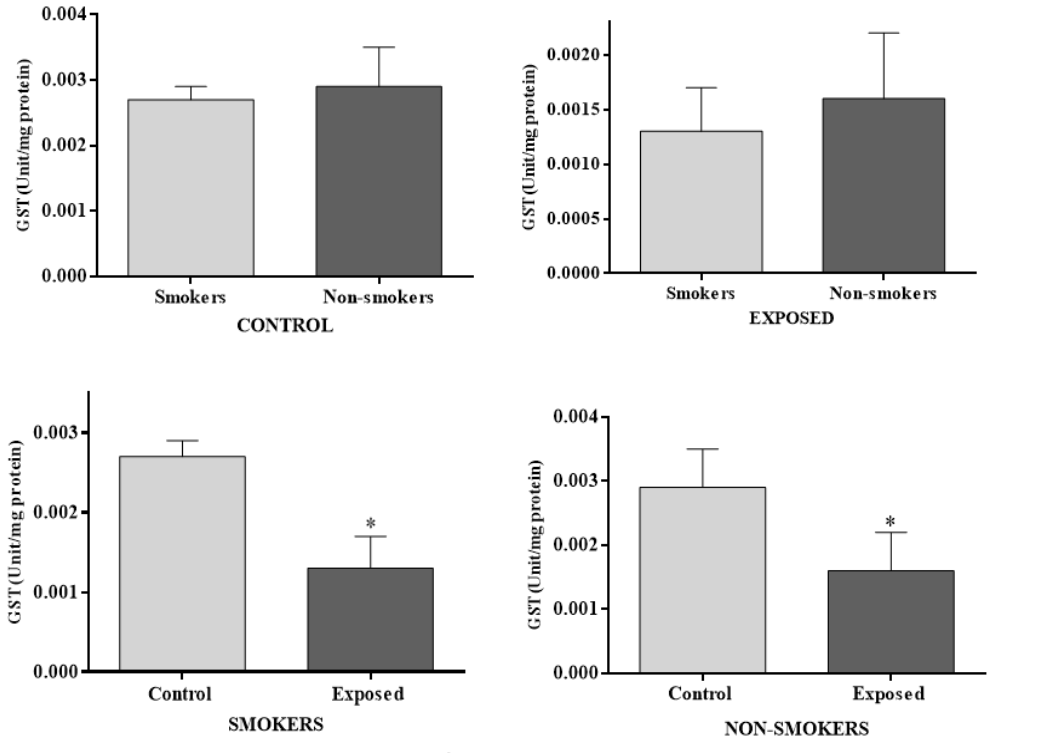
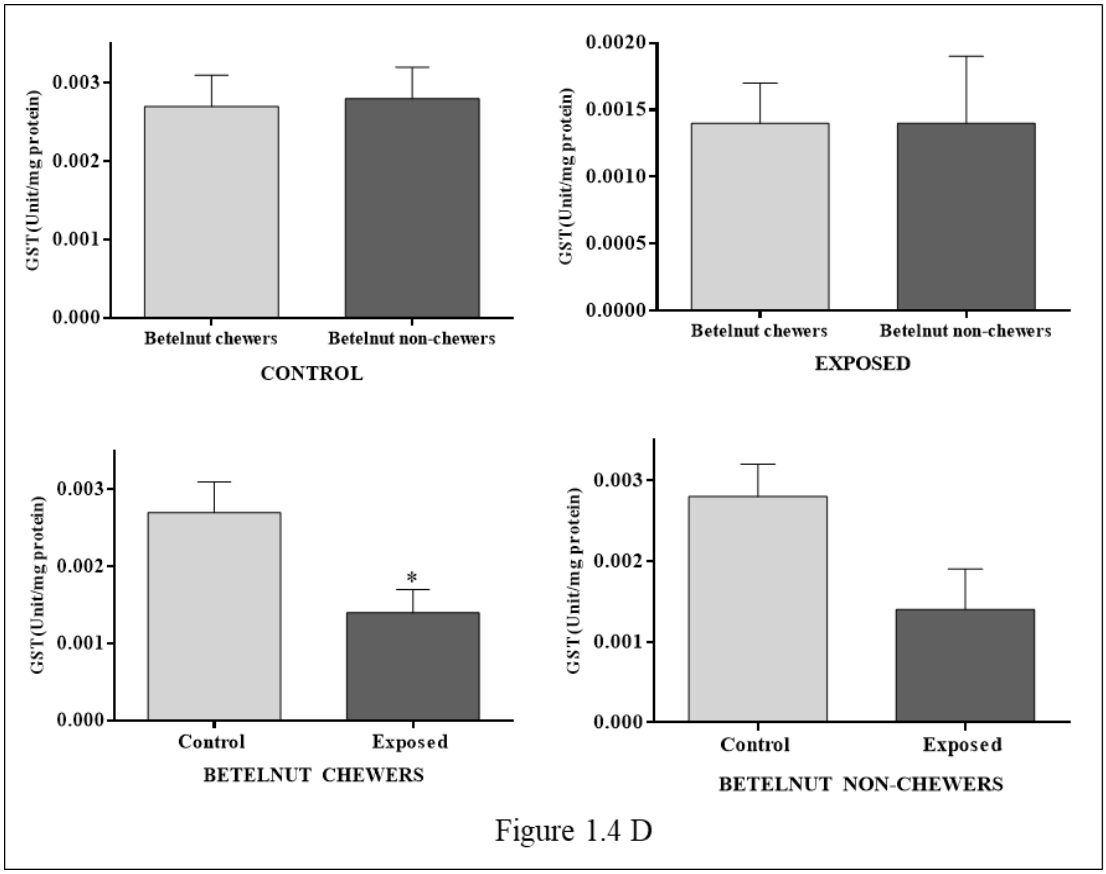


Figure 1.4 C



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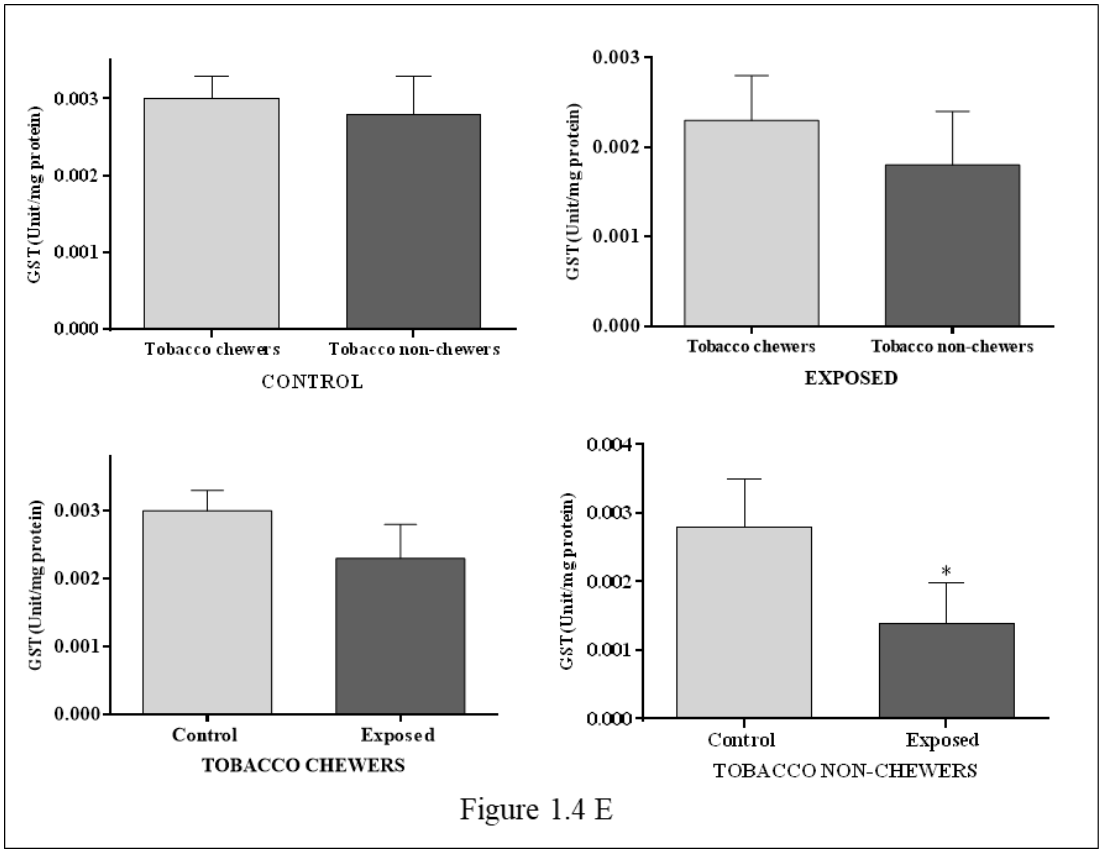

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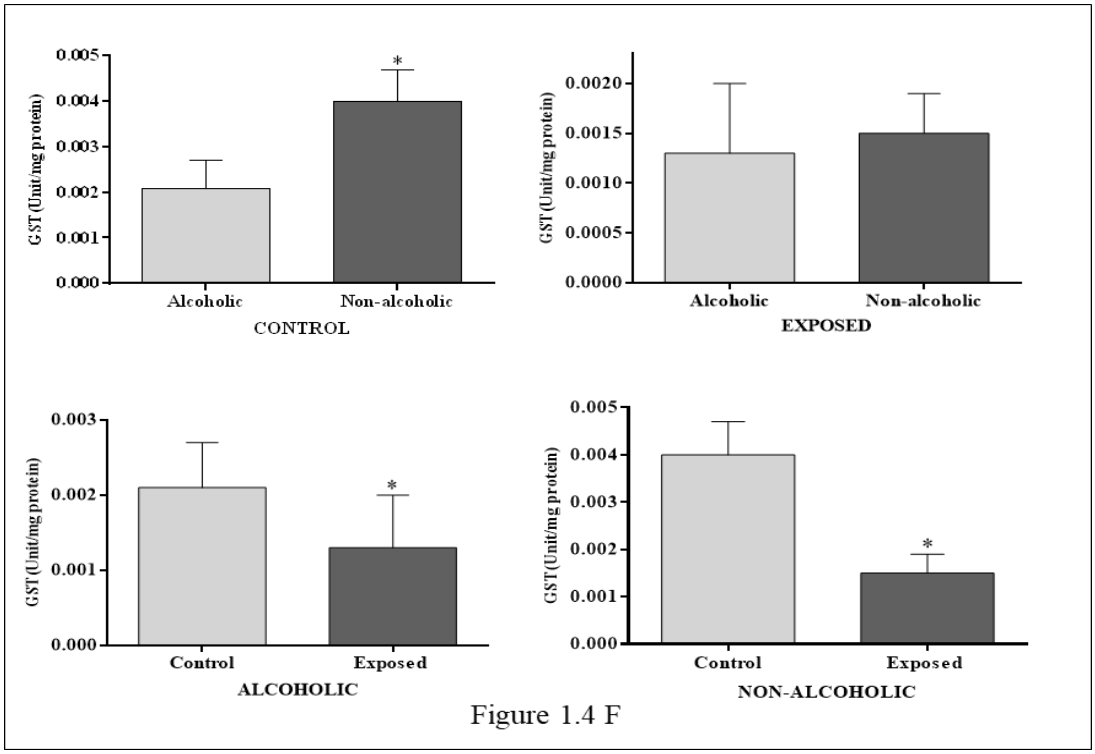

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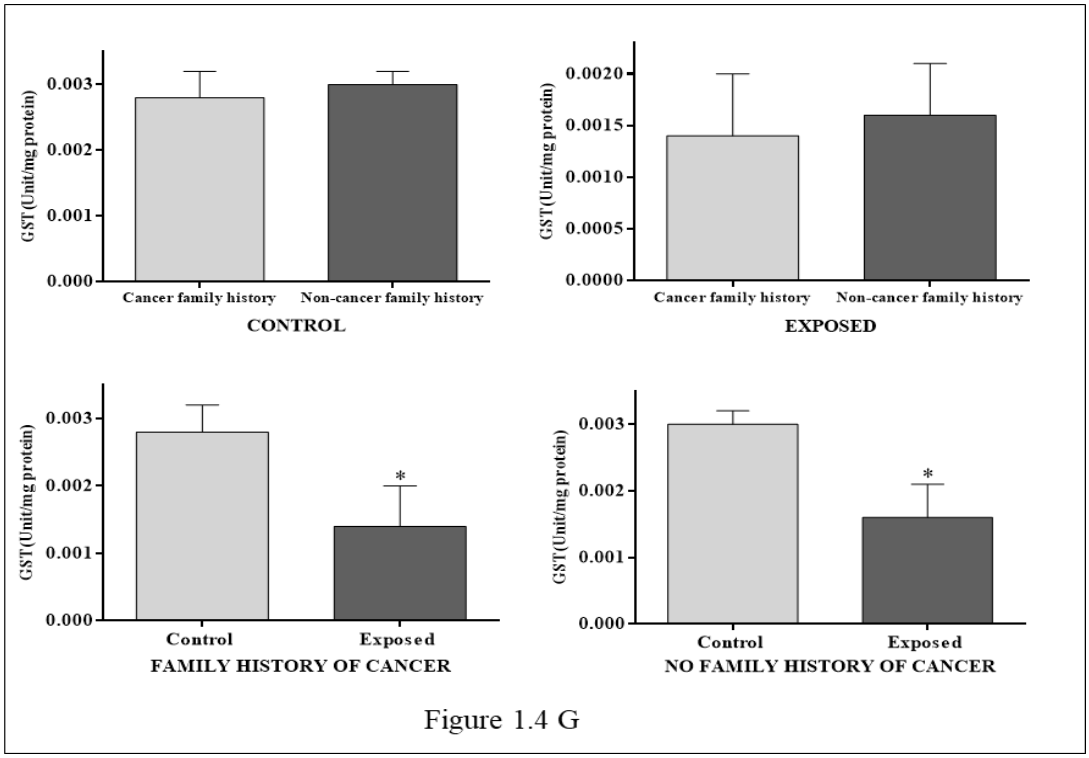


Figure 1.4 G

Figure 1.4: Functions of the demographic characteristics on glutathione-s-transferase (GST) activity (Unit/mg protein) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

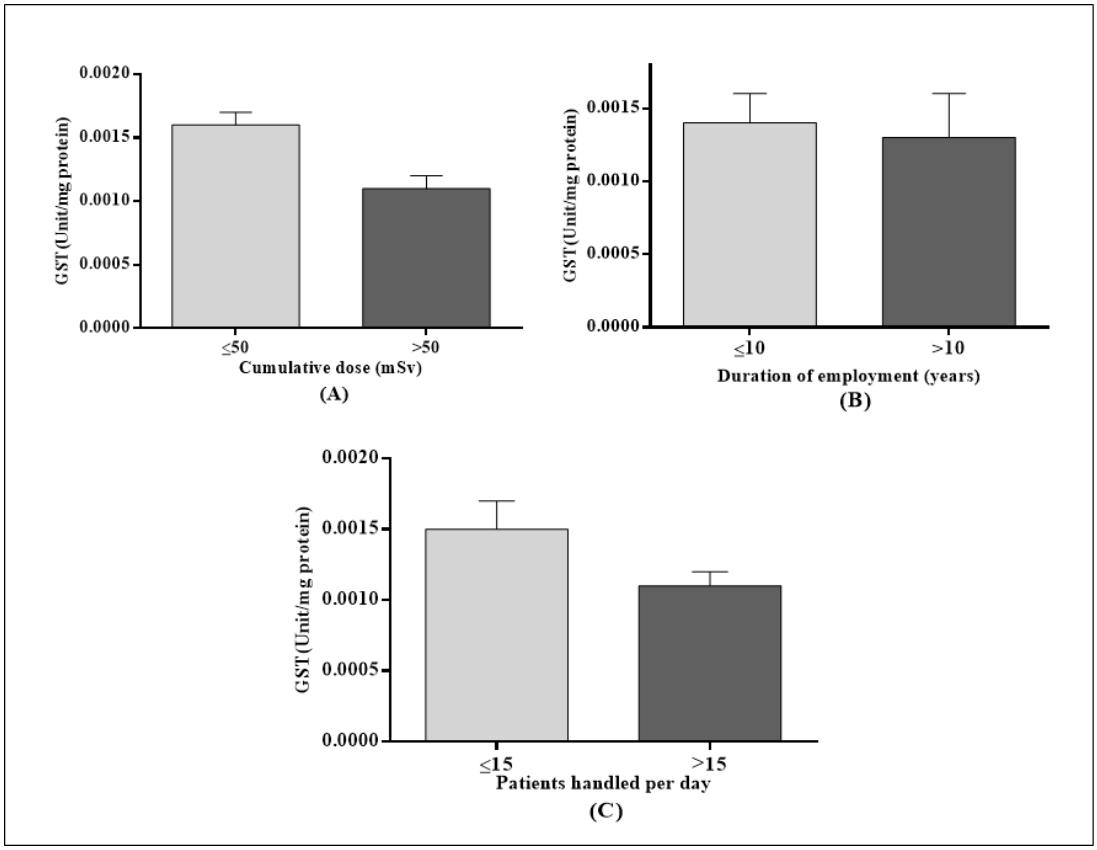


Figure 1.5: Functions of the demographic characteristics on glutathione-s-transferase (GST) activity (Unit/mg protein) of the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

3.3. Effects of demographic characteristics on the activity of superoxide dismutase among the exposed and control groups:

Males of the exposed group showed a significantly lower SOD activity when compared with males of control group. Significant variation in SOD activity was not observed in females of both groups (Figure 1.6 A). The elder members (41-60 years) of the exposed group showed significantly lower SOD activity when compared with elder members of control group (Figure 1.6 C). Smoking, betelnut chewing, tobacco chewing, alcohol consumption and family history of cancer did not significantly affect the activity of SOD in both exposed and control groups (Figure 1.6 D-G).

Among the occupationally exposed group, technicians who have a cumulative

effective dose of >50 mSv showed a significantly lower SOD activity when compared with workers having a cumulative effective dose of ≤50 mSv. Duration of employment and number of patients handled per day did not show a significant effect on SOD activity (Figure 1.7). Multiple linear regression analyses revealed a significant association between the reduced activity of GST and cumulative dose of the exposed group (Table 2).

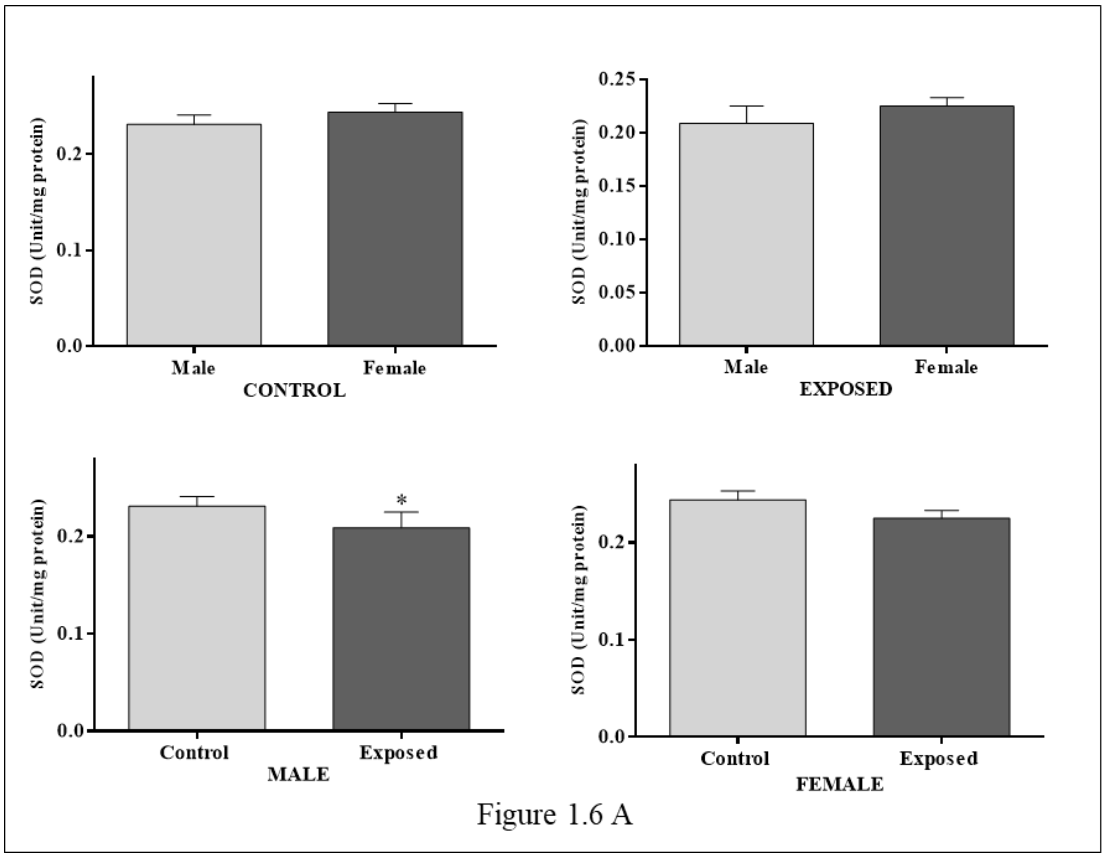
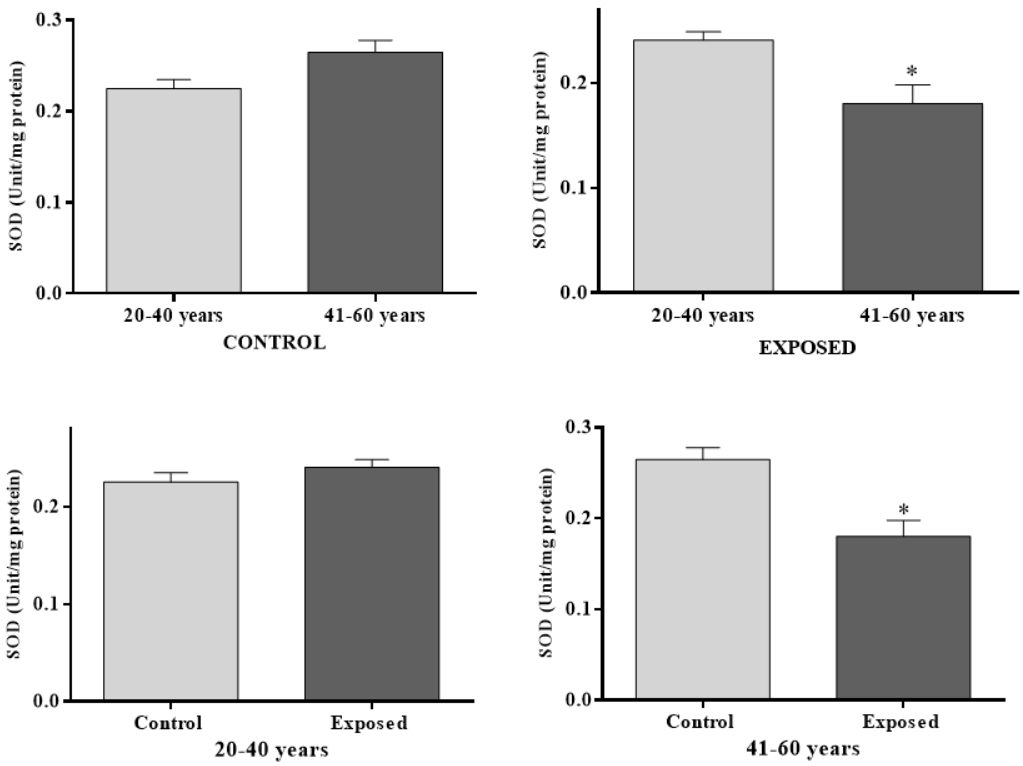


Figure 1.6 A



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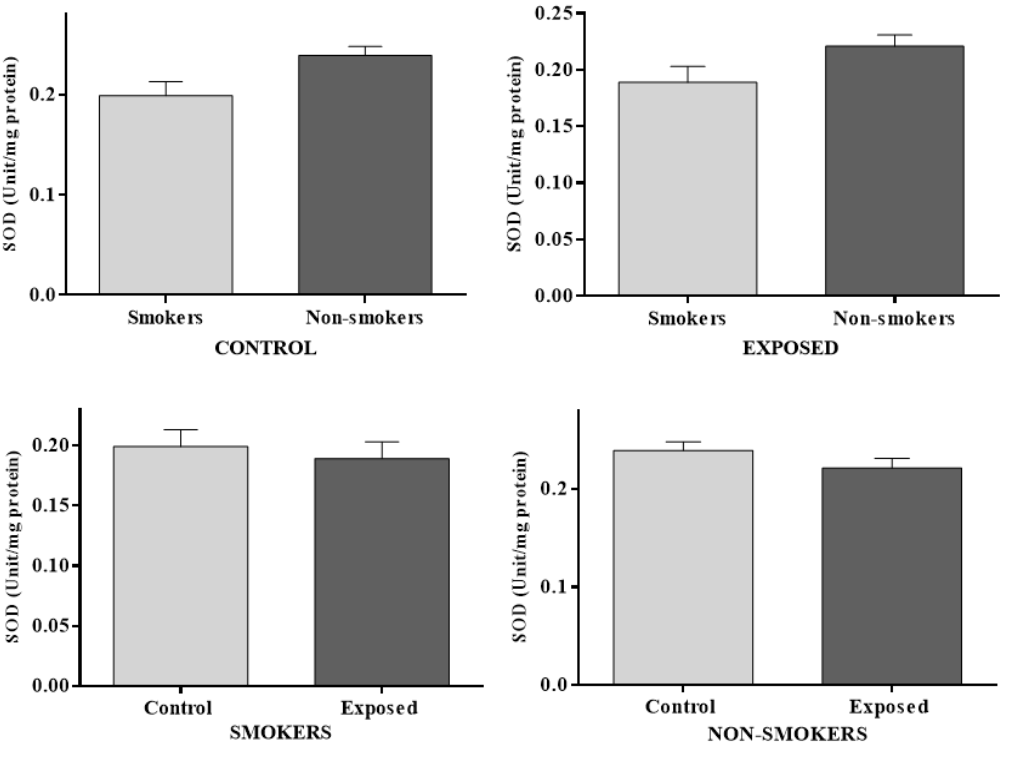
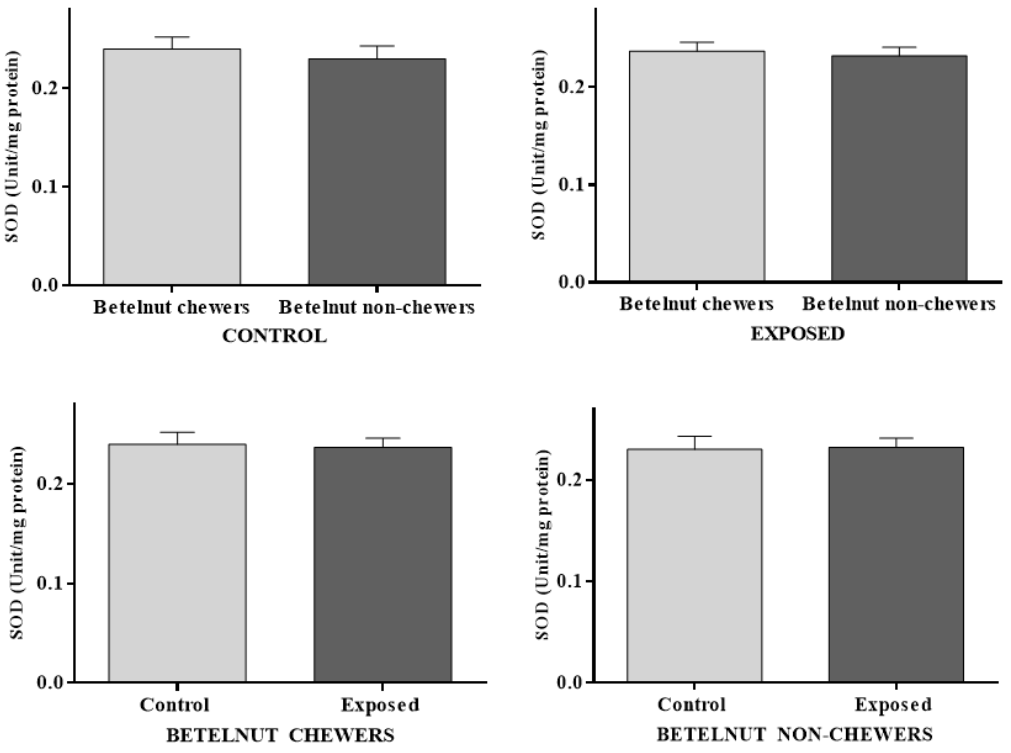


Figure 1.6 C



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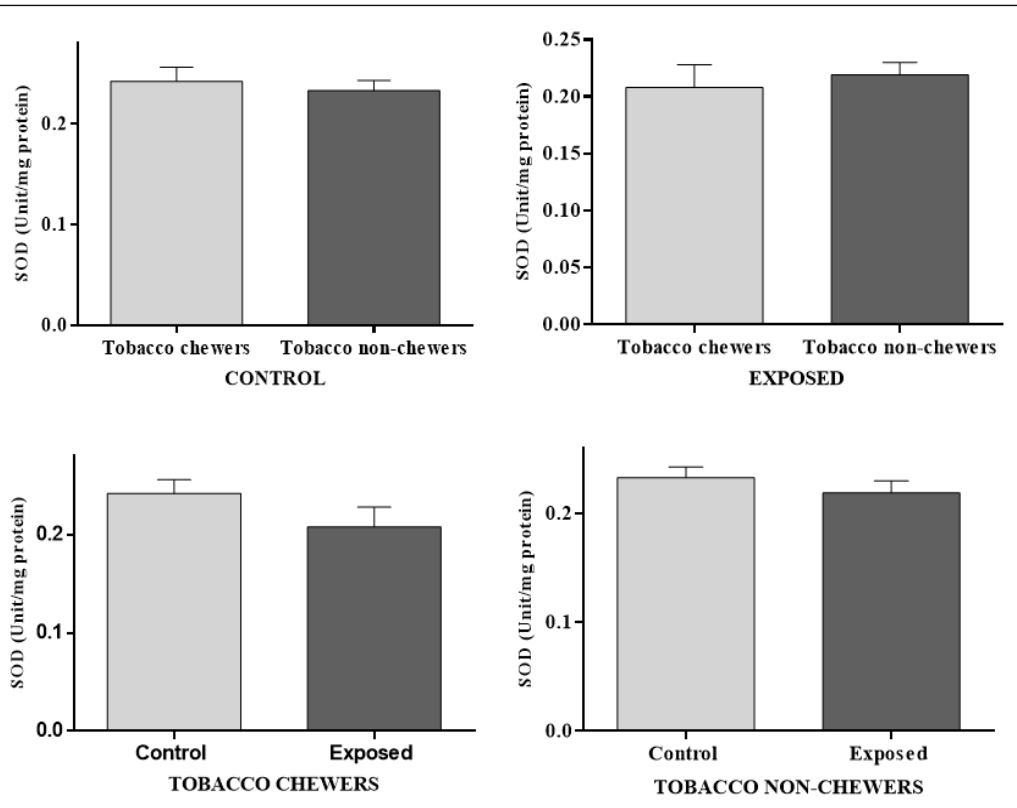

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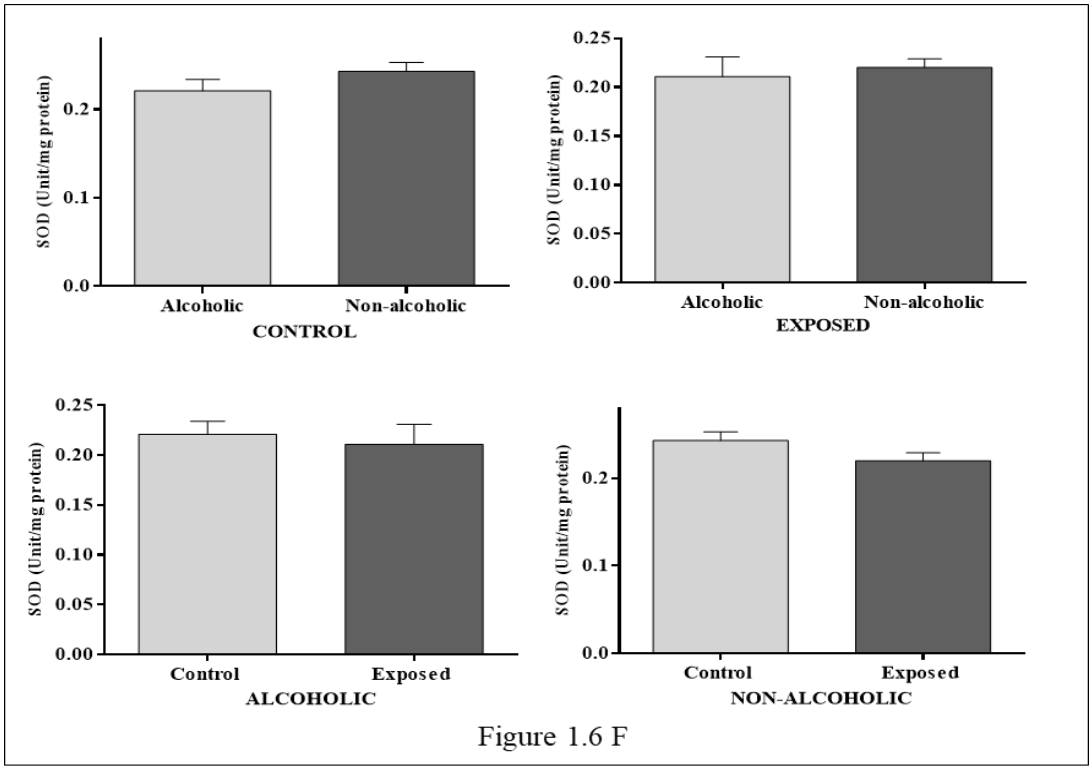


Figure 1.6: Functions of the demographic characteristics on superoxide dismutase (SOD) activity (Unit/mg protein) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

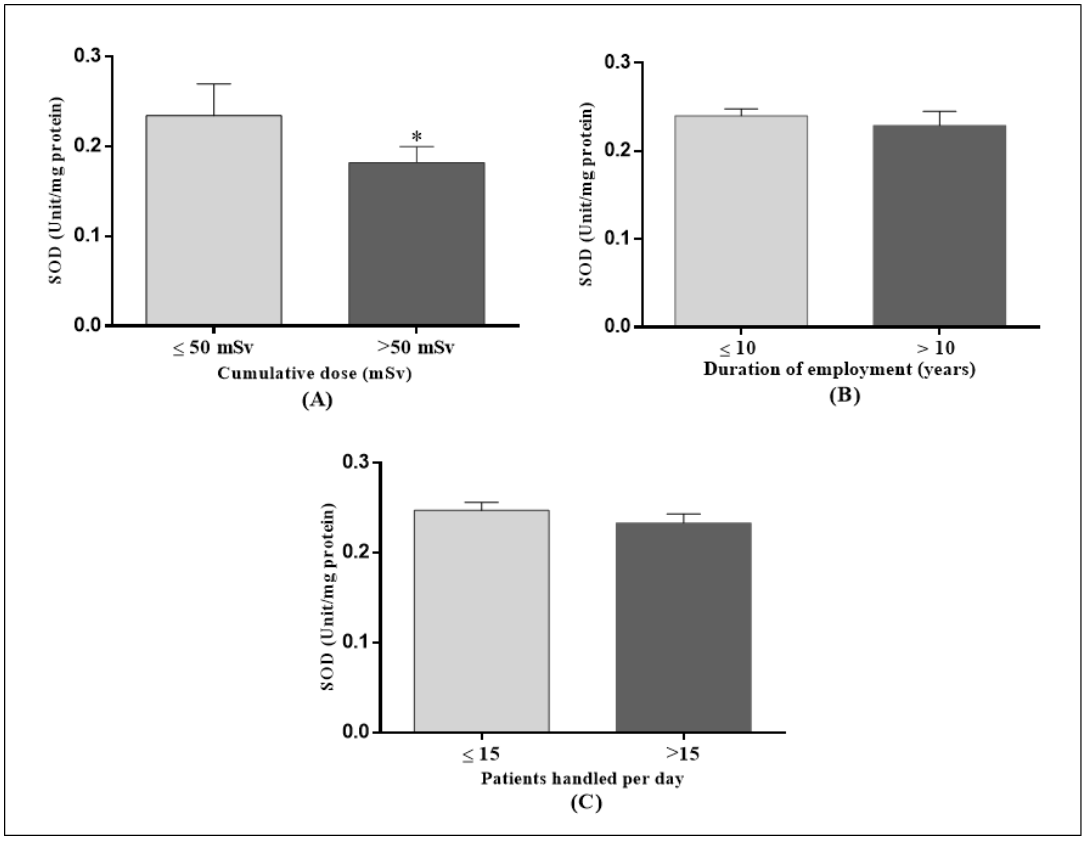


Figure 1.7: Functions of the demographic characteristics on superoxide dismutase (SOD) activity (Unit/mg protein) of the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

3.4. Effects of demographic characteristics on the activity of catalase among the exposed and control groups:

There was a significant decline in the activity of CAT in males and females of the exposed group when compared with their respective gender of the control group (Figure 1.8 A). Also, the younger members (20-40 years) and elder members (41-60 years) of the exposed group showed a significant decline in CAT activity when compared with their respective age groups of the control group. (Figure 1.8 B). Smokers had significantly lower CAT activity than non-smokers among the exposed and control group. Similarly, smokers of the exposed group showed significantly lower CAT activity when compared with control group (Figure 1.8 C). Occupational exposure results in a decline in catalase activity regardless of betelnut chewing, tobacco chewing, alcohol consumption and family history of cancer (Figure 1.8 D-

G). Multiple linear regression analyses revealed a significant association between the reduced activity of CAT and smoking habits (Table 2).

Cumulative dose (mSv), duration of employment (years) and the number of patients handled per day significantly affect CAT activity among individuals working with X-rays (Figure 1.9). Multiple linear regression analyses revealed a significant association of reduced activity of CAT and the number of patients handled per day and cumulative dose (Table 2).

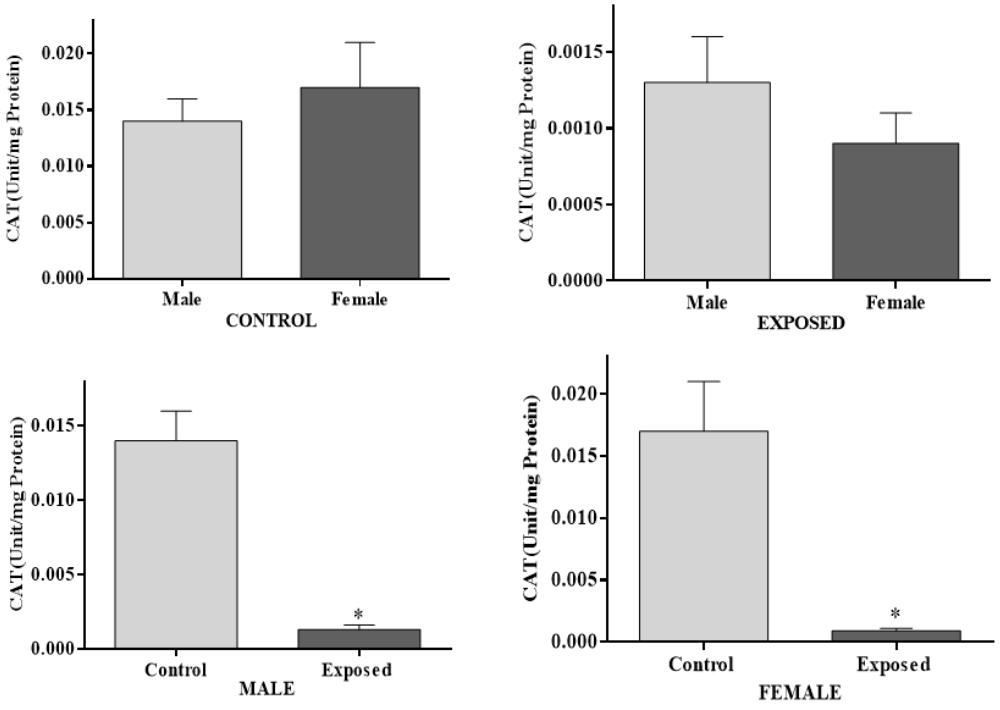


Figure 1.8 A

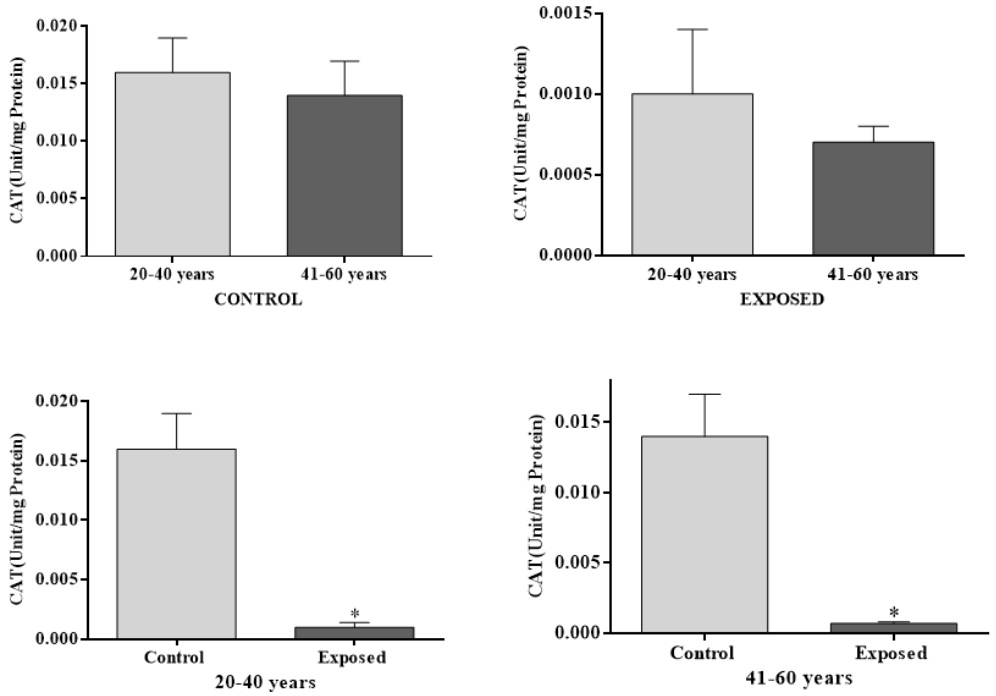


Figure 1.8 B

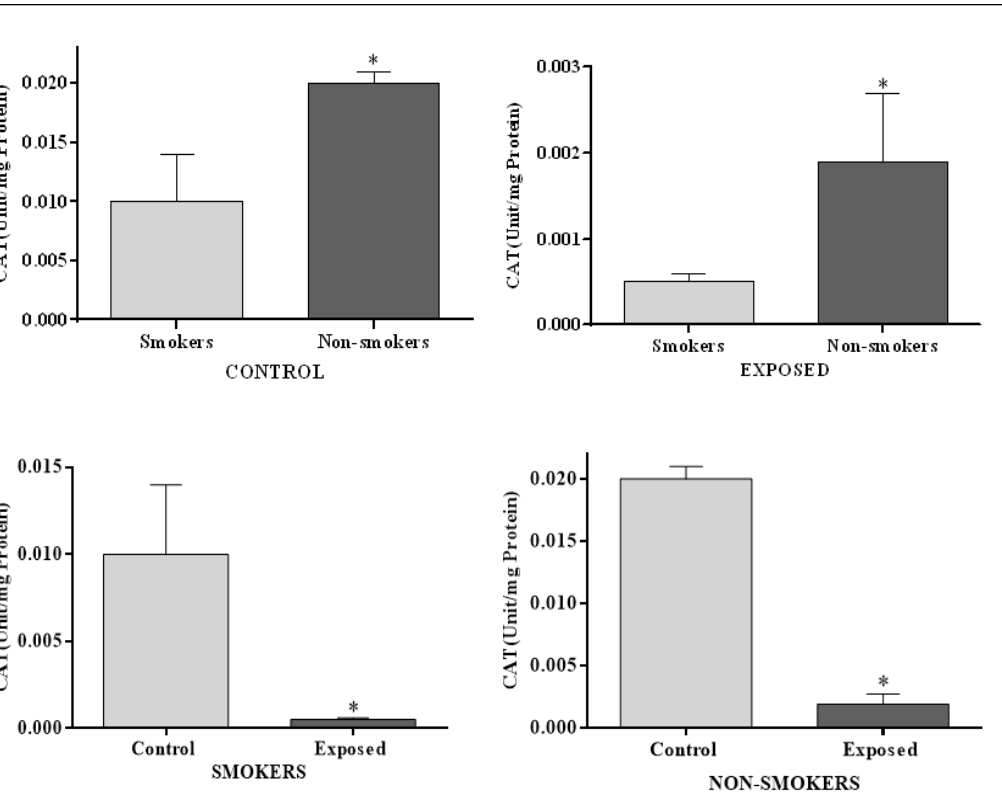
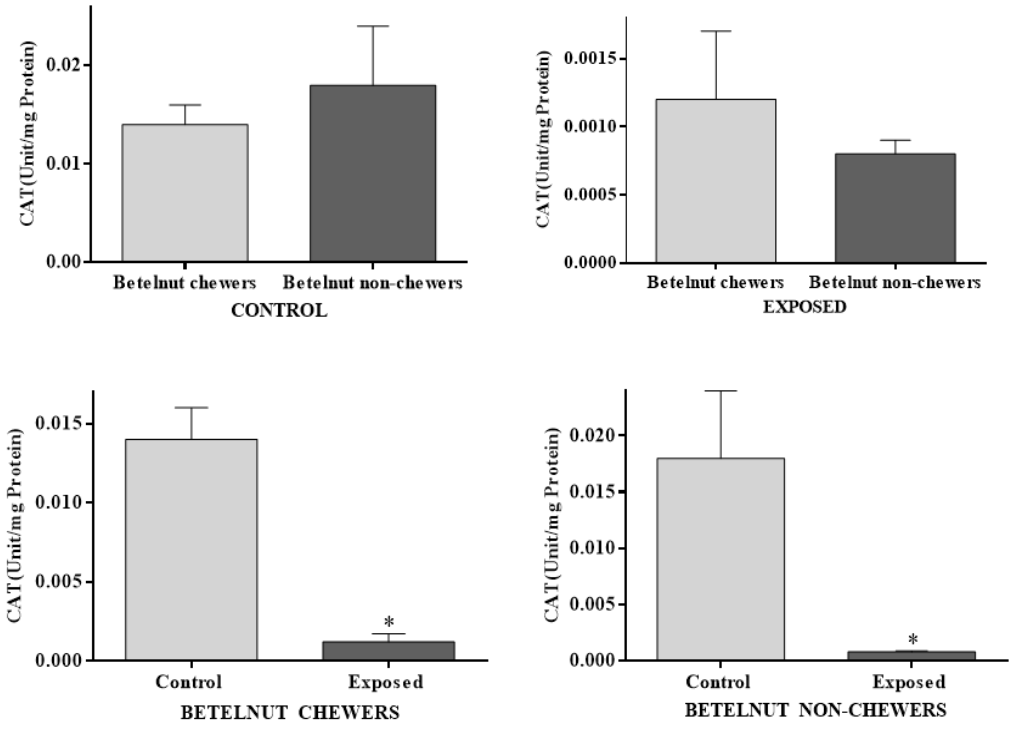


Figure 1.8 C



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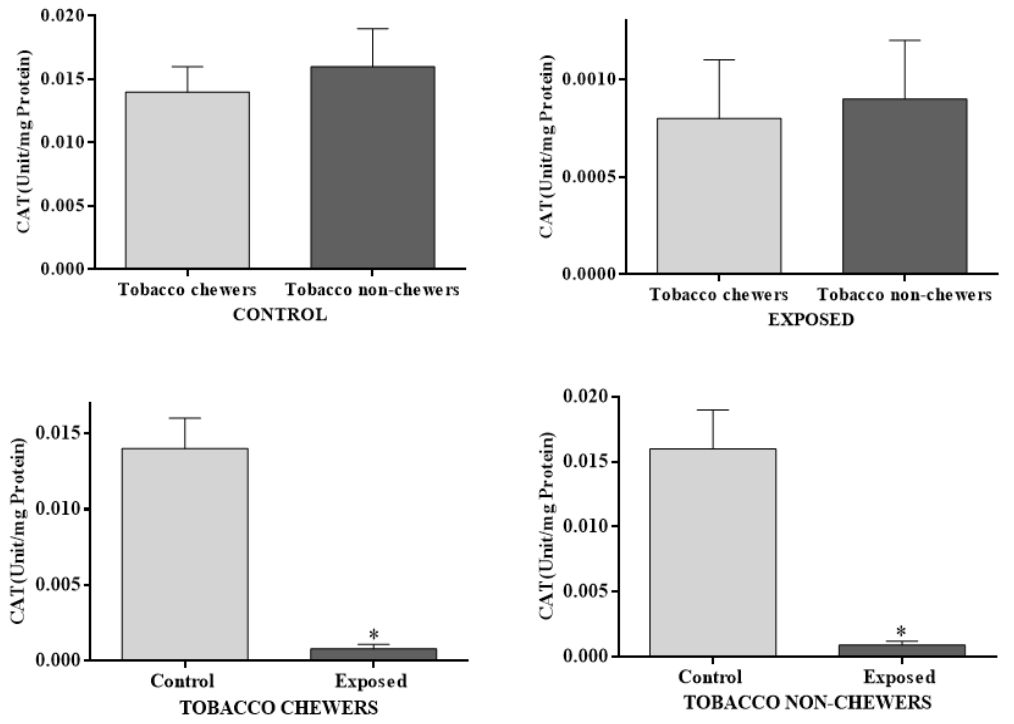

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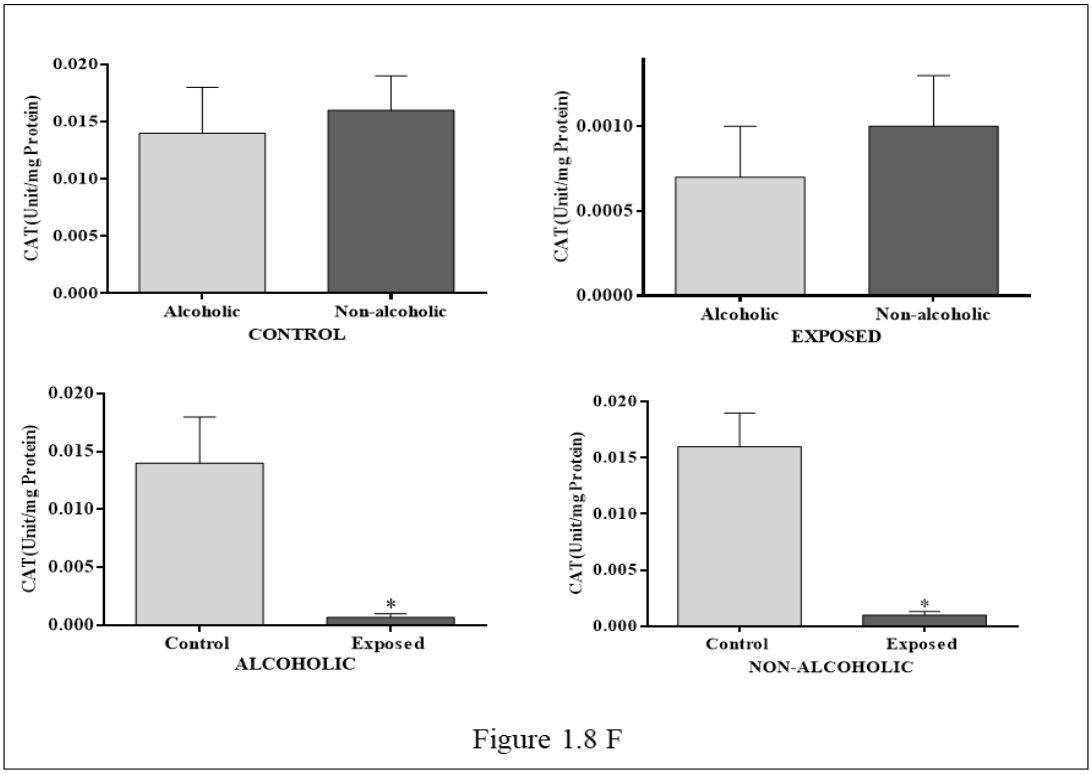

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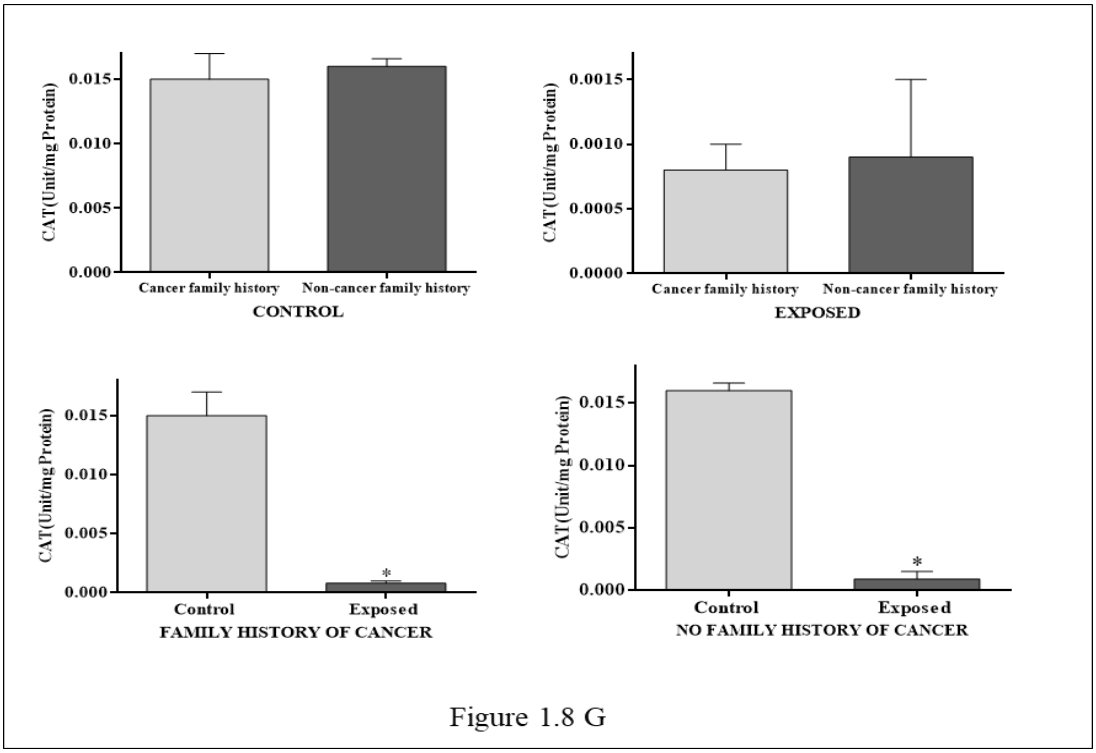


Figure 1.8 G

Figure 1.8: Functions of the demographic characteristics on catalase (CAT) activity (Unit/mg protein) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

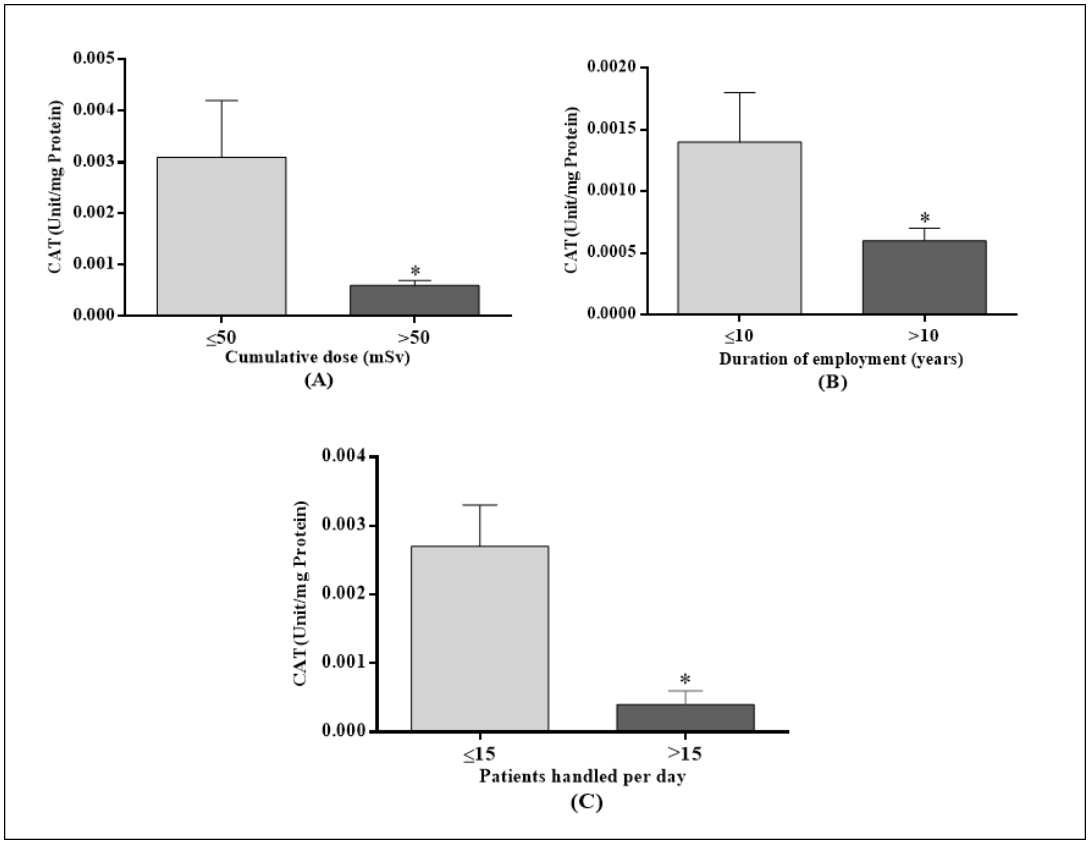
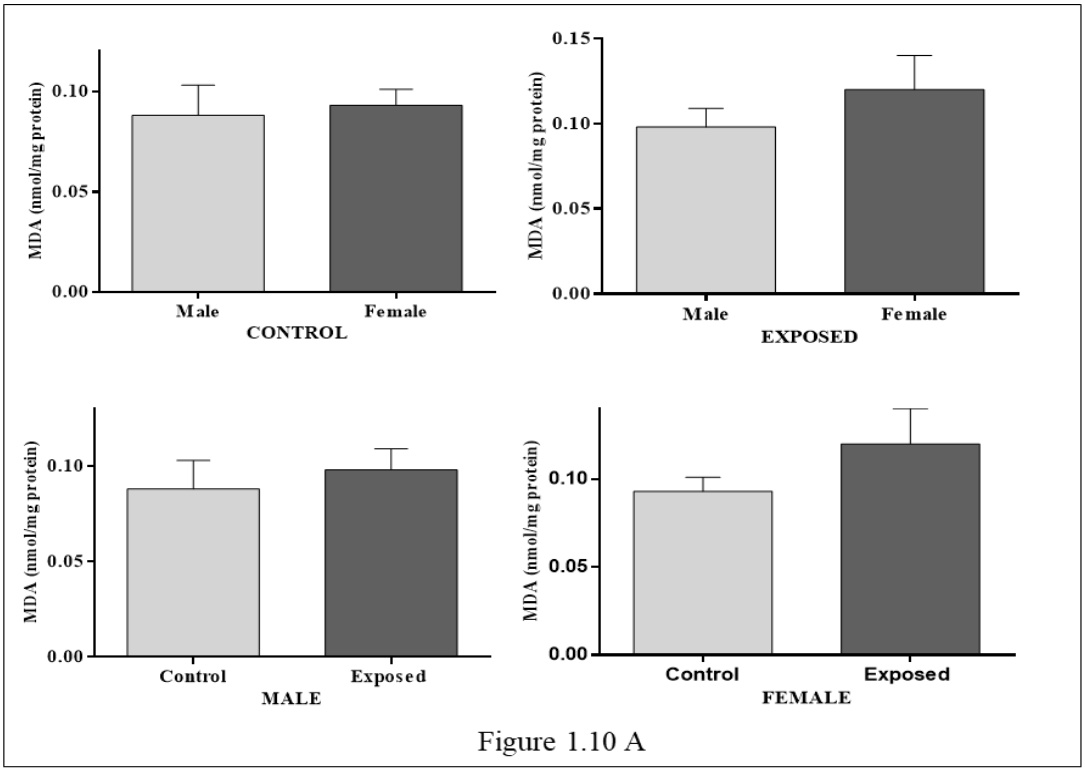


Figure 1.9: Functions of the demographic characteristics on catalase (CAT) activity (Unit/mg protein) of the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

3.5. Effects of demographic characteristics on the level of malondialdehyde among the exposed and control groups:

Tobacco chewers belonging to the exposed group also showed a significant increase on MDA level when compared with those belonging to the control group (Figure 1.10 E). Similarly, among the non-alcoholic, participants belonging to exposed group showed significant increase in MDA levels when compared to those belonging to the control group (Figure 1.10 F). Gender, age, smoking habits, betelnut chewing and family history of cancer did not show significant effect on MDA level in both the groups (Figure 1.10 A-D & G).

Cumulative dose (mSv), duration of employment (years) and the number of patients handled per day did not show a significant effect on MDA level on individuals working with X-ray (Figure 1.11).



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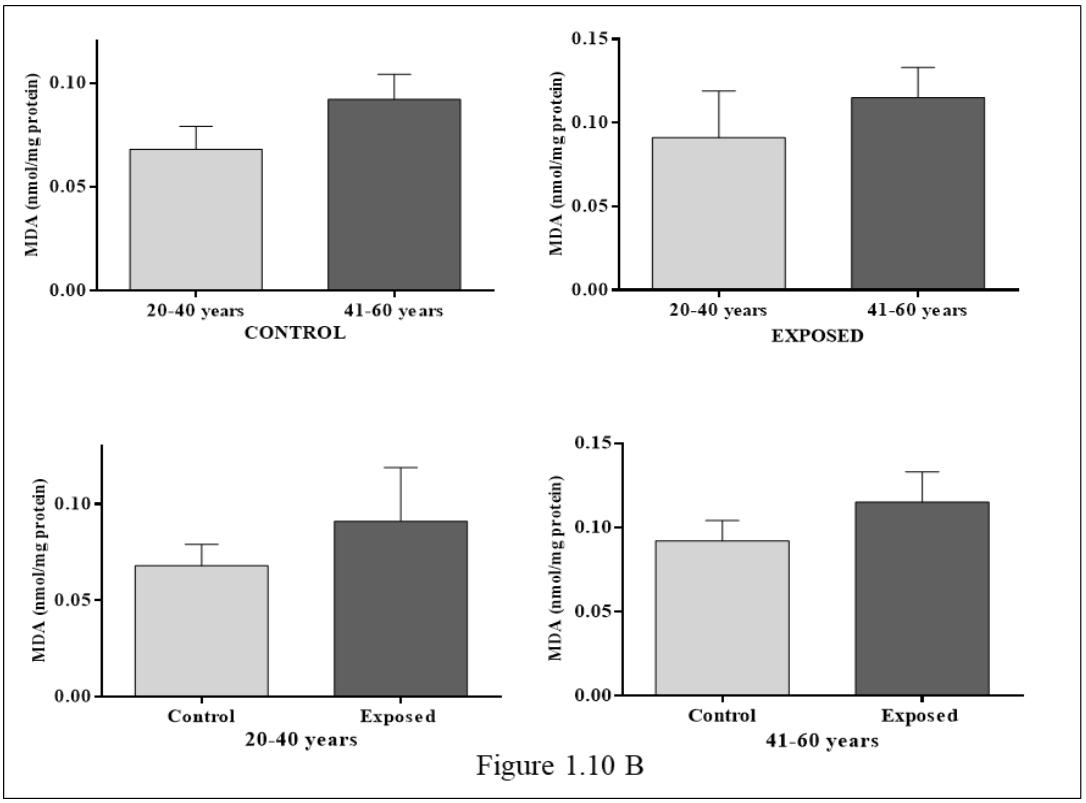

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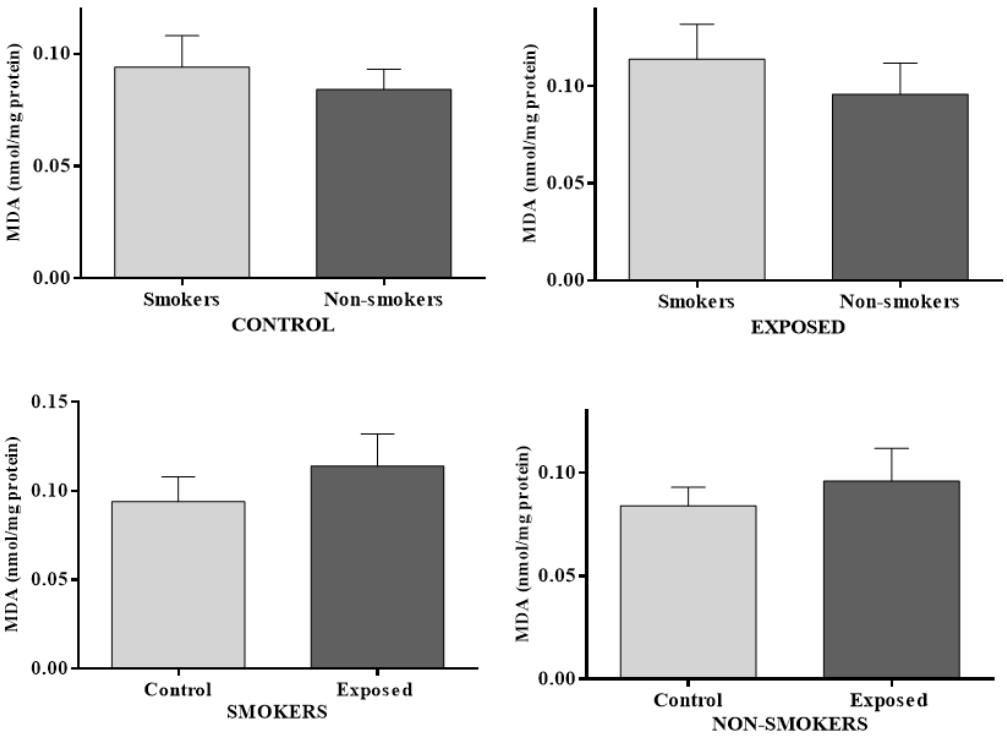

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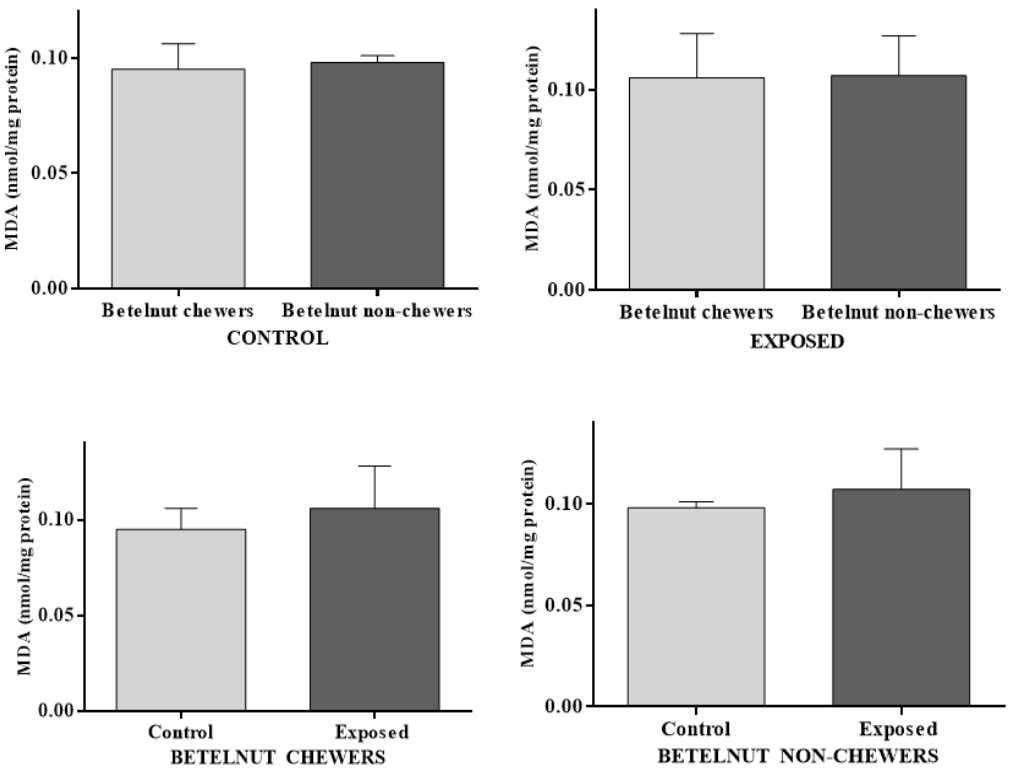


Figure 1.10 D

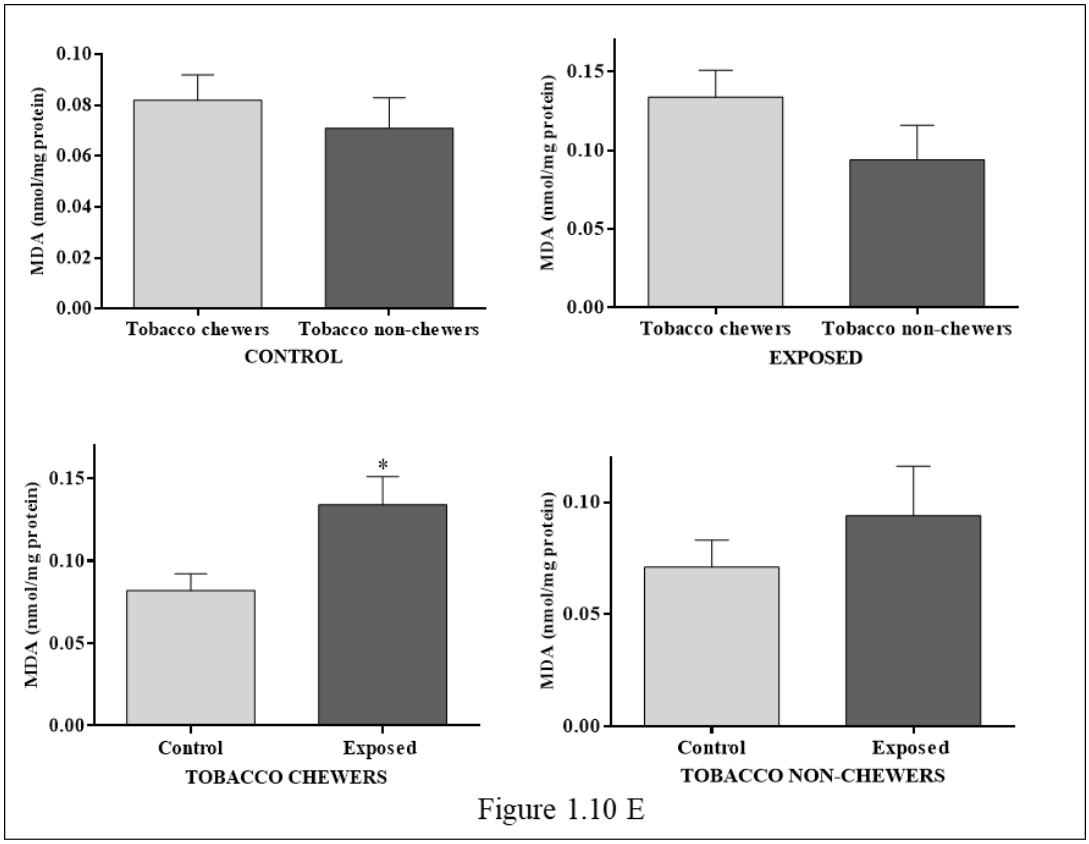


Figure 1.10 E

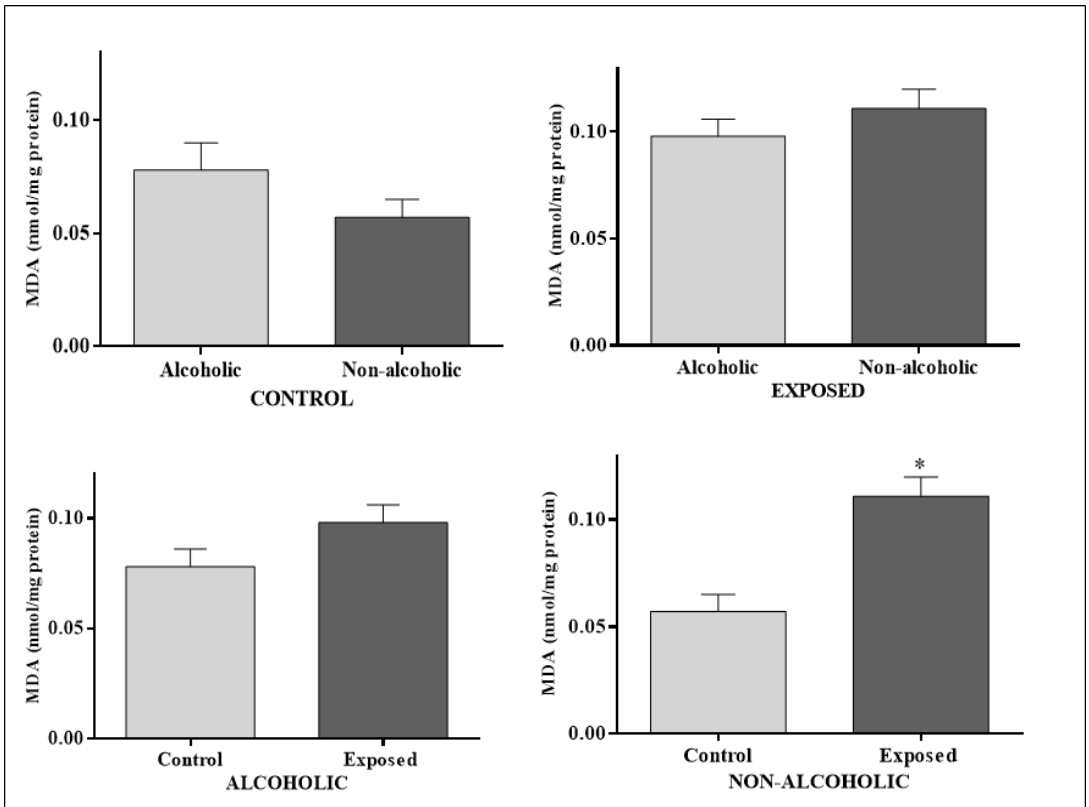


Figure 1.10 F

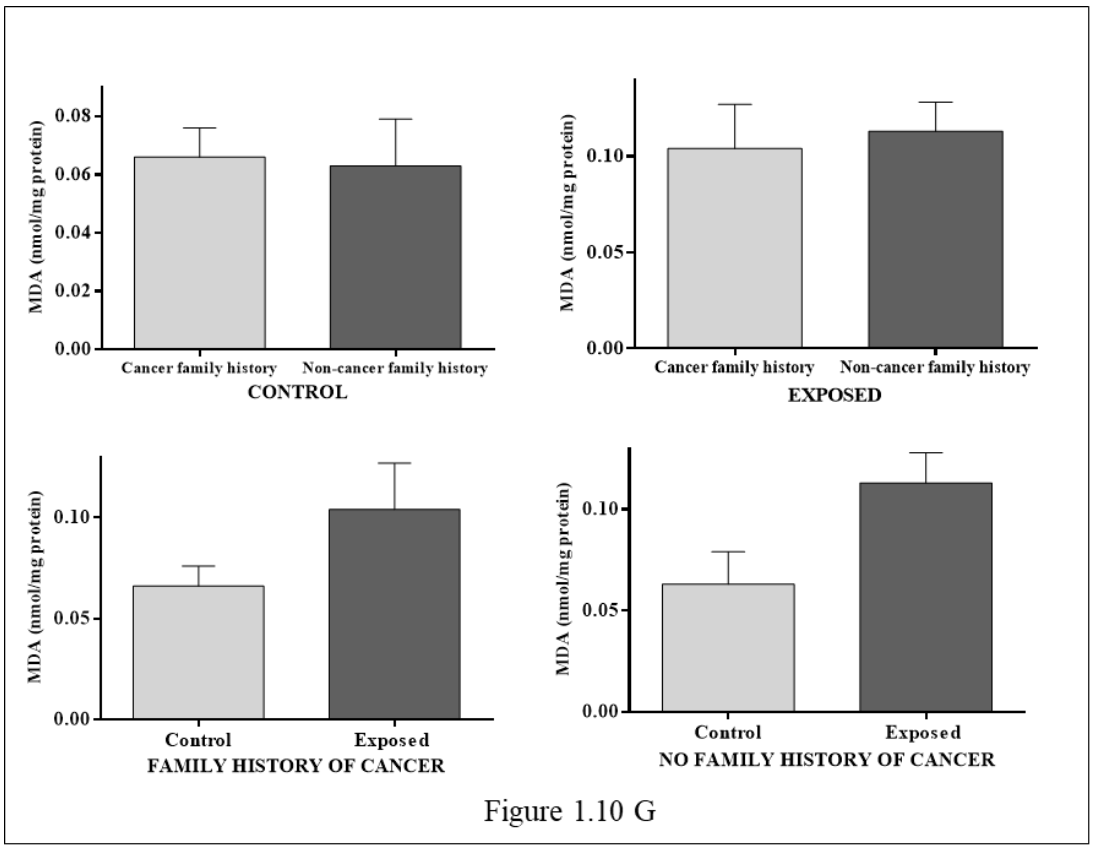


Figure 1.10: Functions of the demographic characteristics on the level of malondialdehyde (MDA) (nmol/mg protein) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer.* *indicates significant variations between the group ($p<0.05$).*

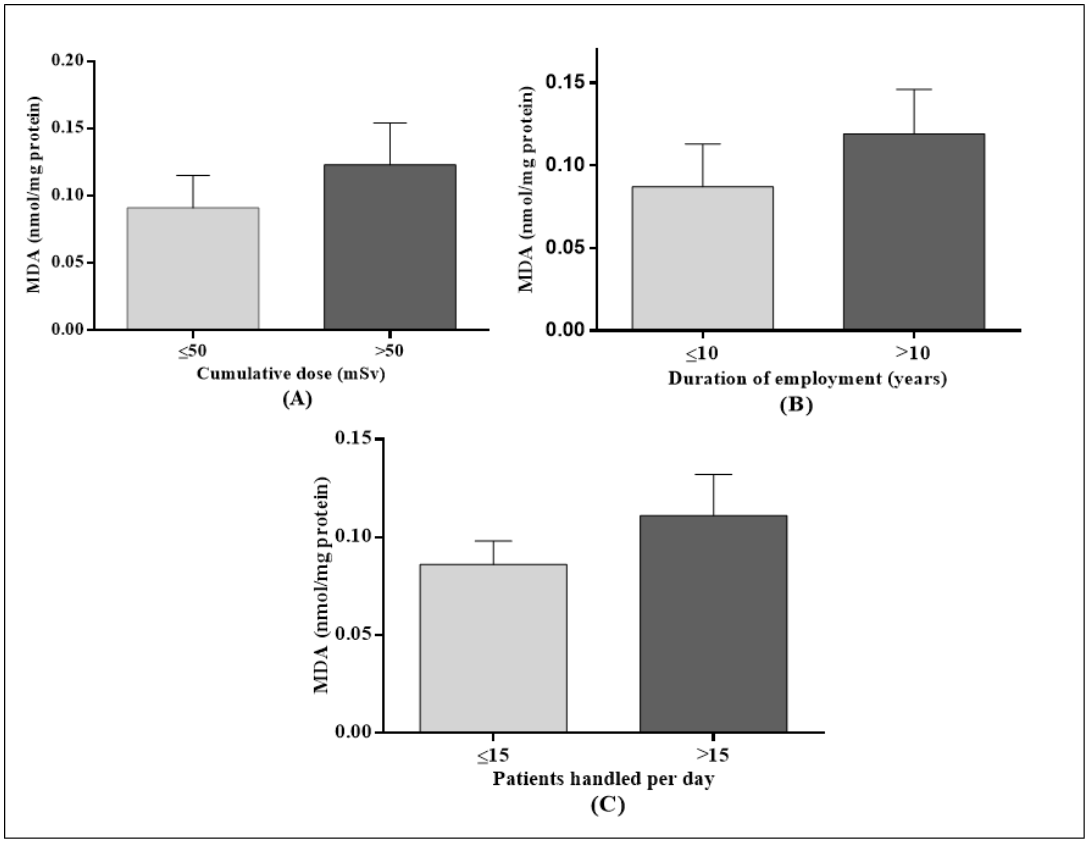


Figure 1.11: Functions of the demographic characteristics on the level of malondialdehyde (MDA) (nmol/mg protein) of the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

Table 2: Influence of demographic characteristics on the activities of antioxidant enzymes and lipid peroxidation in the occupationally exposed and control group.

Characteristics	Durbin Watson	Beta-value	t-value	p-value
Glutathione				
Gender	1.884	-0.82	-3.07	**0.01**
Age		-0.39	-2.26	**0.03**
Smoking habits		0.55	1.95	0.06
Betelnut chewing		-0.22	-1.20	0.241
Tobacco chewing		-0.22	-1.11	0.28
Alcohol consumption		-0.21	-0.84	0.41
Family history of cancer		0.17	1.03	0.32
Time since working (years)		0.31	1.72	0.10
No. of patients handled per day		0.14	0.82	0.42
Cumulative dose (mSv)		-0.65	-2.95	**0.01**
Glutathione-s-transferase				
Gender	1.234	-0.247	-0.61	0.55
Age		-0.672	-0.58	0.13
Smoking habits		0.23	0.70	0.49
Betelnut chewing		-0.161	-0.66	0.52
Tobacco chewing		-0.056	-0.24	0.81
Alcohol consumption		-0.201	-0.67	0.51
Family history of cancer		0.24	1.01	0.32
Time since working (years)		0.35	1.11	0.28
No. of patients handled per day		0.24	1.02	0.32
Cumulative dose (mSv)		-0.22	-0.95	0.35
Superoxide dismutase				
Gender	1.629	0.33	1.26	0.22
Age		0.03	0.17	0.87
Smoking habits		-0.05	-0.17	0.87
Betelnut chewing		-0.06	-0.31	0.76
Tobacco chewing		-0.04	-0.22	0.83
Alcohol consumption		-0.14	-0.57	0.57
Family history of cancer		0.37	2.24	0.07
Time since working (years)		0.31	1.77	0.09
No. of patients handled per day		-0.03	-0.19	0.85
Cumulative dose (mSv)		-0.61	-2.79	**0.01**
Catalase				
Gender	2.195	0.06	0.31	0.76
Age		-0.25	-1.27	0.22
Smoking habits		-0.47	-2.11	**0.04**
Betelnut chewing		0.026	1.60	0.13
Tobacco chewing		-0.07	-0.43	0.67

Alcohol consumption		0.11	0.54	0.60
Family history of cancer		-0.10	0.55	0.59
Time since working (years)		-0.18	-0.80	0.44
No. of patients handled per day		-0.61	-4.22	**0.00**
Cumulative dose (mSv)		-0.12	-0.39	**0.02**
Lipid peroxidation				
Gender	1.724	0.03	0.07	0.95
Age		-0.10	-0.28	0.79
Smoking habits		-0.13	-0.32	0.75
Betelnut chewing		0.07	0.27	0.79
Tobacco chewing		0.35	-1.34	0.20
Alcohol consumption		0.07	0.24	0.82
Family history of cancer		0.10	0.40	0.70
Time since working (years)		-0.26	-0.89	0.39
No. of patients handled per day		0.32	1.17	0.26
Cumulative dose (mSv)		0.01	0.02	0.99

Bold value signify $p < 0.05$.

4. DISCUSSION

Ionizing radiation is a well-known environmental toxic agent that elevates free oxygen radical generation and affects antioxidant status by directly interacting with certain molecules in a cell including water (Han and Yu 2010). The majority of the energy of ionizing radiation deposited in cells results in the ejection of electrons from water which subsequently causes the formation of several ROS including the highly reactive hydroxyl radicals (·OH) that inflict instant oxidative damage upon various biomolecules (Adams 1986; Le Caer 2011). Ionizing radiation in the presence of molecular oxygen converts hydroxyl, superoxide, and organic radicals into hydrogen peroxide and organic peroxides which are longer-lived ROS that continue to damage the cellular genome and other important biomolecules. Moreover, hydrogen peroxide reacts with redox-active metal ions, such as Fe^{2+} and Cu^{2+}, via Fenton's reactions and generates the most deleterious ·OH radical and thus intensifies cellular oxidative stress thereby leading to certain pathophysiological conditions (Halliwell and Gutteridge 1985; Biaglow et al., 1992). Cells have evolved sophisticated antioxidant defense mechanisms to deal with the negative effect of oxidative stress. The endogenous enzymatic antioxidant system is one such mechanism that helps to directly or sequentially remove ROS, thereby terminating the build-up of oxidative stress.

Many of the biological effects resulting from exposure to ionizing radiation are well-known to be mediated via oxidative stress. In this study, the exposed group showed higher MDA levels indicating increased lipid peroxidation (LPO) and reduced enzymatic activities of GST and CAT. The occupational workers exposed to ionizing radiation for 15-35 years have shown increased LPO in an earlier study (Durovi et al., 2004). An increase in the LPO and superoxide dismutase activity has been observed in medical workers occupationally exposed to ionizing radiation; however, the decline in CAT they observed was not statistically significant (Ahmad et al., 2016). Radiological workers who had been working with ionizing radiation for 1-30 years and exposed to less than 20 mSv have shown a decline in the CAT activity which was similar to our study. In contrast, a depletion in LPO and a rise in superoxide dismutase and glutathione peroxidase was reported earlier (Eken et al., 2012). The interventional cardiologists working for 3-19 years with ionizing

radiation have shown non-significant attrition in the activities of CAT whereas this decline was significant for superoxide dismutase (Russo et al., 2012). An increase in LPO and reduction in CAT activity was observed in intestinal cells of mice exposed to 2 Gy of γ-rays and 1.6 Gy of ^{56}Fe radiation collected after one year of irradiation. The decline in CAT after ^{56}Fe radiation was significant but non-significant after γ -irradiation (Datta et al., 2012). A number of studies have revealed biochemical changes associated with prolonged exposure to ionizing radiation and their relationship to the antioxidant system (Achudume et al., 2010; Atasoy et al., 2013). This decrease in the GST and CAT in the occupationally exposed workers seems to be due to the persistence of oxidative stress with each exposure, which may not get neutralized with subsequent protracted low dose irradiation. It has also been indicated that in addition to the rapid burst of ROS observed immediately following irradiation, cells can exhibit more persistent and prolonged increases in ROS over time ranging from several minutes to several days post-irradiation (Yoo et al., 2000). This may lead to prolonged oxidative cellular damage such as various DNA lesions including most toxic double-strand breaks. DNA damage produced by ROS is considered to be the most frequently occurring damage (De Bont and Larebeke 2004; Yu et al., 2016). Total body irradiation has been shown to increase markers of LPO, including thiobarbituric acid reaction products (TBARS), 4-hydroxynonenal (4-HNE) and hexane in animal models and in patients (Umegaki et al., 2001; Lonergan et al., 2002; Jagetia and Reddy 2005; Jagetia and Shetty 2016). Polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation. This process produces lipid hydroperoxides that are either reduced by glutathione peroxidases to less reactive fatty acid alcohols or react with metals to produce epoxides, aldehydes etc. Malondialdehyde, the most mutagenic product of lipid peroxidation reacts with DNA causing adduct formation and strand breaks (Lonergan et al., 2002; Blair 2008).

Ionizing radiation induces different ROS, mostly as free radicals, directly or indirectly including ·OH, H_2O_2, lipid peroxides, etc. which lead to oxidative damage in DNA bases and sugar moieties, various DNA lesions including single and double strand breaks (Santivasi and Xia 2014). Sustained oxidative stress due to chronic exposure to ionizing radiation would have led to DNA damage and reduction in

antioxidant enzymes activities in the present study. Furthermore, reduction in antioxidant enzymes as a consequence of increased ROS production (cellular oxidative stress state) in turn exacerbates DNA damage without the effective quenching of ROS thereby potentially contributing to the formation of micronuclei as reported in other chapters. Radiation-induced ROS is the principal cause of oxidative stress that in turn activates signal transduction pathways via NF-κB, COX-II and PARP at the molecular level implicating in various biological processes (Watters 1999; Meng et al., 2003; Tessner et al., 2004; Datta et al., 2012).

CHAPTER IV

ASSESSMENT OF DNA DAMAGE AMONG MEDICAL WORKERS EXPOSED TO IONIZING RADIATION USING COMET ASSAY

Abstract

Long-term exposure to low doses of ionizing radiation causes a wide variability of DNA damage. Ionizing radiation directly interacts with DNA to cause ionization, thus initiating the chain of events that leads to biological changes. Biophysical interaction of DNA with ionizing radiation can lead to DNA damage including primary lesions, double-strand breaks (DSBs), single-strand breaks (SSBs), alkali-labile sites, and damage to pyrimidine and purine bases, DNA-protein and DNA-DNA crosslinks. Studies have reported that DNA damage can be influenced by radiation exposure in medical radiation workers. Therefore, the study was conducted to examine the levels of DNA damage among the group of professionals working with low doses of medical X-rays using the alkaline comet assay. Blood samples were collected from the exposed and control group for analysis. A total of 100 randomly captured cells from each slide were examined using a fluorescence microscope. DNA damage caused by exposure radiation is indicated by comet tail length, tail area, tail DNA %, and olive moment of the tail. The comet tail length, tail area and olive moment of the tail in the exposed group were significantly higher when compared to the control group. However, no significant difference was observed in the percentage of tail DNA between the exposed and control groups. Multiple linear regression analysis reveals a significant association of increased DNA damage with tobacco chewing and alcohol consumption. Among the occupationally exposed group, cumulative dose and duration of employment showed a significant association with the levels of DNA damage. The study confirmed radiations-imposed DNA damage in radiology workers during their occupational exposure.

1. INTRODUCTION

Ionizing radiation is a ubiquitous environmental agent that has damaging effects on DNA. Biophysical interaction of DNA with ionizing radiation can lead to DNA damage including primary lesions, double-strand breaks (DSBs), single-strand breaks (SSBs), alkali-labile sites, and damage to pyrimidine and purine bases, DNA–protein and DNA–DNA crosslinks (Natarajan 1993; Kruszewski et al., 1998; Chaubey et al., 2001). The cell repair mechanisms are activated when DNA damage is induced and stop the cell cycle at certain checkpoints to repair the damage. If cells damaged by radiation are repaired efficiently, resistance to radiation develops, enabling cells survival and replication. If not repaired, damaged DNA will be directed to apoptosis, a programmed cell death to prevent accumulations of mutations in daughter cells (Deckbar et al., 2011; Guo and Zhang 2011). If the cell with damaged DNA manages to escape apoptosis and continue to divide, it can lead to the development of cancer1 and other genetic diseases (Christmann et al., 2003; Jeggo et al., 2016; Valko et al., 2004; Petrini and Stracker 2003).

X-ray is one of the most critically used diagnostic components in medical technology that has been use for diagnosis of various human ailments (Padmaja et al., 2006). However, the effects of low levels of ionizing radiation are of great concern to occupationally exposed medical workers. The government of the United States and the International Agency for Research on Cancer (IARC) classified X-ray as carcinogenic (Roobottom et al., 2010). Use of medical X-rays has been reported to be the cause of 0.6 to 3% of all types of cancers incidence worldwide, and at least 20% of cancer in developed countries (Berrington et al., 2004; Picano 2004). The acute harmful effects of high doses of ionizing radiation were discovered during the 19th century (Vaiserman et al., 2018). However, the detrimental health effects of radiation with low-dose radiation still remain debatable. Generally, radiation at a cumulated dose of 100 mSv is referred to as low-dose radiation, although sometimes relevant doses increase. The increasing use of ionizing radiation (IR) in medical diagnosis and treatment has raised many concerns about its potential long-term impacts on health, including genomic instability and cancer (Visweswaran et al., 2019; Basheerudeen et al., 2017; Grosse et al., 2009). Thus, the effects of low-level exposure to ionizing

radiation has become great concern to a large number of people including workers occupationally exposed to medical X-ray.

Several research methods including sister chromatid exchange and chromosomal aberration are normally used for investigating genetic damage induced by medical X-ray (Tice et al., 1991; Olive 1999; Ostling and Johanson 1984). However, these methods are economically costly, time-consuming and require proliferating cells. Therefore, the use of single-cell gel electrophoresis (SCGE) or the comet assay for genotoxicity studies has greatly increased during the past few decades. The comet assay is a very sensitive, rapid, and simple technique that detects DNA damage within individual cells (Olive 1999). The alkaline comet assay can be adapted for various exposure conditions and applies to almost all types of tissue or cells. The alkaline version has been used for detection of direct effects of DNA damage from radical-forming chemicals, alkylating agents, stirring chemicals, various metals, and UV or ionizing radiation (Reus et al., 2013; Vasquez 2010).

The single cell gel electrophoresis (SCGE) or Comet assay has been widely used for genotoxicity testing (Singh et al., 1988; Tice 1990; Fairbairn et al., 1995; Olive 1999). In molecular epidemiology studies, DNA damage evaluated by the Comet assay has been used as a biomarker of exposure to various forms of ionizing radiations (Betti et al., 1994; Collins et al., 1997; Garaj-Vrhovac and Kopjar 1998; Sram et al., 1998; Piperakis et al., 1999; Kopjar and Garaj-Vrhovac 2001; Maluf et al., 2001; Garaj-Vrhovac et al., 2002). The Comet assay permits the detection of primary DNA damage and the study of repair kinetics at the level of single cells (Singh et al., 1988; Olive et al., 1990; Hellman et al., 1995; Olive 1999). A variety of possible modifications of the assay facilitate the detection of SSBs, alkali-labile sites, DSBs, incomplete excision repair sites and interstrand crosslinks. Furthermore, DNA fragmentation associated with cell death or related to apoptosis can be evaluated with the Comet assay (Piperakis et al., 1999; Olive 1999). Cells with damaged DNA give a ‘comet’, consisting of ‘head’ nuclear matrix and ‘tail’. Under electrophoretic conditions, the extent of DNA migration is correlated with the damage unless the DNA cross-links are involved. The length of tail multiplied by fraction of DNA present in the tail is usually a measure of DNA damage. Therefore, considering the potential hazardous effects of X-rays and lack of awareness among radiation personnel, this

study aims to examine the levels of DNA damage among the group of professionals working with low doses of medical X-rays using comet assay.

2. MATERIALS AND METHODS

2.1. Chemicals

RPMI-1640 medium, sodium chloride (NaCl), Triton X-100, normal-melting point agarose (NMPA), ethidium bromide and dimethyl sulphoxide (DMSO) were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Histopaque-1077, low-melting point agarose (LMPA), di-sodium EDTA (Na_2EDTA), Trizma base and Trizma hydrochloride were purchased from Sigma Chemical Co., Bangalore, India. FBS (Fetal Bovine Serum) was purchased from ThermoFisher Scientific, Bangalore, India.

2.2. Selection of subjects

The volunteers were divided into two groups as described in Chapter 2.

***2.2.1. Exposed* group:** The group comprised of 40 healthy individuals that are occupationally exposed to X-rays for medical diagnostic purposes, working in different hospitals in Aizawl, Mizoram.

***2.2.2. Control group*:** The group comprised of 40 healthy individuals matched for various demographic data, residing in the same urban area. The volunteers did not have any work-related exposure to ionizing radiation or any known genotoxic agent. After identifying suitable volunteers for the study, each individual was asked to sign a consent form and questionnaire as described in Chapter 2.

2.3. Blood sample collection and lymphocytes isolation

Approximately 3 ml of whole blood were collected from each volunteer of both the groups by venepuncture and were stored in individual sterile heparinized tubes. Separation of lymphocytes from the whole blood was done using Histopaque-1077. Briefly, blood was diluted with RPMI-1640 media (1:1) and layered over 1 ml Histopaque and centrifuged at 800 Xg for 20 min. The buffy coat was aspirated into 3 ml of RPMI-1640 media and centrifuged for 10 min. The pellet containing lymphocytes was resuspended in 1 ml of RPMI-1640. Cells counting was performed using haemocytometer.

2.4. Assessment of DNA damage using the alkaline comet assay

The alkaline single-cell gel electrophoresis (comet assay) was used to detect both single and double-strand breaks (SSBs and DSBs, respectively). The assay was performed according to Singh et al. (1998) with minor modifications. Briefly, the isolated cells (~2 x 10^4 cells) from each individual of both exposed and control groups were suspended in 75 µl of 0.5% low-melting point agarose (LMPA) prepared in 1X PBS and spread onto a frosted slide precoated with 1% normal-melting point agarose (NMPA) and covered with a coverslip. Once the gel gets solidified following incubation of the slide at 4°C, the coverslip was gently removed and the third layer of 90 µl 0.5% LMPA was added. The slides were then incubated for 2 h in a freshly prepared lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Trizma base, 1% Triton X-100 and 10% DMSO, pH 10). After lysis, slides were placed on a horizontal electrophoresis tank filled with freshly prepared alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH13) for 20 min to allow the unwinding of DNA. Electrophoresis was then carried out for 30 min at 24 V and 300 mA. The slides were then neutralized by washing with neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 5 min. After neutralization, slides were washed with distilled water and then stained with ethidium bromide (EtBr) solution (2 µg/ml) for 5 min. Each slide was prepared in duplicate and 100 randomly selected cells from each slide were examined using florescence microscope with a magnification of 200X. Image capture and analysis were performed with Tritek CometScore 2.0.0.38 software.

2.5. Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). Student "t" test was performed to determine the statistical significance on the extent of DNA damage between the groups. Multiple linear regression analyses were carried out for the parameters of comet assay with the different demographic characteristics. Statistical analyses were conducted using Graph Pad Prism ver. 6.0 and SPSS Ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

DNA damaging effects of low doses of ionizing radiation among the occupationally exposed group were studied using Comet assay. The comet tail length (range; 1.77 to 14.87 µm. Mean ± SEM; 5.414 ± 0.635 µm) in the exposed group was found to be significantly higher when compared with the comet tail length of the control group (range; 1.18 to 9.21 µm. Mean ± SEM; 3.68 ± 0.385 µm) (Figure 2.1 A). The comet tail area measured in the exposed group (range; 81.56 to 1126.2. Mean ± SEM; 342.72 ± 48.72) was also significantly higher than that of the control group (range; 60.42 to 487.31. Mean ± SEM; 198.4 ± 23.71) (Figure 2.1 B). Similarly, the observed olive tail moment in the exposed group (range; 1.01 to 5.28. Mean ± SEM; 1.701 ± 0.176) was significantly higher when compared to the control group (range; 0.35 to 2.98. Mean ± SEM; 1.264 ± 0.091) (Figure 2.1 D). However, no significant difference was observed in the percentage of tail DNA between the exposed and control groups (Figure 2.1 C).

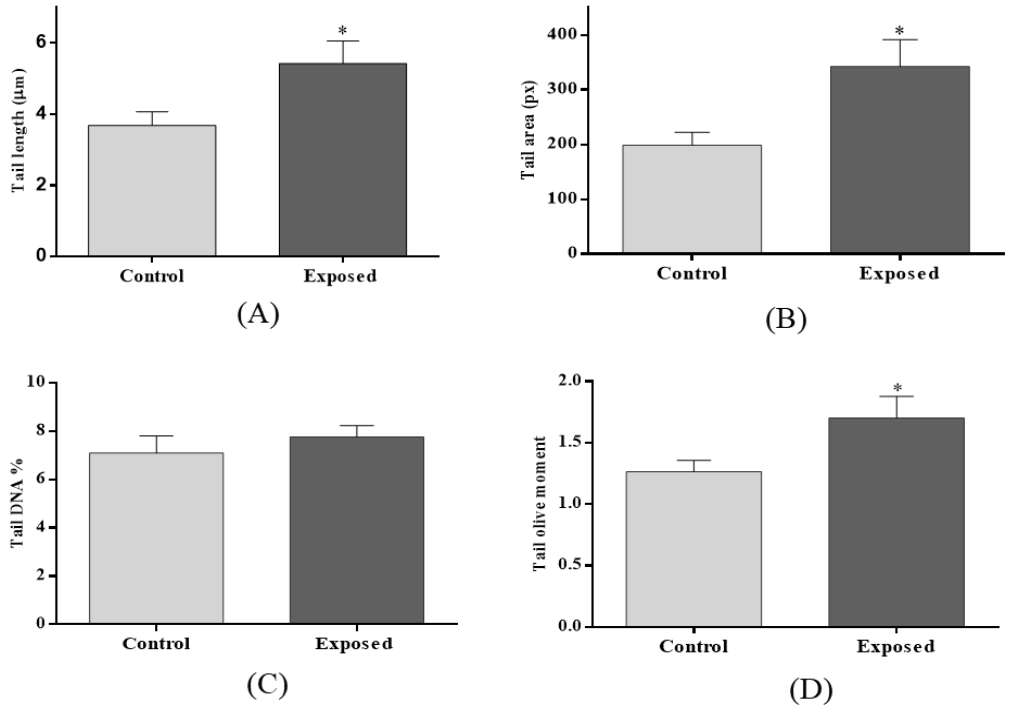


Figure 2.1: Overall comparison of the extent of DNA damage in the exposed and control groups expressed in terms of (A) Tail length (µm); (B) Tail area (px); (C) Tail DNA (%); (D) Tail olive moment. ** indicates significant variations between the groups ($p<0.05$).*

3.1. Effects of demographic characteristics on the extent of DNA damage expressed in terms of tail length among the exposed and control groups:

Gender-wise analysis revealed that significant variation was not observed in the comet tail length between males and females of both exposed and control groups. However, females of exposed group showed a significant increase in comet tail length when compared with females of control group (Figure 2.2 A). Among betelnut chewers and tobacco chewers, participants belonging to the exposed group showed an increase in comet tail length when compared with the control group (Figure 2.2 D & E). However, it was observed that age, smoking habits, alcohol consumption and family history of cancer did not significantly influence the levels of comet tail length between the exposed and control groups (Figure 2.2 B, C, F & G). Multiple linear regression analyses revealed a significant association of increased comet tail length with gender, tobacco chewing and alcohol consumption (Table 3).

Among the occupationally exposed group, technicians who have a cumulative effective dose of >50 mSv showed significant increase in comet tail length than those workers having a cumulative effective dose of ≤50 mSv. However, duration of employment and number of patients handled per day did not show significant effect on comet tail length (Figure 2.3). Multiple linear regression analyses revealed a significant association of increased comet tail length with cumulative dose, and duration of employment (Table 3).

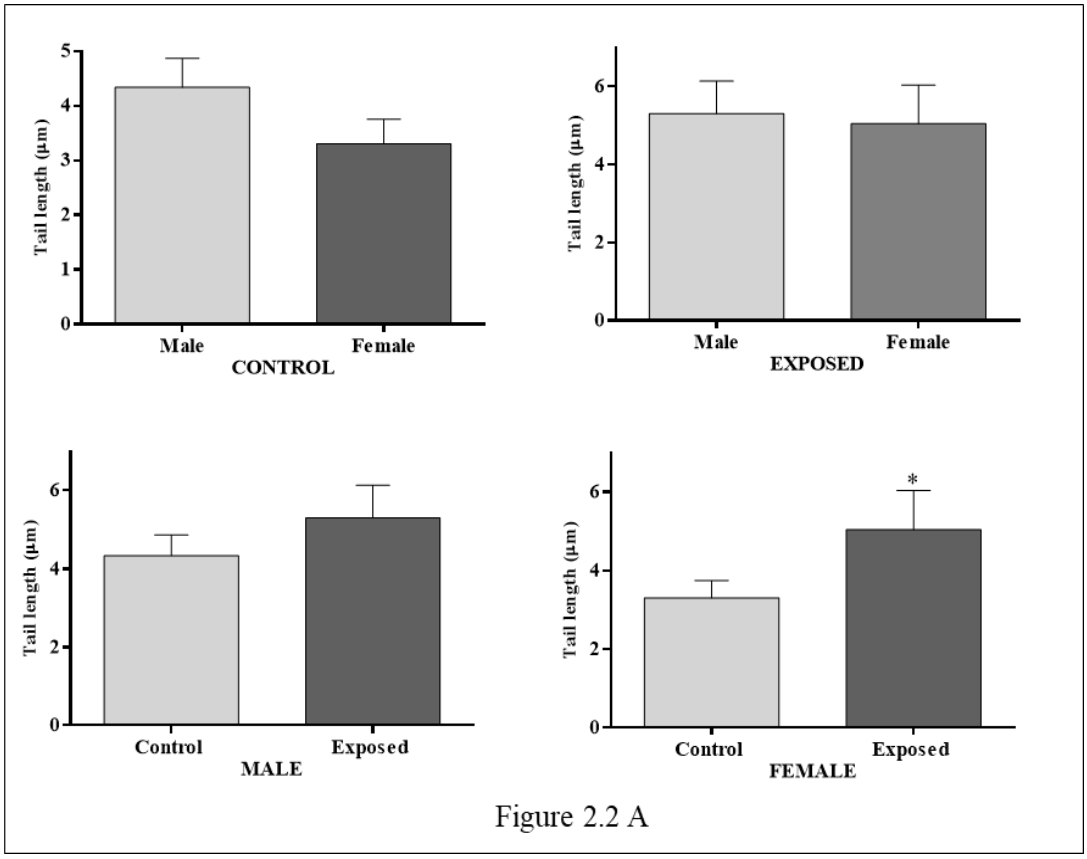


Figure 2.2 A

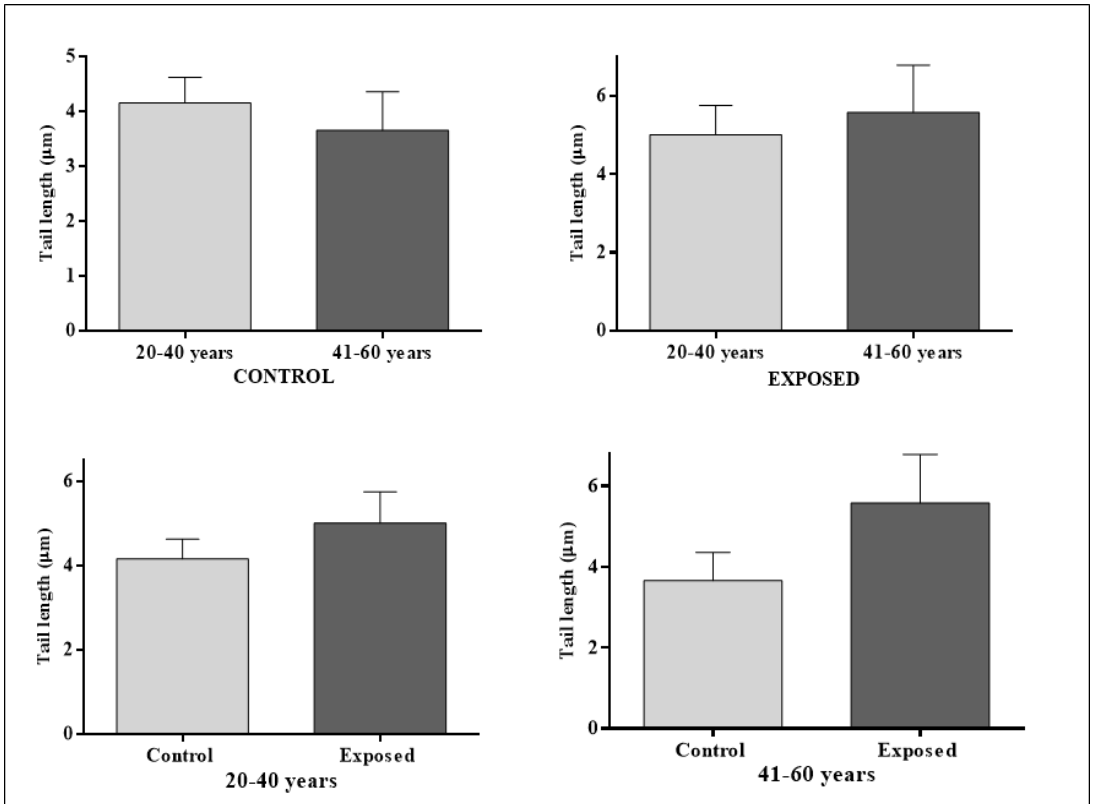


Figure 2.2 B

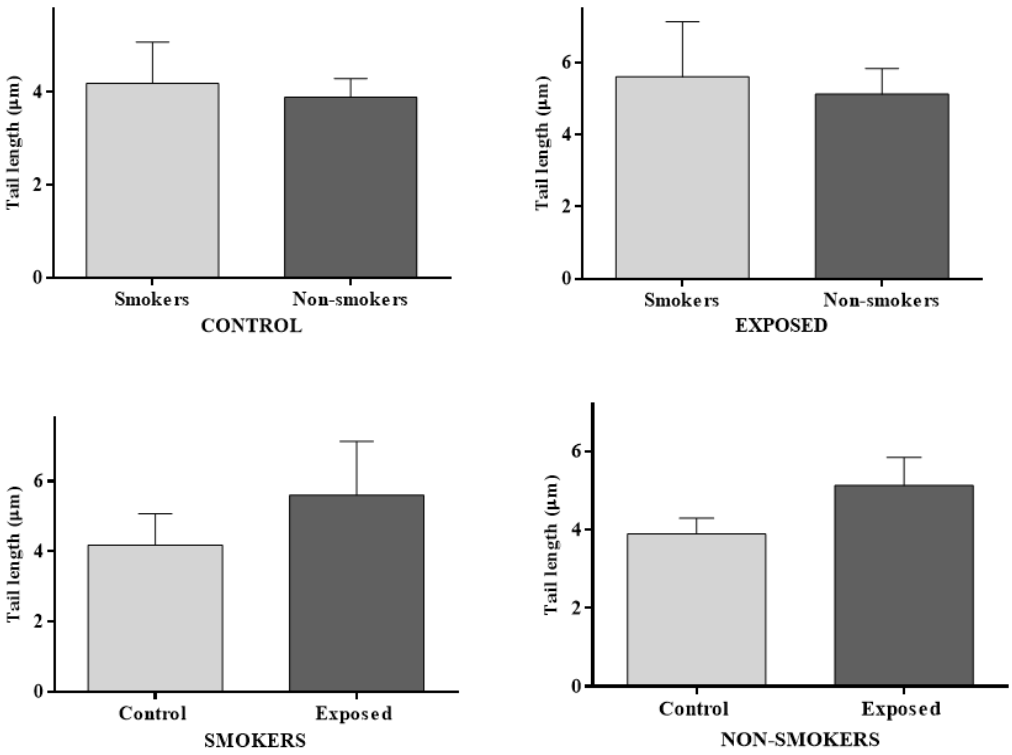


Figure 2.2 C

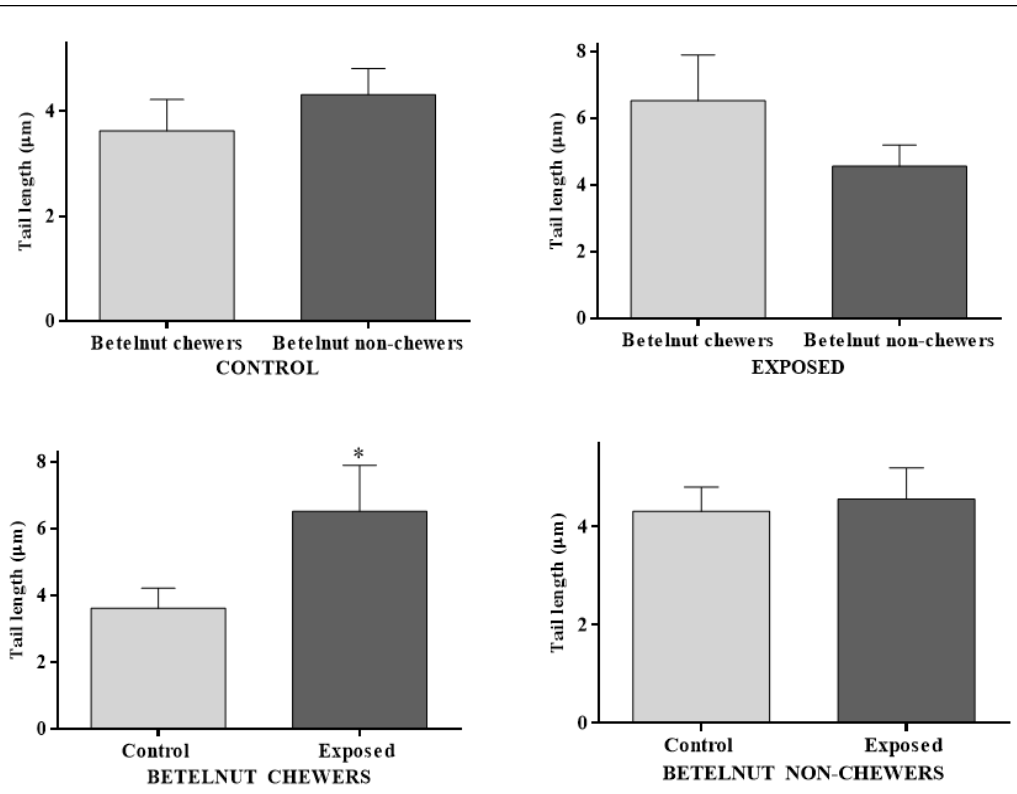


Figure 2.2 D

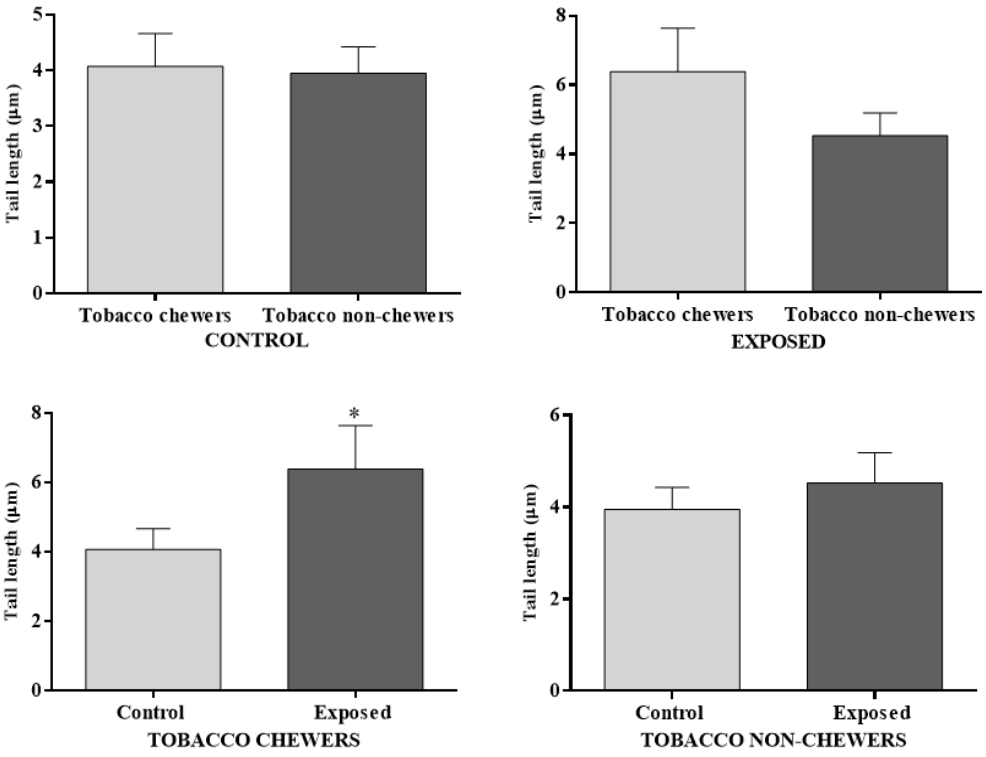


Figure 2.2 E

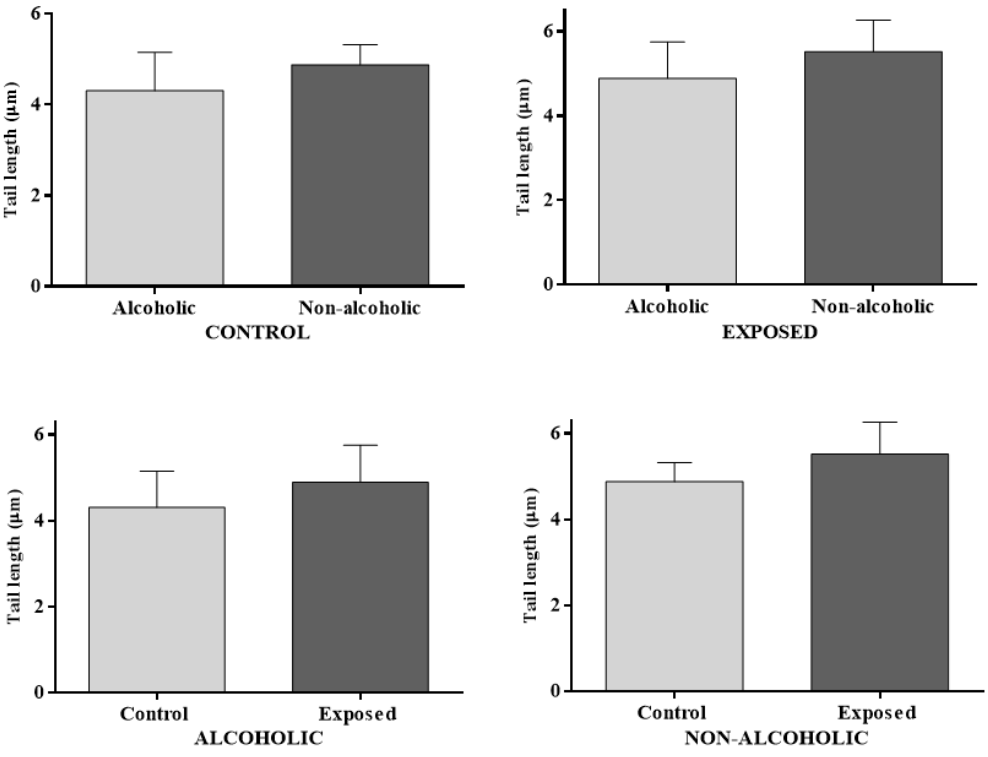


Figure 2.2 F

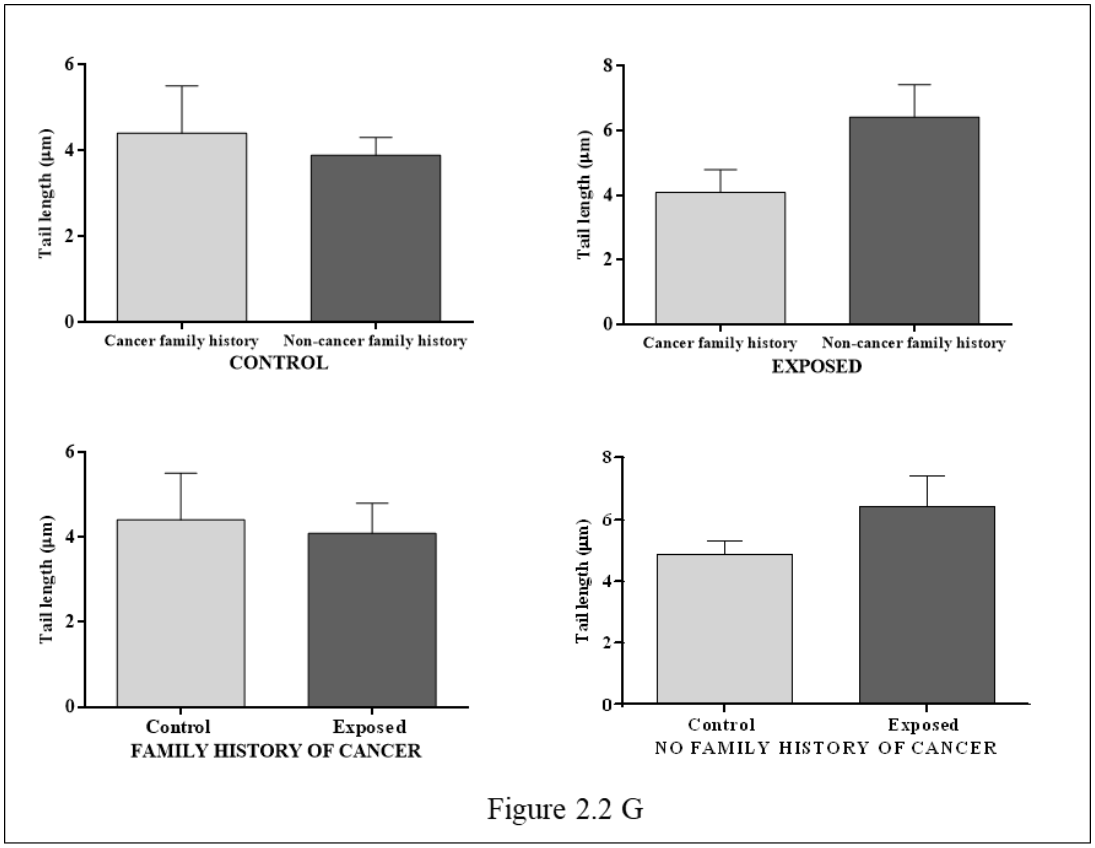


Figure 2.2: Functions of the demographic characteristics on comet tail length (µm) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

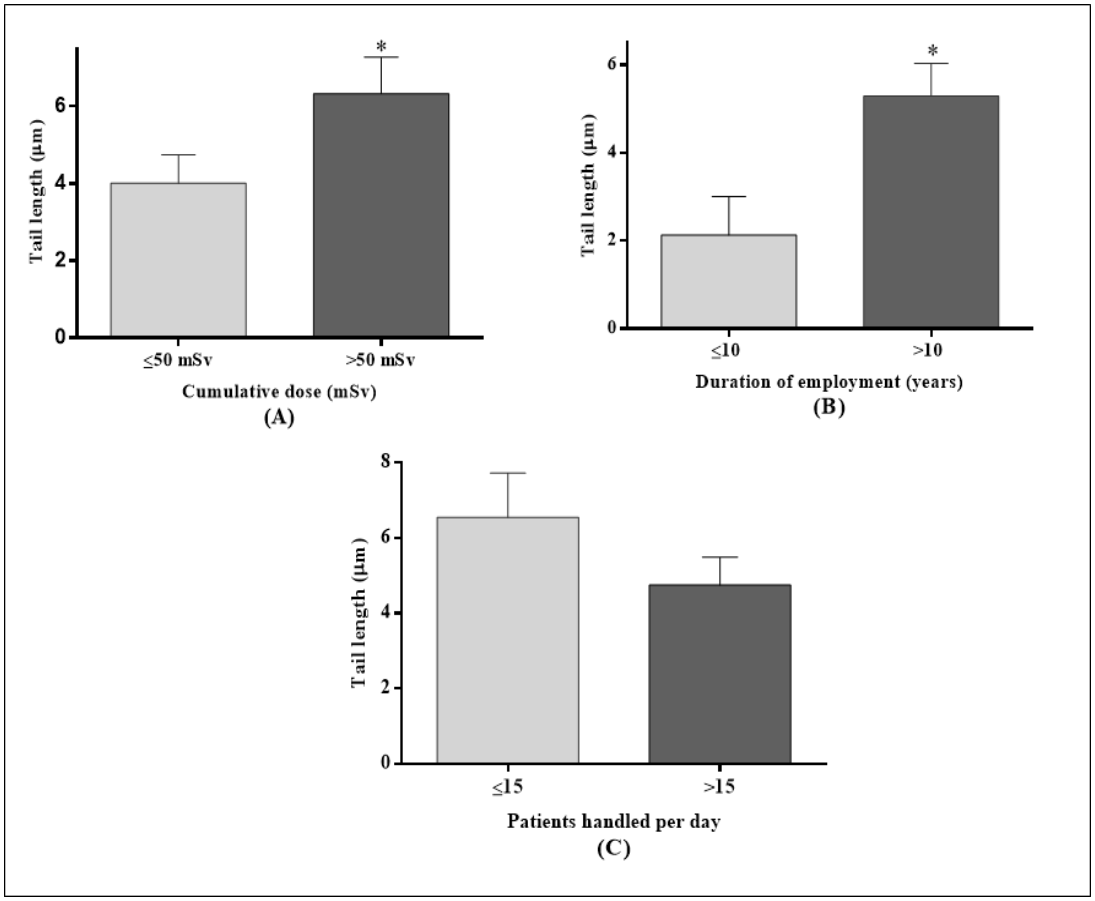


Figure 2.3: Functions of the demographic characteristics on comet tail length (µm) among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

3.2. Effects of demographic characteristics on the extent of DNA damage expressed in terms of tail area among the exposed and control groups:

Females and males of the exposed group showed significant increase in comet tail area when compared with the respective gender in control group. Also, males of control group showed significant increase in comet tail area when compared with females of control group (Figure 2.4 A). The younger members (20-40 years) and elder members (41-60 years) of exposed group also showed significant increase in the comet tail area when compared with their respective age group of the control (Figure 2.4 B). Occupational exposure to ionizing radiation results in an increase in the comet tail area regardless of their smoking habits, betelnut chewing and tobacco chewing (Figure 2.4

C, D & E). Similarly, among the non-alcoholic and individuals having no family history of cancer, participants belonging to the exposed group showed a significant increase in the comet tail area when compared to those belonging to the control group (Figure 2.4 F & G). Multiple linear regression analyses revealed a significant association of increased comet tail area with gender, tobacco chewing and alcohol consumption (Table 3).

Among the occupationally exposed group, technicians who have a cumulative effective dose of >50 mSv showed a significant increase in the comet tail area when compared with workers having a cumulative effective dose of ≤50 mSv. However, duration of employment and number of patients handled per day did not show a significant effect in the comet tail area (Figure 2.5). Multiple linear regression analyses revealed a significant association of increased comet tail area with cumulative dose and duration of employment (Table 3).

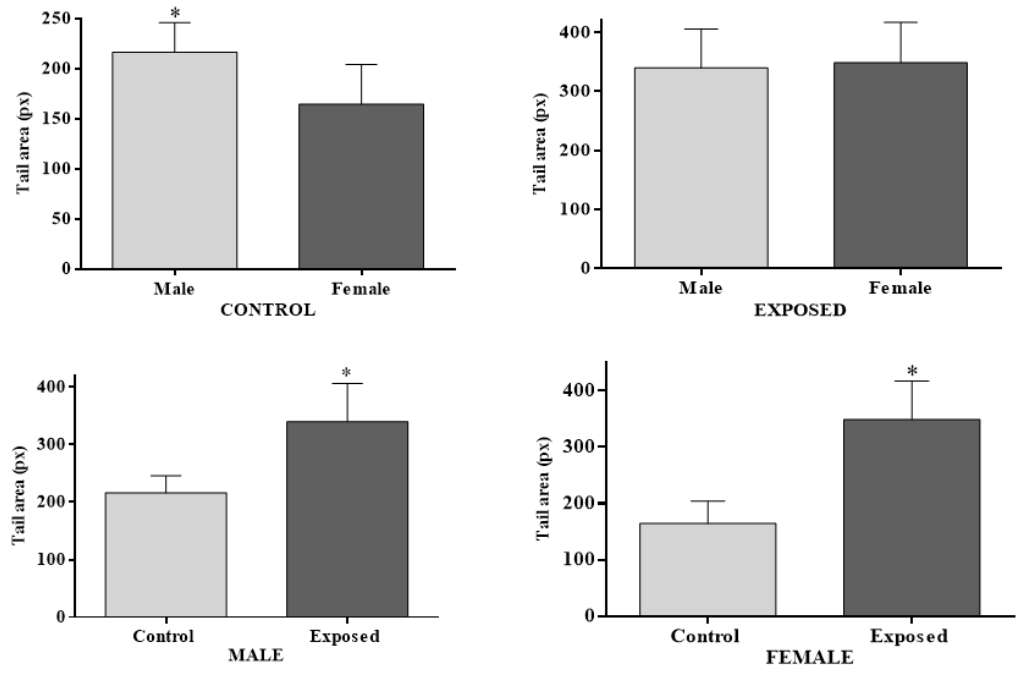


Figure 2.4 A

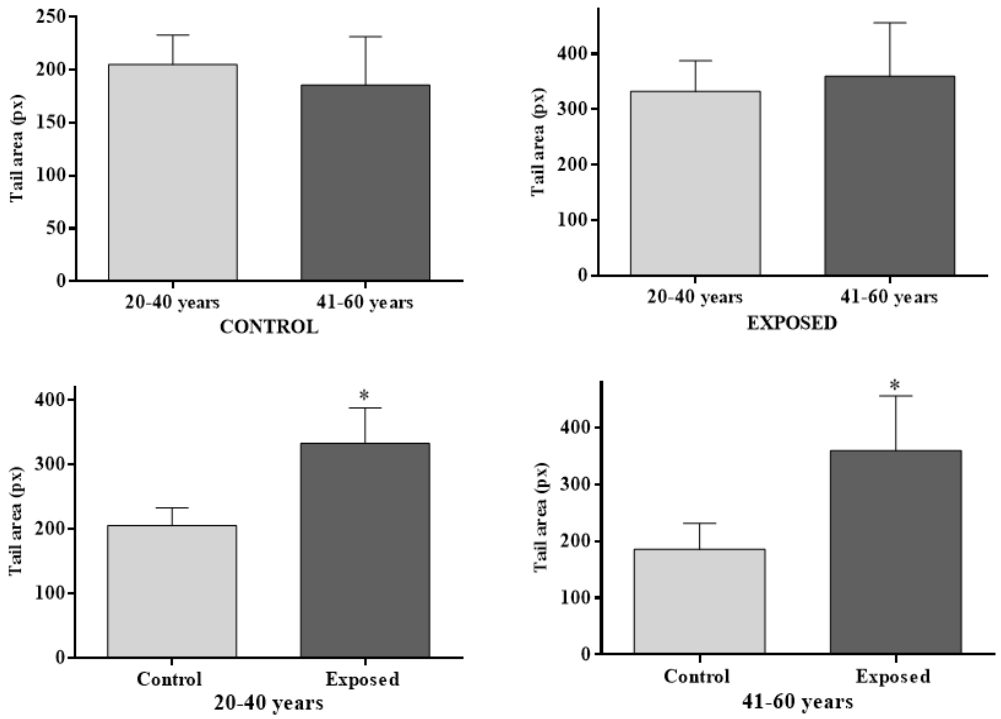


Figure 2.4 B

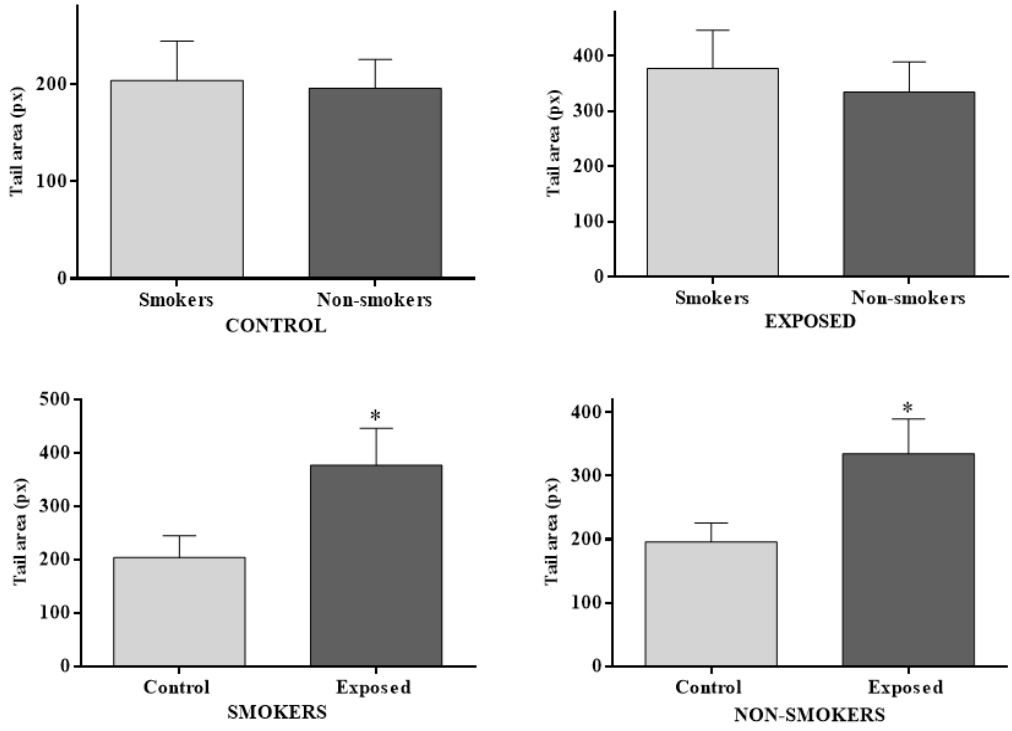


Figure 2.4 C

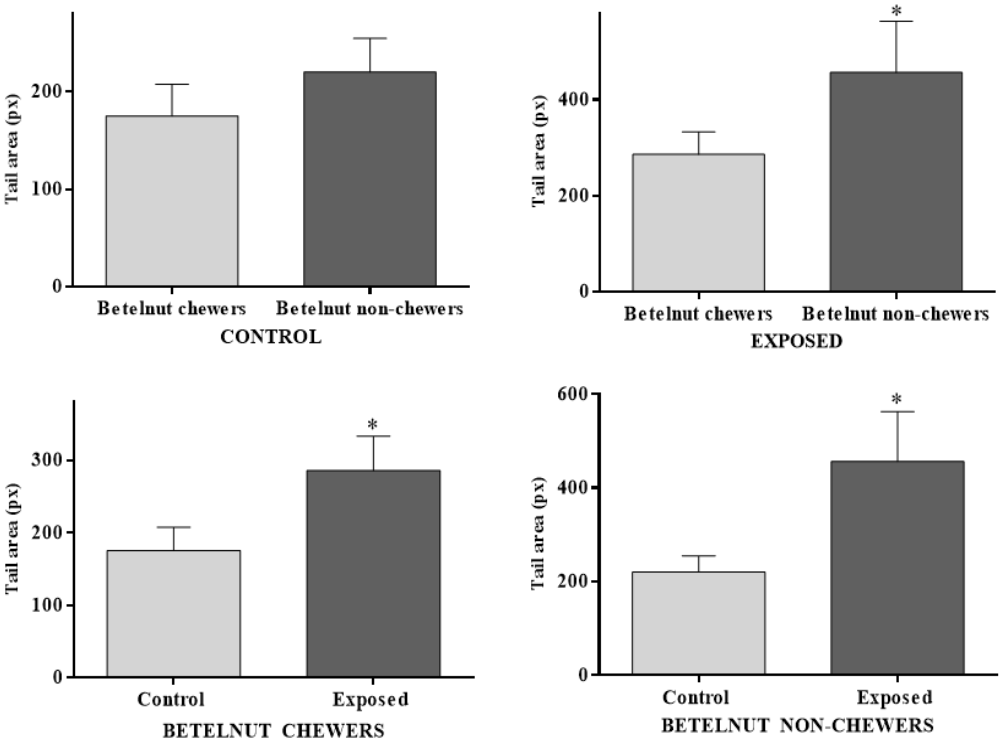


Figure 2.4 D

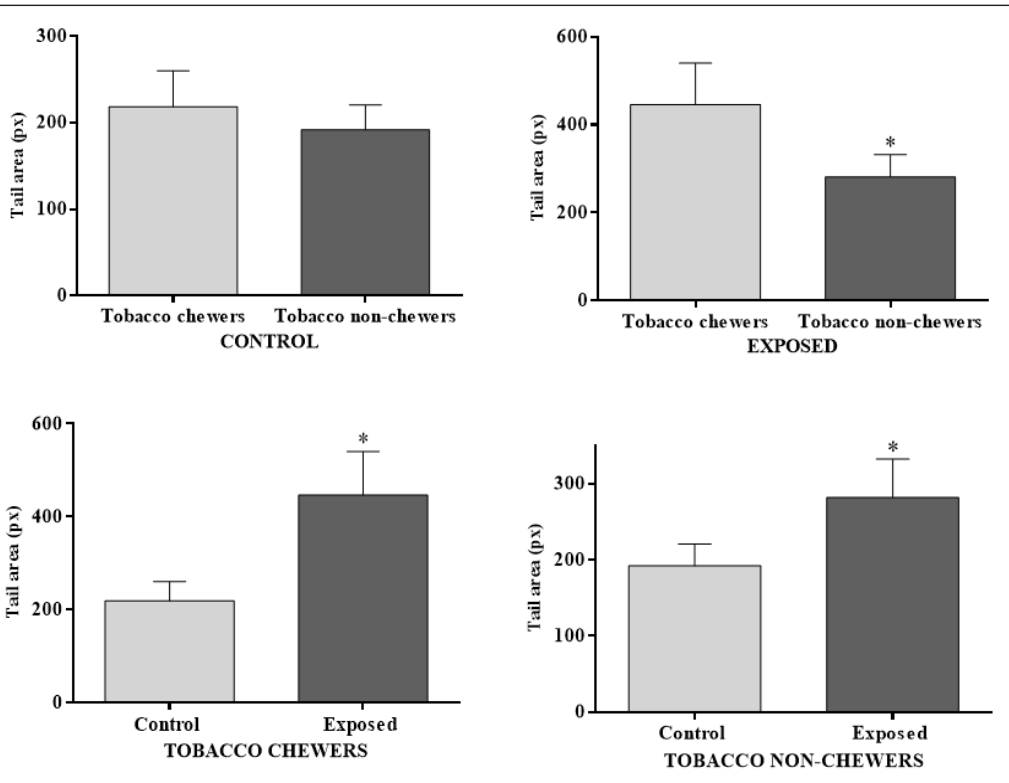


Figure 2.4 E

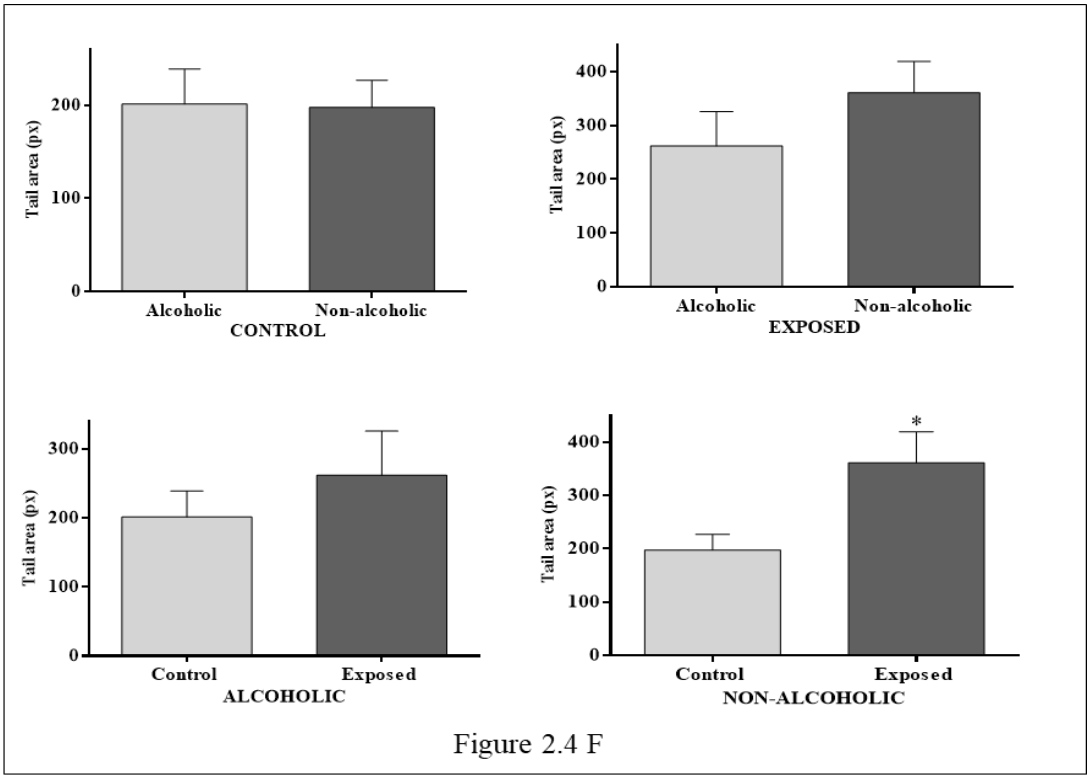


Figure 2.4 F

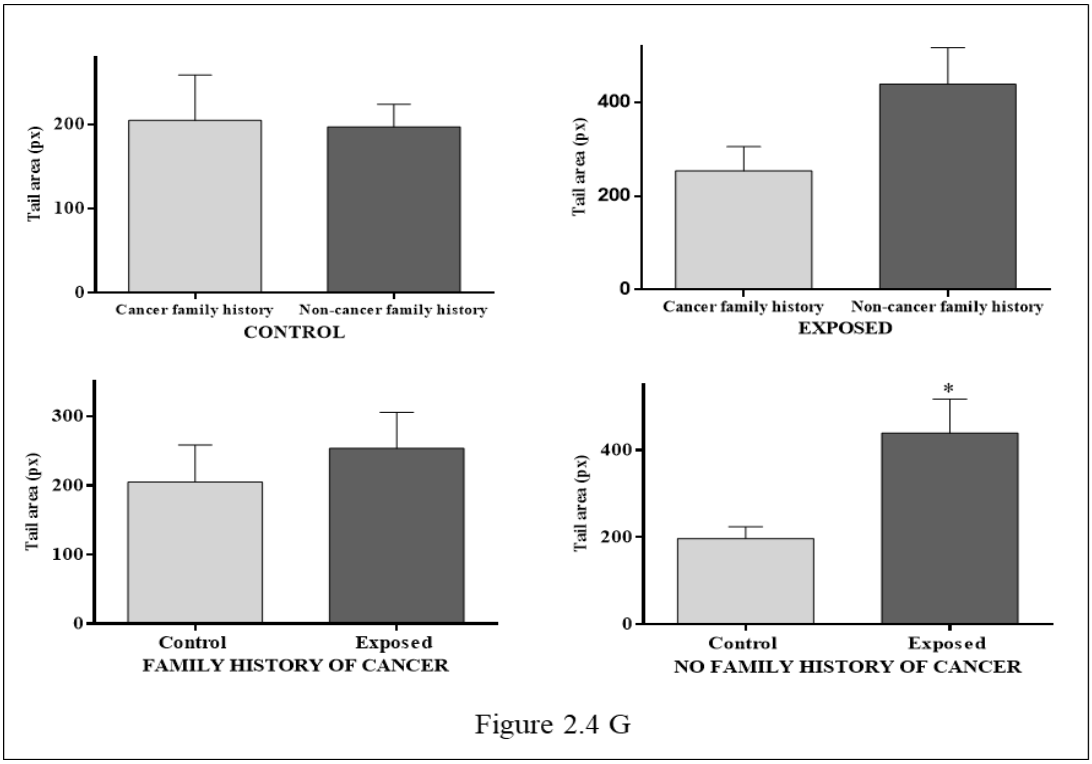


Figure 2.4 G

Figure 2.4: Functions of the demographic characteristics on comet tail area (px) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

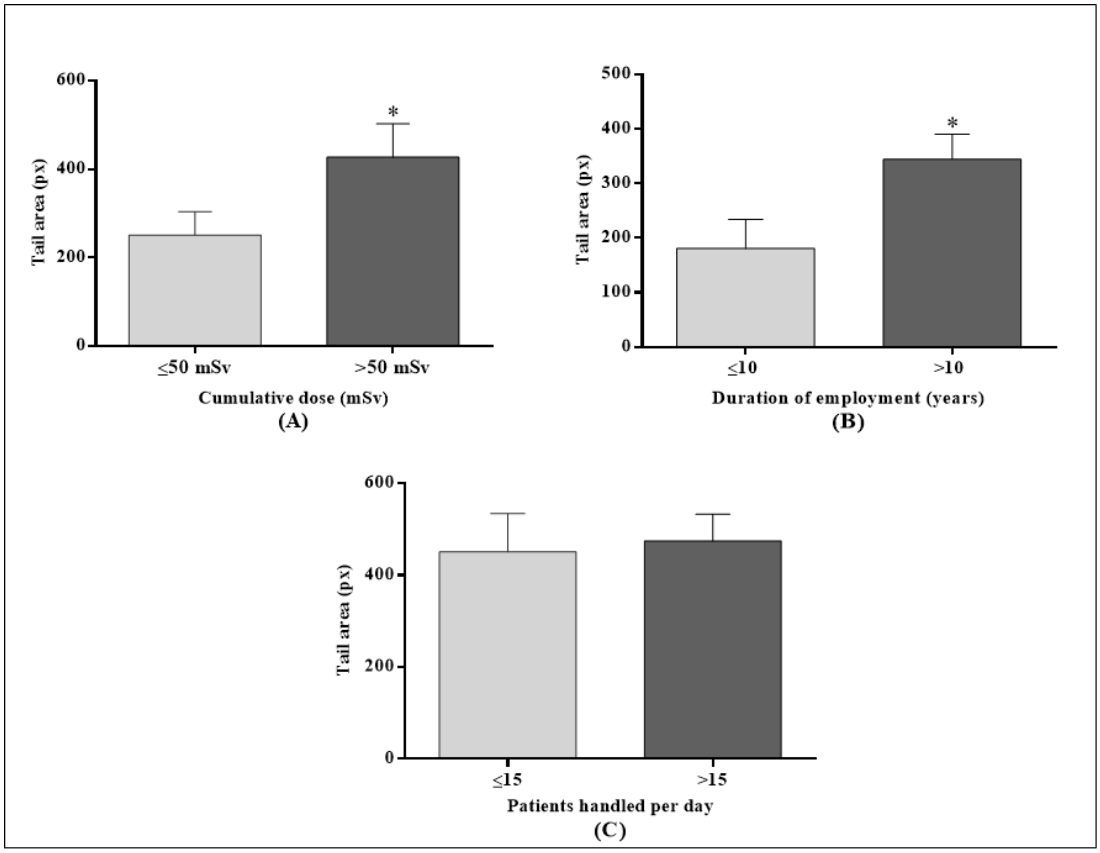


Figure 2.5: Functions of the demographic characteristics on comet tail area (px) among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group (p<0.05).*

3.3. Effects of demographic characteristics on the extent of DNA damage expressed in terms of tail DNA % among the exposed and control groups:

Tobacco chewers of the exposed group showed a significant increase in the percentage of DNA in comet tail when compared with tobacco chewers of the control group. However, a significant difference was not observed between chewers and non-chewers of both exposed and control groups (Figure 2.6 E). Furthermore, gender, age, smoking habits, betelnut chewing, alcohol consumption and family history of cancer did not significantly influence the percentage of DNA in comet tail in both the exposed and control groups (Figure 2.6 A-D, F & G). Multiple linear regression analyses revealed a significant association between the increase in the percentage of tail and tobacco chewing (Table 3).

Cumulative dose, duration of employment and the number of patients handled per day did not show significant effect on the percentage of DNA in comet tail among individuals working with X-rays (Figure 2.7).

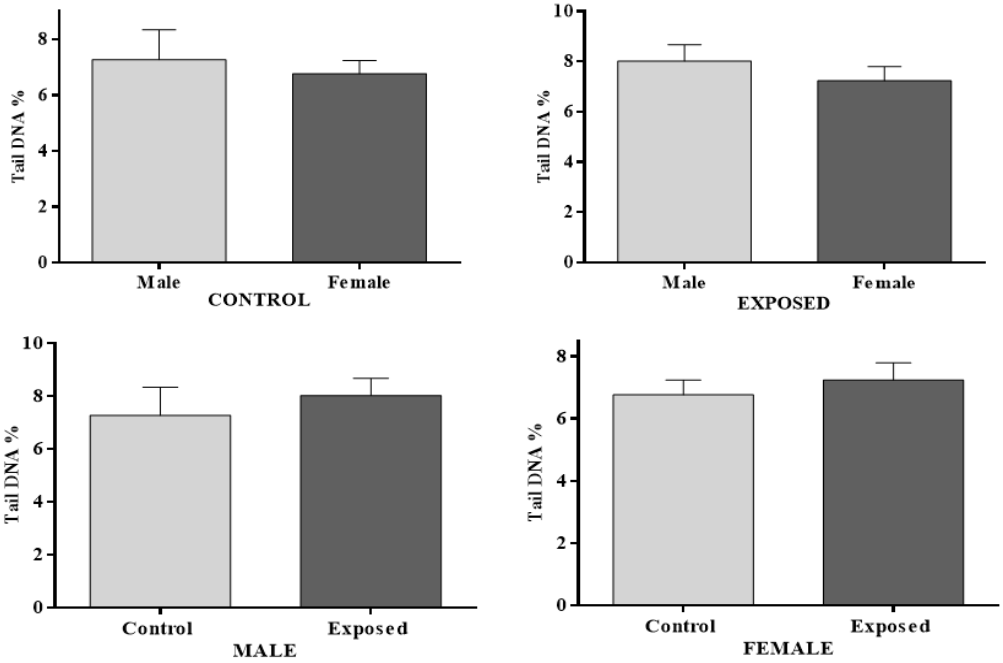


Figure 2.6 A

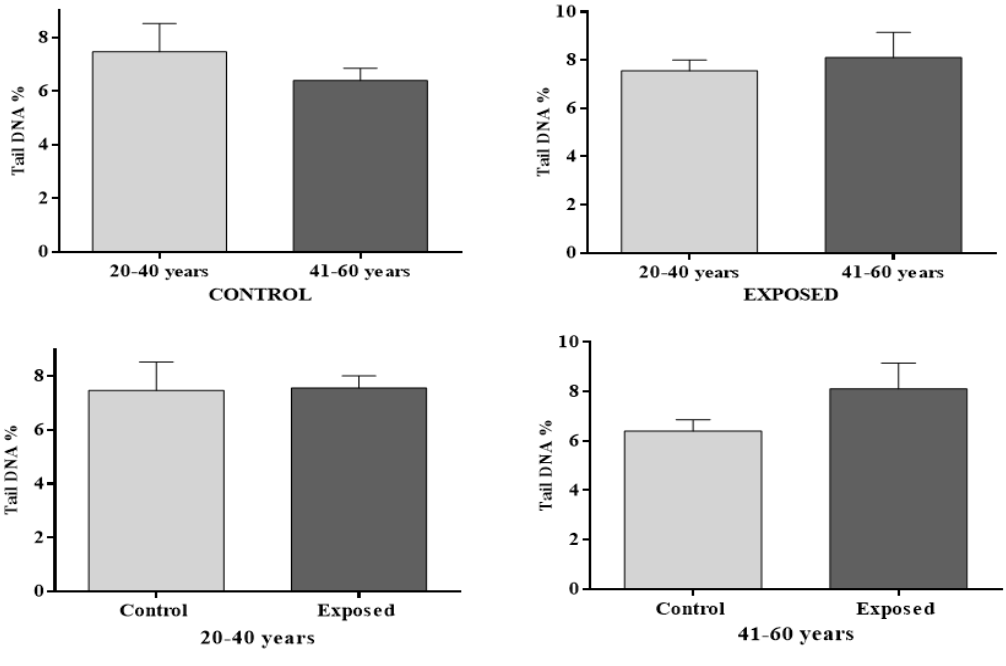


Figure 2.6 B

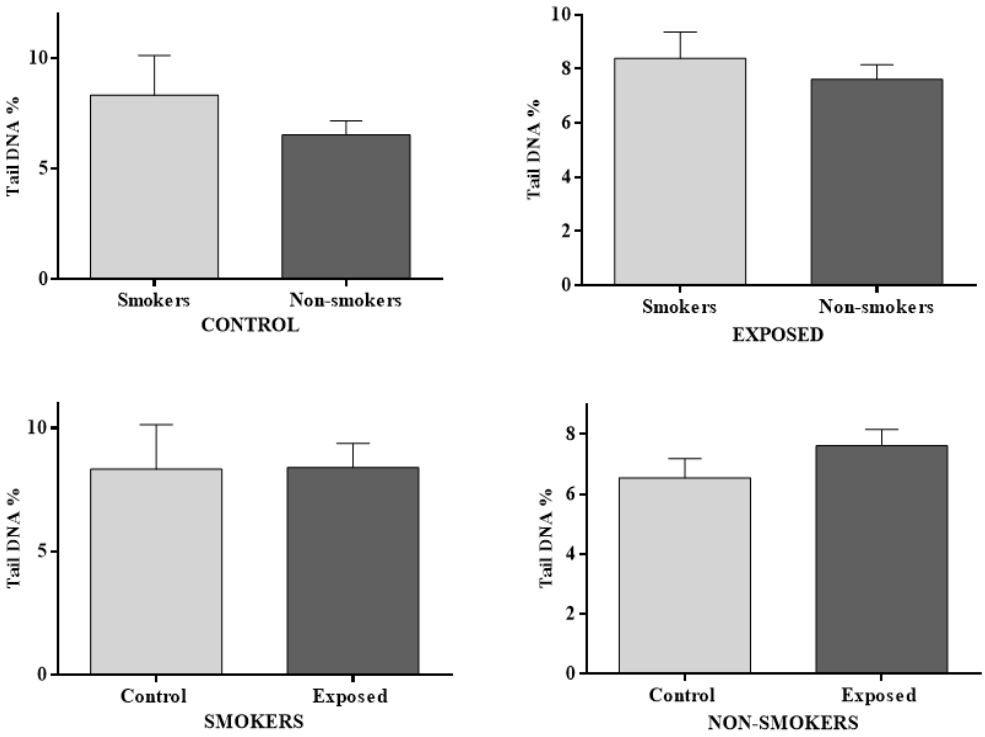


Figure 2.6 C

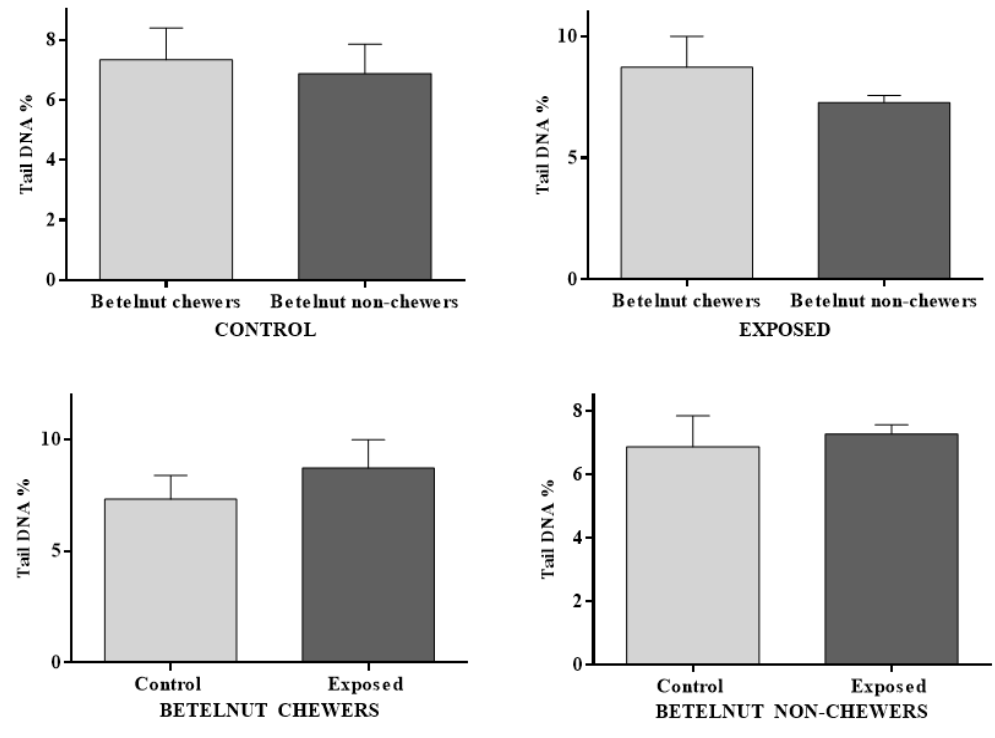


Figure 2.6 D

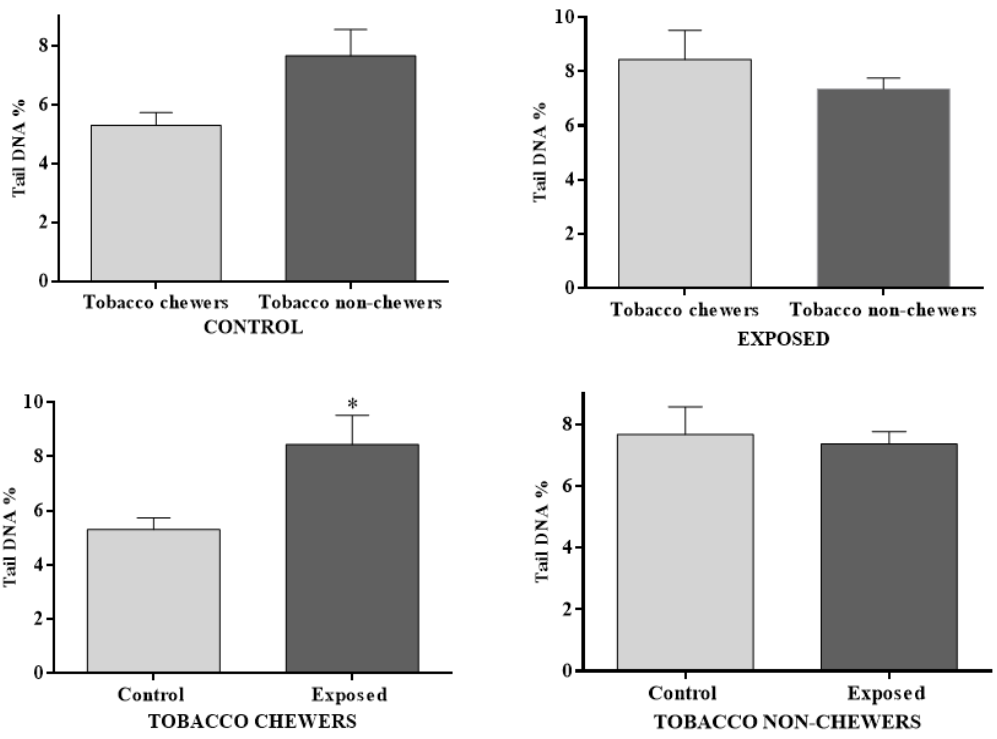


Figure 2.6 E

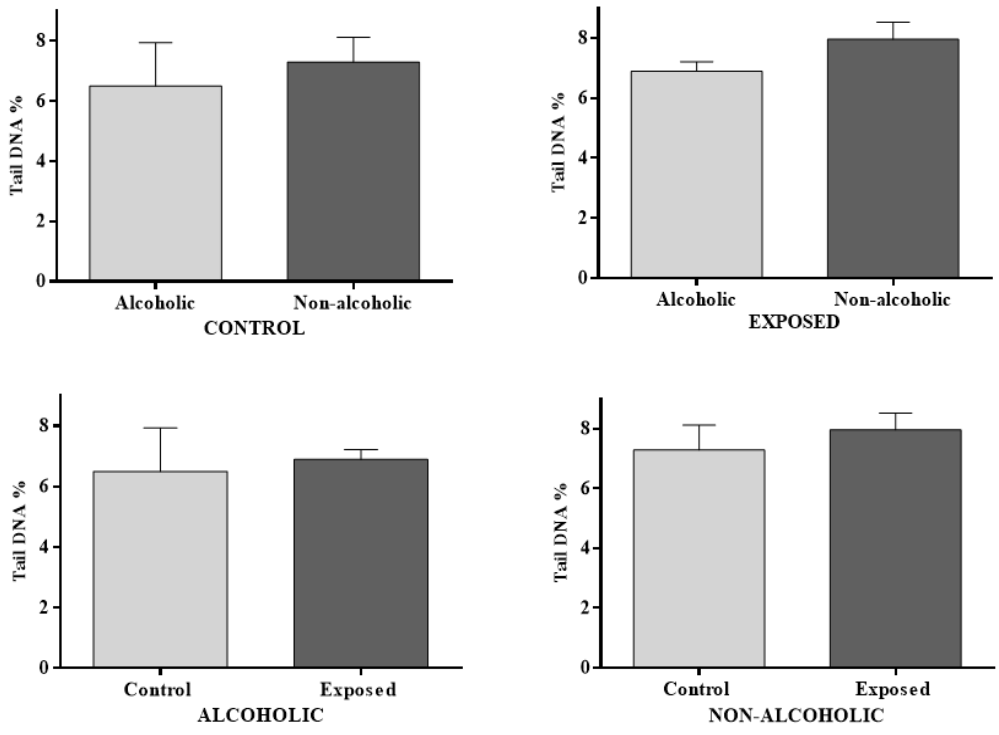


Figure 2.6 F

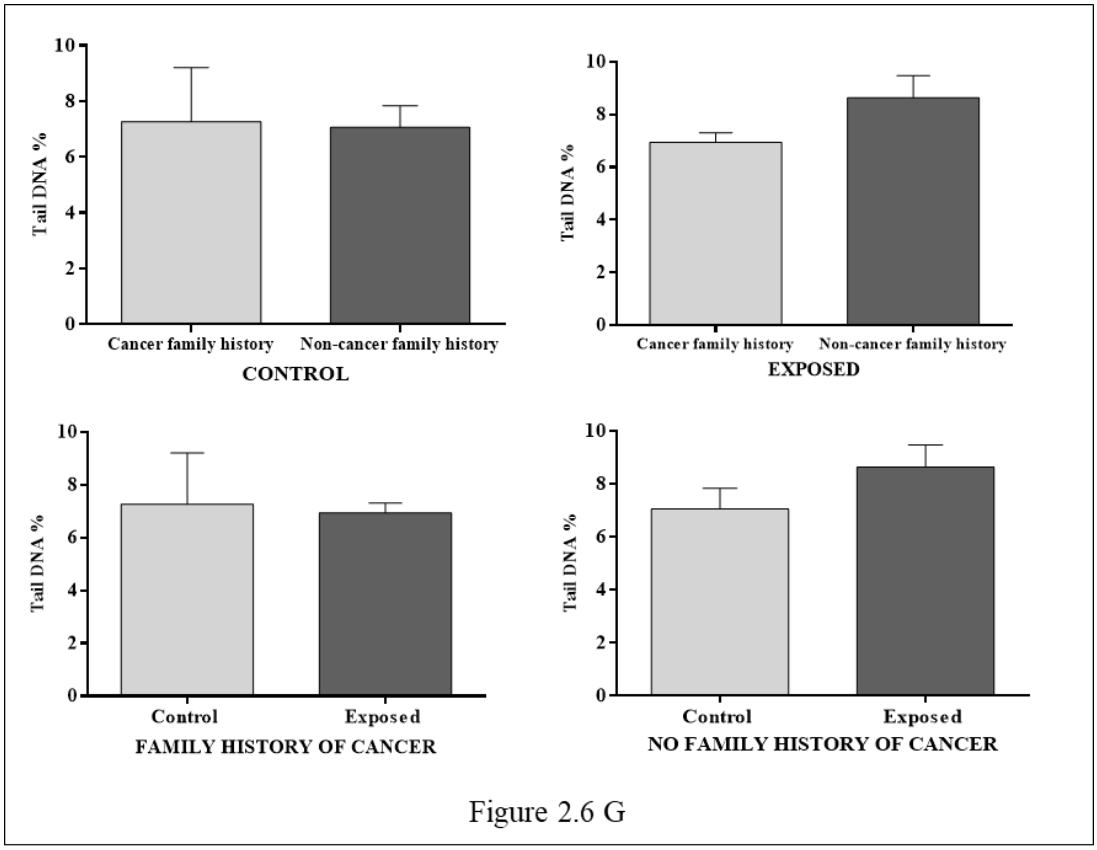


Figure 2.6: Functions of the demographic characteristics on the percentage of tail DNA in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the group ($p<0.05$).*

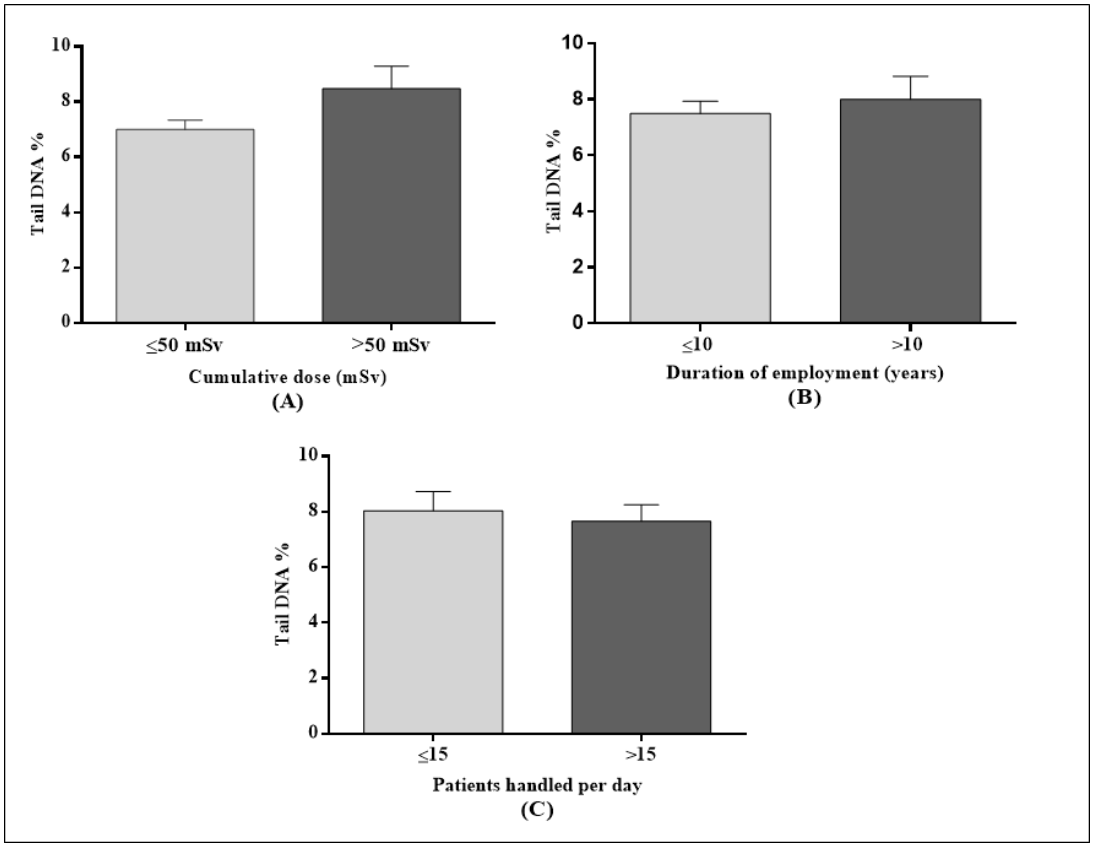


Figure 2.7: Functions of the demographic characteristics on the percentage of tail DNA among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

3.4. Effects of demographic characteristics on the extent of DNA damage expressed in terms of the olive tail moment among the exposed and control groups:

No significant variation was observed in the olive tail moment measured in males and females of the exposed and control groups (Figure 2.8 A). The elder members (41-60 years) of exposed group showed a significant increase in the olive tail moment when compared with control group. However, significant variation was not observed in the younger members in both exposed and control groups (Figure 2.8 B). Smokers, betelnut chewers and tobacco chewers belonging to the exposed group also showed a significant increase in olive tail moment when compared with those belonging to the control group (Figure 2.8 C, D & E). Similarly, among the non-alcoholic and

individuals having no family history of cancer, participants belonging to exposed group showed a significant increase in the olive tail moment when compared to those belonging to the control group (Figure 2.8 F & G). Multiple linear regression analyses revealed a significant association of increased olive tail moment with gender, tobacco chewing and alcohol consumption (Table 3).

Among the occupationally exposed group, technicians who have a cumulative effective dose of >50 mSv showed a significant increase in the olive tail moment when compared with workers having a cumulative effective dose of ≤50 mSv. Duration of employment and number of patients handled per day did not show a significant effect in the olive tail moment (Figure 2.9). Multiple linear regression analyses revealed a significant association of increased olive tail moment with cumulative dose and duration of employment (Table 3).

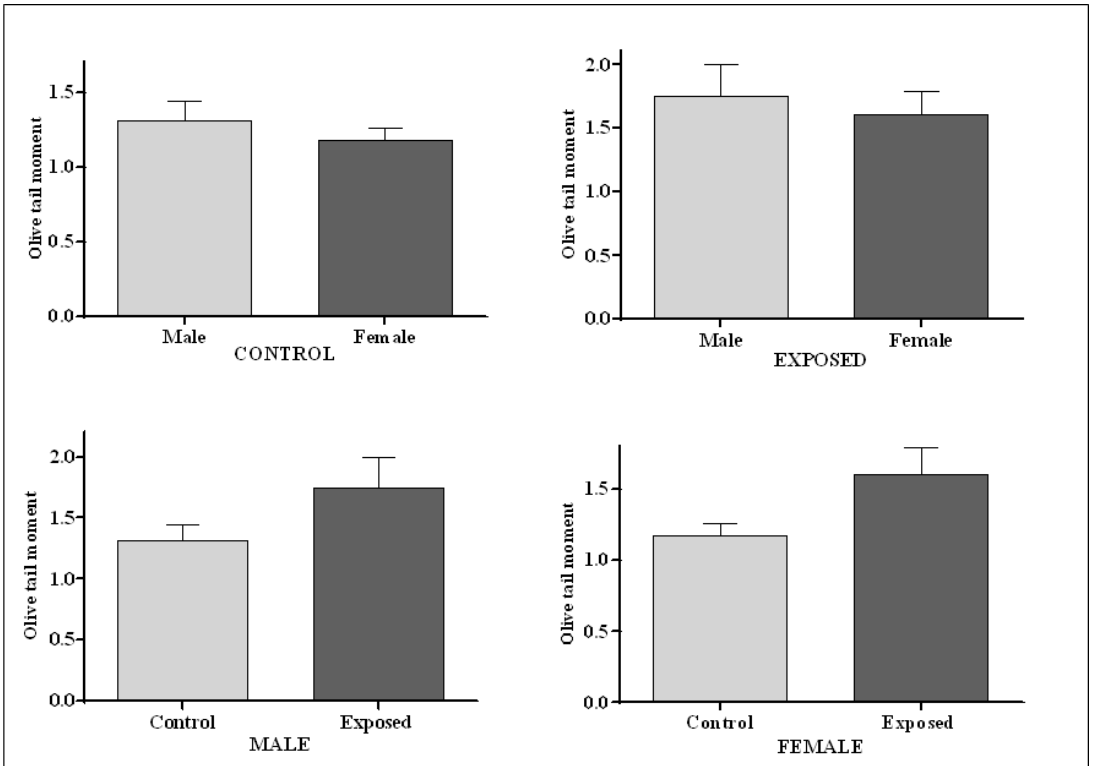


Figure 2.8 A

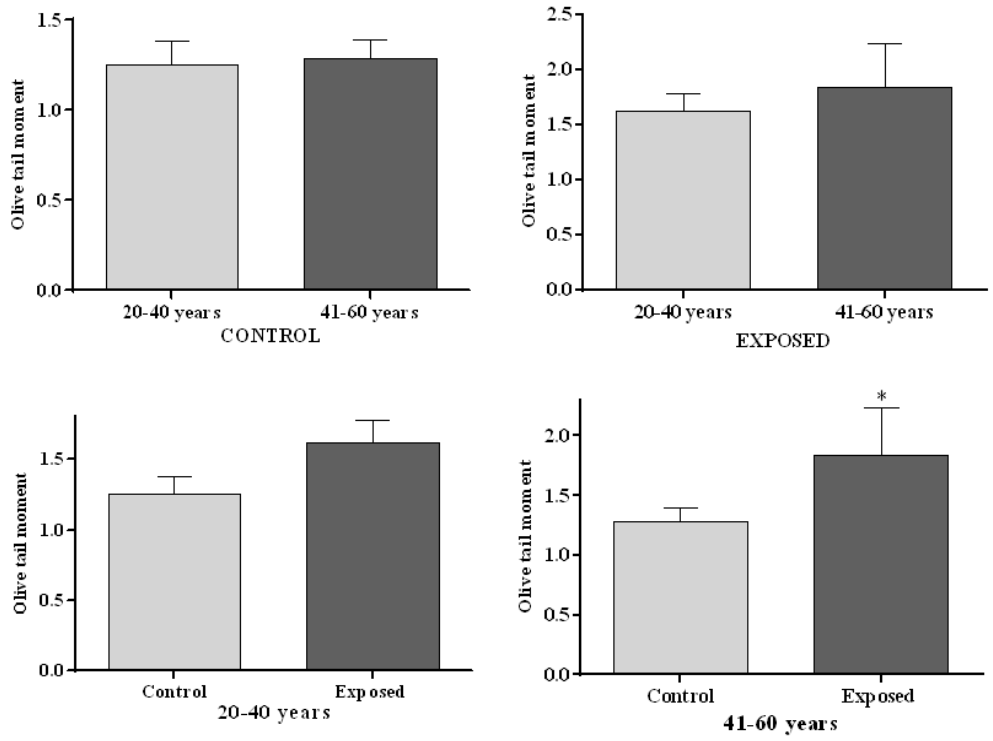


Figure 2.8 B

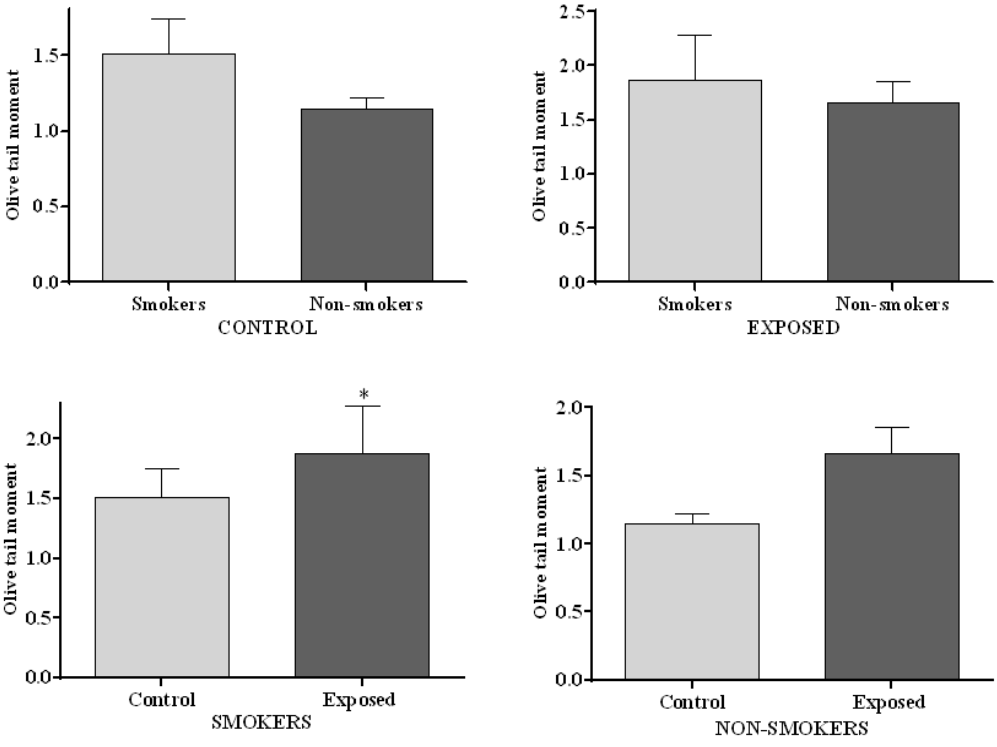


Figure 2.8 C

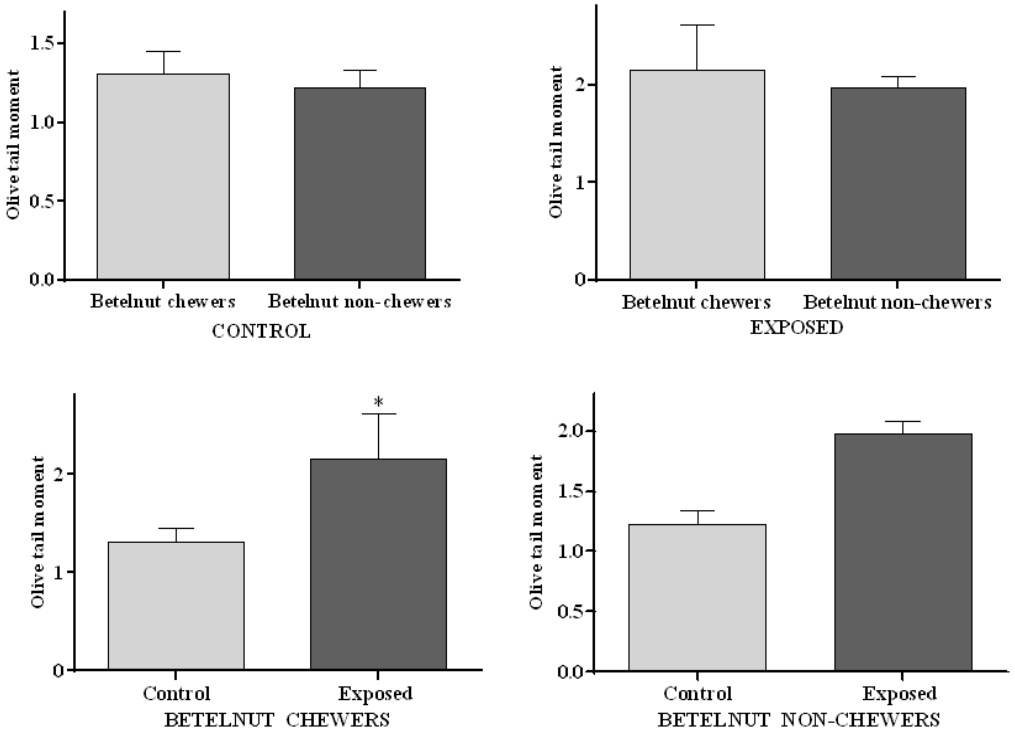


Figure 2.8 D

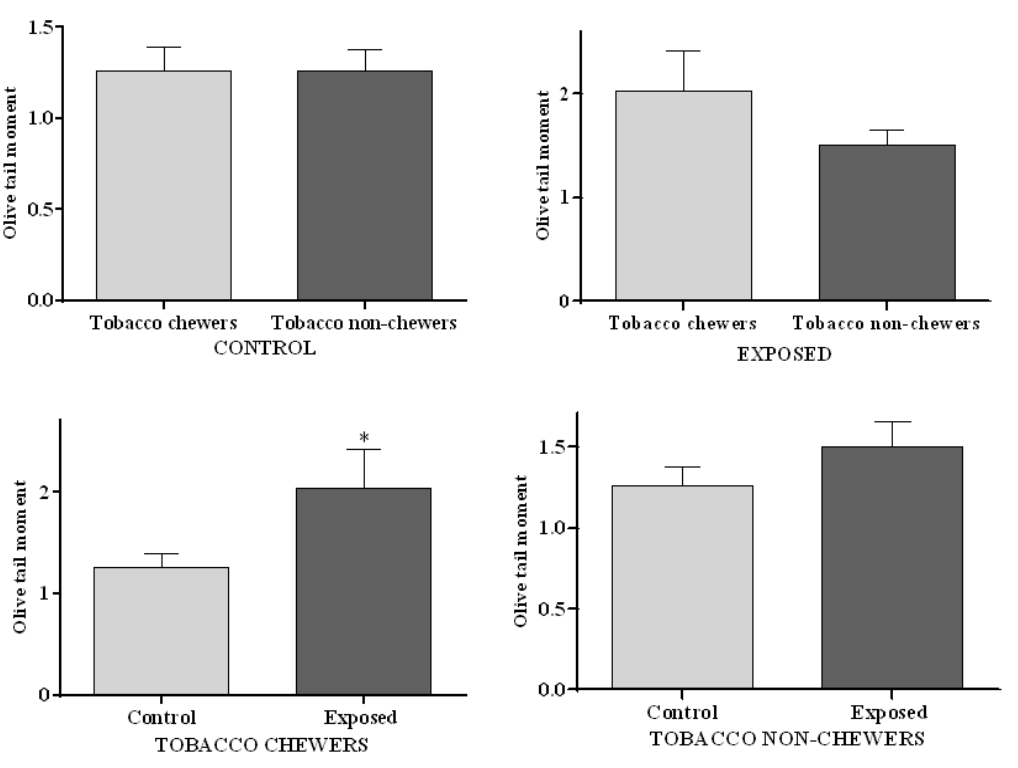


Figure 2.8 E

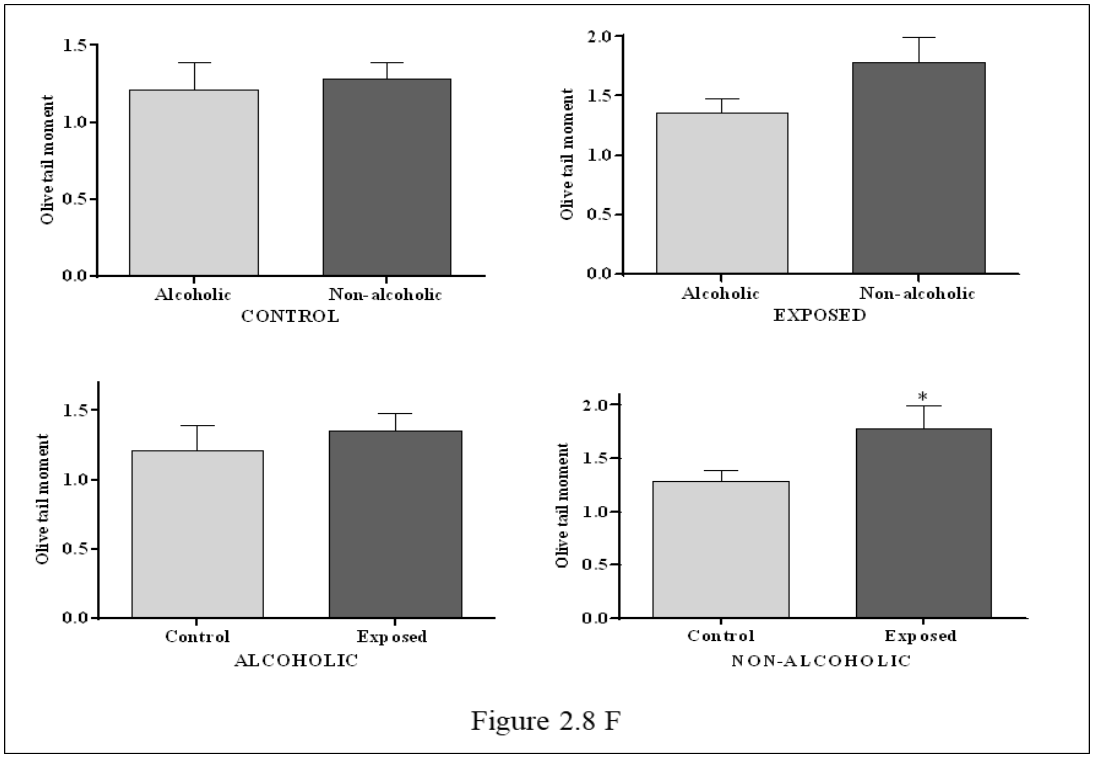


Figure 2.8 F

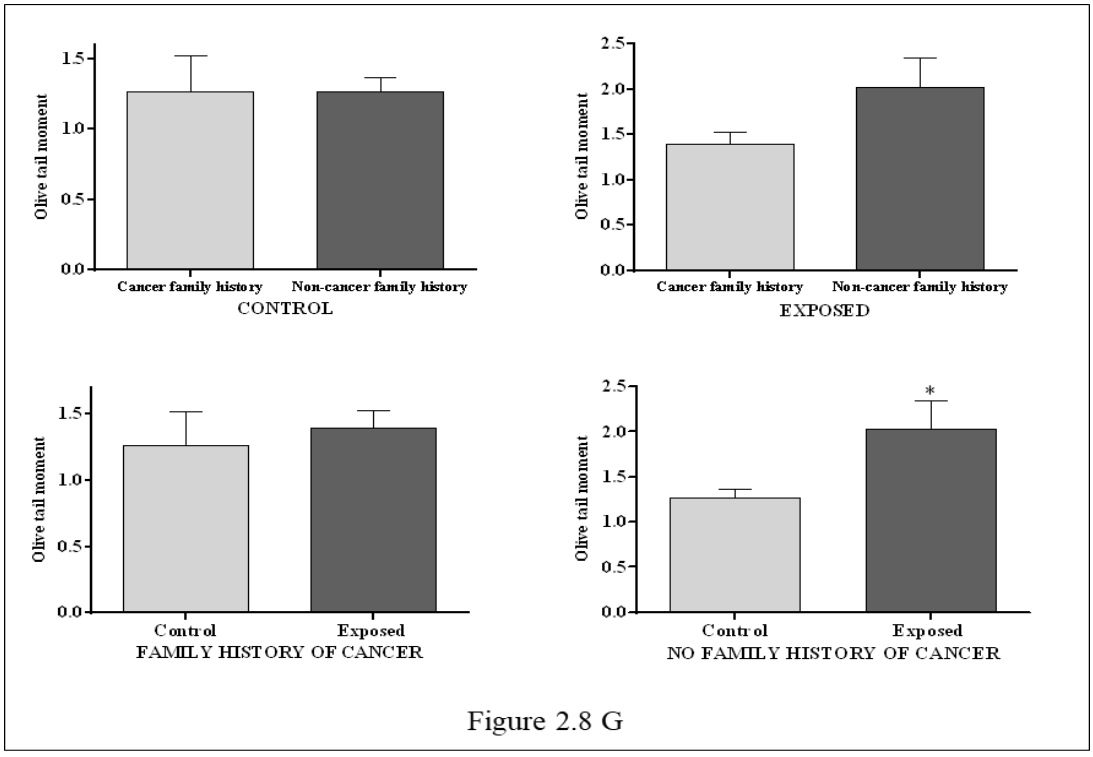


Figure 2.8 G

Figure 2.8: Functions of the demographic characteristics on the olive tail moment in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer.* *indicates significant variations between the group ($p<0.05$).*

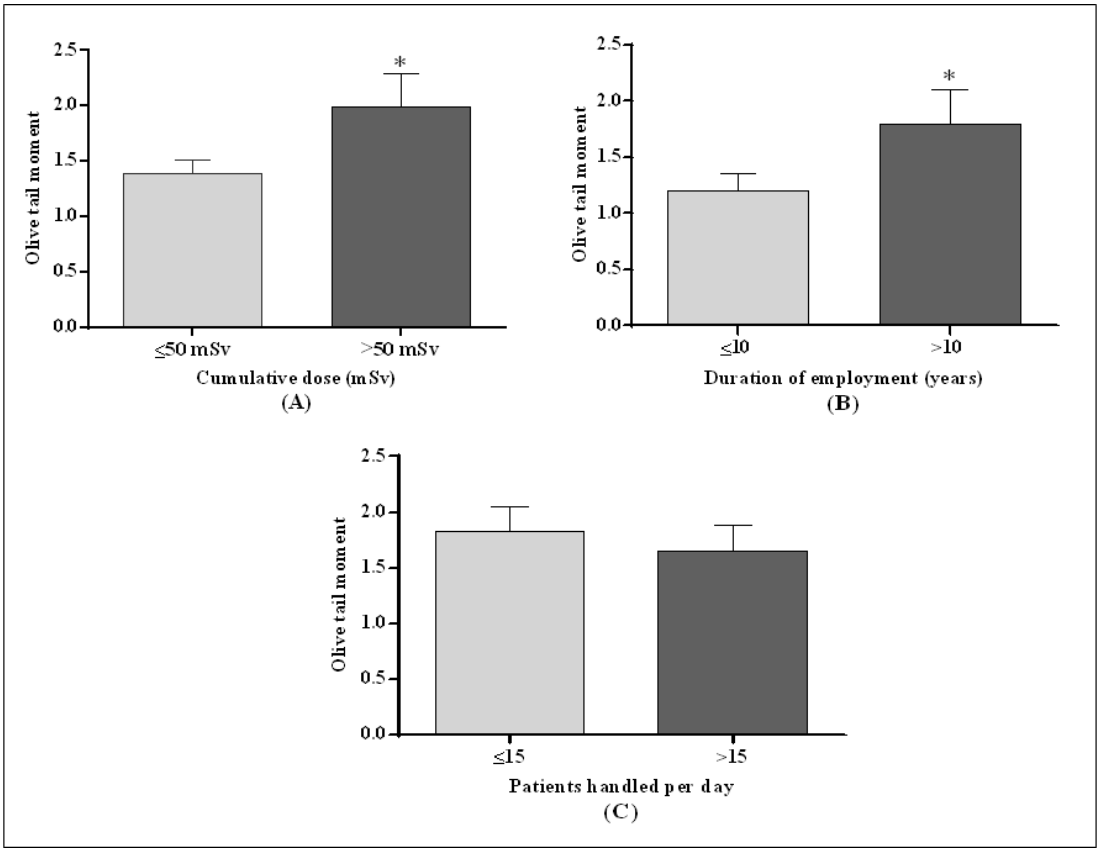


Figure 2.9: Functions of the demographic characteristics on the olive tail moment among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the group ($p<0.05$).*

Table 3: Influence of demographic characteristics on comet tail length, tail area, tail DNA %, and olive moment of the tail of the exposed and control groups.

Characteristics	Durbin Watson	Beta-value	t-value	p-value
Tail length				
Gender	1.84	-0.609	-2.646	0.058
Age		-0.347	-1.267	0.223
Smoking habits		-0.194	-1.215	0.242
Betelnut chewing		0.246	1.388	0.184
Tobacco chewing		-0.761	-3.532	**0.003**
Alcohol consumption		0.677	3.759	**0.002**
Family history of cancer		0.511	2.929	0.060
Time since working (years)		0.075	0.264	**0.040**
No. of patients handled per day		-0.363	-2.028	0.060
Cumulative dose (mSv)		0.529	2.527	**0.022**
Tail area				
Gender	1.774	-0.540	-2.260	0.078
Age		-0.348	-1.224	0.239
Smoking habits		-0.226	-1.358	0.193
Betelnut chewing		0.195	1.061	0.305
Tobacco chewing		-0.783	-3.496	**0.003**
Alcohol consumption		0.585	3.128	**0.006**
Family history of cancer		0.512	2.828	0.052
Time since working (years)		0.022	0.074	0.942
No. of patients handled per day		-0.301	-1.617	0.125
Cumulative dose (mSv)		0.526	2.415	**0.028**
Tail DNA %				
Gender	1.99	-0.799	-3.042	0.068
Age		-0.494	-1.580	0.134
Smoking habits		-0.209	-1.142	0.270
Betelnut chewing		0.133	0.655	0.522
Tobacco chewing		-0.859	-3.489	**0.003**
Alcohol consumption		0.572	2.784	0.073
Family history of cancer		0.406	2.039	0.058
Time since working (years)		0.180	0.552	0.589
No. of patients handled per day		-0.142	-0.695	0.497
Cumulative dose (mSv)		0.479	2.000	0.063

Tail olive moment

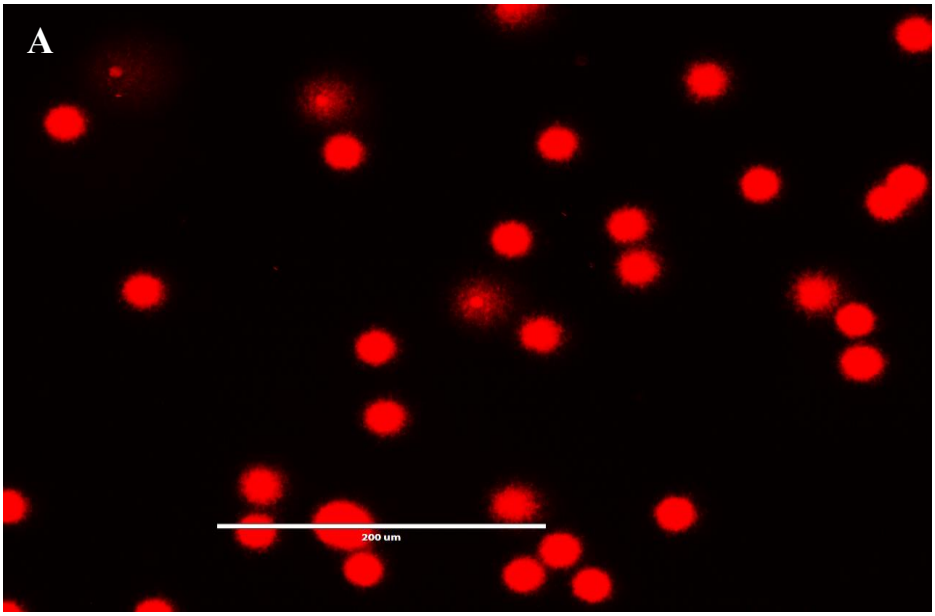
Gender	1.908	-0.680	-2.611	0.089
Age		-0.408	-1.318	0.206
Smoking habits		-0.211	-1.166	0.261
Betelnut chewing		0.054	0.272	0.789
Tobacco chewing		-0.835	-3.424	**0.003**
Alcohol consumption		0.557	2.732	**0.015**
Family history of cancer		0.428	2.171	0.095
Time since working (years)		0.130	0.403	**0.039**
No. of patients handled per day		-0.116	-0.570	0.576
Cumulative dose (mSv)		0.447	1.885	**0.045**

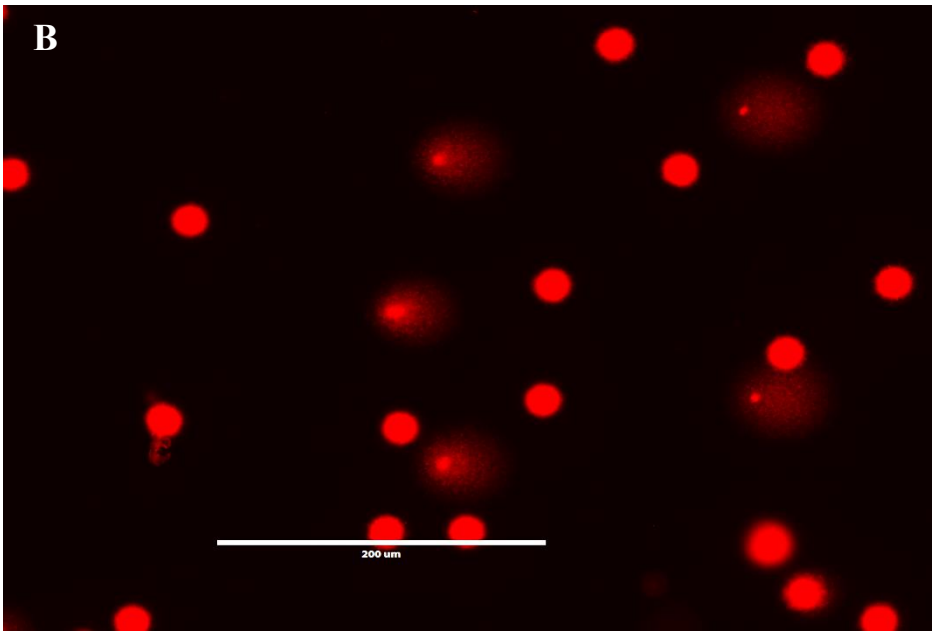
Bold value signify $p<0.05$.

PHOTO PLATES

Plate A: Fluorescence microscopy visualization of DNA damage observed in control group.

Plate B: Fluorescence microscopy visualization of DNA damage observed in exposed group.





4. DISCUSSION

Modern radiobiology has adopted the principle that DNA molecule is the critical target for lethal, carcinogenic, and mutagenic effects of ionizing radiation (Chaubey et al., 2001). Ionizing radiation causes a wide variability of DNA damage (Ward 1988; Natarajan 1993; Kruszewski et al., 1998*;* Chaubey et al., 2001). Various studies have indicated the DNA damaging effect of ionizing radiation even among the medical radiation workers (Maluf et al., 2001, Khrisroon et al., 2015; Garaj-Vrhovac and Kopjar 2003; Barquinero et al., 1993; Bonassi et al., 1997; Rozgaj et al., 1999; Sakly et al., 2012; Choi et al., 2013; Wang et al., 2016). Increased frequencies of chromosome aberrations and micronuclei are well known among individuals occupationally exposed to ionizing radiation (Evans et al., 1979; Jha and Sharma 1991; Thierens et al., 1996; Vera et al., 1997). A variety of research methods including sister chromatid exchange, chromosomal aberration and micronucleus assay are normally used for investigating the effects of human exposure to genotoxic agents (Tice et al., 1991; Olive 1999; Ostling and Johanson 1984). However, these techniques are not sensitive enough to detect single-strand breaks and alkali-labile sites. Furthermore, investigating low doses effects of ionizing radiation is limited as the above-mentioned techniques cannot be used to investigate DNA damage after exposures to doses under 2 Gy and at the single cellular level (Rosenberger et al., 2011). Therefore, an alkaline comet assay, which is known to be a more sensitive technique was performed in this study for the detection of double and single-strand breaks and alkali-labile sites. Comet assay is a biomarker assay that has been widely applied as a "golden standard" for screening the genotoxic effects of various agents including ionization radiation (Valverde and Rojas 2009). Its novel applications include monitoring occupational exposure to genotoxic chemicals or radiation (Somorovská et al., 1999). With the alkaline comet assay, an irradiation dose ranging from 100 mGy to 8 Gy can be investigated along with the DNA repair capacity (Rosenberger et al., 2011). Age, sex, and many environmental exposures have been reported to induce DNA damage that can be detected using comet assay (Møller et al., 2000). In case of chronic exposure,

comparing the levels of damage with comet assay provide information about concurrent and past exposure levels.

In the present study, the DNA damaging effects of X-ray irradiation among the hospital workers were represented by comet tail length, tail area, tail DNA %, and olive moment of the tail. Tail length detects DNA damage at low levels but doesn't change once the tail is established. The tail area gives a total projected area of the comet tail, coupling the length and width of the tail in a single measure. Subsequently, the area of the tail represents the severity of the damage. The percentage of DNA in the comet tail is another useful parameter that is linearly related to the breaking frequency. The tail moment combines tail length and tail intensity in one single value, thus, it is the most reliable and most frequently used parameter. The comet tail increases in intensity but not in length as the dose of damage increases (Collins 2004). The influence of age, gender, and lifestyle including betelnut chewing, tobacco usage, smoking habit and alcohol consumption and family history of cancer on DNA damage in both the exposed and control groups were also determined using multiple linear regression analysis. This study indicates significantly higher DNA damage in the exposed group when compared with the controls irrespective of their demographic characteristics. Our findings are in agreement with other studies on medical workers exposed to low-dose irradiation (Martinez et al., 2010; Wojewodzka et al., 1998; Surniyantoro et al., 2020; Garaj-Vrhovac and Kopjar 2003; Khrisroon et al., 2015; Maluf et al., 2001). Chromosome damage usually is expected in individuals from the hazard group that could increase the mean comet tail of the cell population. Possibilities could be the decreased ability to repair the spontaneous DNA damage, where they are constantly being induced by endogenous or exogenous factors. Such decreased repairability in individuals from high-risk groups has been proposed as a good indicator to document DNA damage from exposure to mutagens (Plappert et al., 1997). On the other hand, there are assumptions that cells exposed to ionizing radiation respond by an enhanced intracellular oxidative stress which is the cause of chromosomal instability commonly observed in various cell systems (Clutton et al., 1996). This could increase DNA damage that is high enough to be detected by the sensitive comet assay.

Functions of demographic parameters in the level of DNA damage were also estimated using multiple linear regression analysis which reveals a significant association of increased DNA damage with tobacco chewing and alcohol consumption. Among the occupationally exposed group, cumulative dose and duration of employment were observed to show significant association with the levels of DNA damage (Table 3). The present study also observed a significant association between levels of DNA damage, which was expressed by various comet parameters, with the age of the participants. Age as a function of increased DNA damage has been reported earlier by various studies (McCurdy et al., 1997; Barquinero et al., 1993). The increase in chromosomal damage with increasing age was reported in several studies earlier (Fenech and Morley 1985; Migliore et al., 1991; Joseph et al., 2004; Ropolo et al., 2012; Singh et al., 1991) using micronucleus assay. The increase in DNA damage with the age of the exposed group has been reported to be attributed to the cumulative effect of acquired mutations in genes involved in DNA repair, chromosome segregation, and cell cycle checkpoint with increasing age (Fenech and Bonassi 2011). However, the relationship between increasing age and DNA damage is still controversial as several other studies did not find a significant relationship (Maluf 2001; Dhawan et al., 2001; Betti et al., 1994; Frenzili et al., 1997).

Certain demographic factors such as diet, smoking, and alcohol consumption have also been reported to induce DNA damage as indicated by increasing MN frequency (Fenech et al., 1999; An and Kim 2002; Ishikawa et al., 2003; Kim et al., 2005; Maffei et al., 2002; Betti et al., 1994, 1995; Dhawan et al., 2001; Hartmann et al., 1994). Boyaci et al. (2004) have reported a significant association between tobacco consumption with DNA damage in the lymphocytes of cardiologists exposed to radiation. Furthermore, Martínez et al. (2010) demonstrated the DNA damaging effects of alcohol consumption among the individuals exposed to low doses of radiation which was similar to the current findings where radiology workers who consume alcohol and tobacco have significantly higher DNA damage when compared with that of the control group showing the same demographic characteristics. In the present study, smoking also significantly increases DNA damage levels in radiology workers which is in accordance with the previous findings (Frenzili et al., 1997; Maluf et al., 2001, Betti et al., 1994; Fuchs et al., 1995). However, contradictory results on

the effects of cigarette smoking on DNA damage have also been reported by various studies using comet assay (Hellman et al. 1997, 1999; Wojewodzka et al. 1999; Martínez et al., 2010; Khisroon et al., 2015). Tobacco usage has been reported to trigger the production of free radicals that in turn produce superoxide radicals and hydroxyl radicals (Church and Pryor 1985; Valavanidis et al., 2009). The free radicals induced DNA adducts may be converted into DNA strand breaks. Tobacco is also known to induce sugar damage, apurinic/apyrimidinic sites, small base damages, bulky DNA adducts, DNA cross-links and DNA strand breaks (Hang 2010). Moreover, it has been observed that alcohol induces a significant increase in the percentage of cells with DNA damage in normal individuals (Kalaiselvi et al., 2002). When exposed to ionizing radiation, chemical and physical agents may have additive/synergistic effects in the biological systems (Burkart 2001) which may result in a significant increase of DNA damage in ionizing radiation-exposed radiology workers who consume alcohol and tobacco.

Our results further indicated an association between DNA damage and the cumulative dose of radiology workers. In agreement with our findings, Andreassi et al. (2005) reported a higher MN frequency in interventional cardiologists compared to physicians working in the same hospital not exposed to ionizing radiation. The cumulative annual equivalent dose was 4.06 ± 0.4 mSv and did not correlate with the MN frequency. In a similar study on interventional cardiologists, nuclear medicine physicians, and conventional radiologists whose exposure ranged from 0-48 mSv, the frequency of MN and chromosome aberrations was higher than in controls (Zakeri and Hirobe 2010). Among radiology workers, the DNA damage index was significantly correlated with time of exposure or experience as reported by various studies (Betti et al., 1994; Maluf et al., 2001) which is in agreement with our findings where the duration of employment showed a significant association with increased DNA damage. A similar association between chromosomal aberrations and duration of employment was reported earlier among hospital workers occupationally exposed to ionizing radiation (Mihalache et al., 2007; Vellingiri et al., 2014). The study indicates that occupational exposure to low doses of ionizing radiation could be potentially hazardous if the person is exposed over long periods.

CHAPTER V

INVESTIGATION OF CHROMOSOMAL DAMAGE AMONG THE OCCUPATIONALLY EXPOSED GROUP USING MICRONUCLEUS ASSAY

ABSTRACT

It has been recognized for several decades that ionizing radiation can cause chromosome breakage at any stage of mitosis or meiosis. The individuals working with ionizing radiation in medical radiology may be at a greater disadvantage due to the deleterious and long-term adverse health effects triggered by ionizing radiation which may subsequently induce cancer. Therefore, the present study was conducted to analyze the chromosomal damage associated with ionizing radiation exposure using a micronucleus (MN) assay in hospital workers occupationally exposed to low doses of ionizing radiation. The peripheral blood lymphocytes of the occupationally exposed and control groups matched for age, gender, tobacco usage, and alcohol consumption were cultured and micronuclei frequency was determined. Occupational exposure to ionizing radiation results in a significant rise in micronuclei frequency when compared with the control group. Exposure to ionizing results in a significant rise in micronuclei frequency regardless of their betelnut chewing, tobacco chewing, alcohol consumption and family history of cancer. Among the occupationally exposed group, multiple linear regression analyses revealed a significant association of micronuclei binucleated cell frequency with cumulative dose, duration of employment and number of patients handled per day. We have observed that low dose exposure to ionizing radiation is an inevitable occupational hazard leading to increased genomic instability in the radiological technicians depending on the time spent with X-rays, cumulative dose received and the number of patients handled daily.

1. INTRODUCTION

The increasing use of ionizing radiation in medical diagnosis and treatment has raised concern about its potential long-term effects on human health as prolonged exposure to low-dose radiation has been found to cause adverse health effects (Fazel et al., 2009; Gilbert 2009; Richardson et al., 2015). Ionizing radiation directly interacts with cellular components causing ionization of the important macromolecules and initiating a sequence of events leading to biological changes (Lehnert 2007). Various types of DNA damage such as primary lesions, single-strand breaks (SSBs), double-strand breaks (DSBs), alkali-labile sites, and damage to nitrogenous bases, DNA-protein interaction and DNA-DNA crosslinks have been reported to cause by biophysical interaction of DNA with ionizing radiation (Natarajan 1993; Kruszewski et al., 1998; Chaubey et al., 2001). Such genotoxic effects of ionizing radiation can cause genomic instability and mutations leading to cancer induction among the exposed individuals (Mavragani et al., 2017). Various studies have reported increased levels of chromosomal aberrations in lymphocytes of workers occupationally exposed to ionizing radiation as compared to unexposed controls (Evans et al., 1979; Lloyd et al., 1980; Jha and Sharma 1991; Andreassi et al., 2009; Zakeri and Hirobe 2010; Sakly et al., 2012; Vellingiri et al., 2014). Moreover, some epidemiological studies have also revealed that individuals who are occupationally exposed to ionizing radiation may have an increased risk of leukemia and other cancers (Smith and Doll 1981; Aoyama 1989; Muirhead et al., 1990; IARC 2000; Gilbert 2009; Wang et al., 2016). However, contradictory results have been reported on the genotoxic effects of occupational exposure to low doses of ionizing radiation by various workers (Demirel et al., 1997; Cardoso et al., 2001; Maffei et al., 2002; Thierens et al., 2002; Joseph et al., 2004).

Observation of chromosomal damage caused by exposure to ionizing radiation was among reliable evidence that chemical and physical agents can induce alterations to the genetic material (Evans 1977). Chromosomal aberrations and micronucleus assays are effective tools to evaluate the genotoxic or clastogenic effects of any physical or chemical agent. However, the evaluation of micronuclei frequency is easier, less cumbersome, and allows scoring of a larger number of cells in a short duration than the scoring of the chromosome aberrations. Micronuclei (MN) are small

nuclear bodies in the cytoplasm that contain either chromosome breaks lacking centromeres or whole chromosomes that cannot move to the spindle poles during mitotic cell division. MN provides a reliable index of both chromosome loss and chromosome breakage. MN are scored in binucleated cells as they are expressed in cells that have completed nuclear division. MN can be expressed only in dividing eukaryotic cells, therefore the assay cannot be used efficiently in non-dividing cells. Out of several methods proposed for non-dividing cells, the cytokinesis-block micronucleus (CBMN) assay has been found most favorable due to its simplicity and lack of uncertainty regarding its effect on base-line genetic damage (Fenech and Morley 1985; Fenech and Morley 1986). In CBMN assay, cells that have completed one nuclear division are blocked from performing cytokinesis using cytochalasin-B (Cyt-B) and are consequently readily identified by their binucleated appearance (Carter 1967). Measurement of MN frequency in human peripheral blood lymphocytes using cytochalasin B technique (Fenech and Morley 1985) is extensively used to evaluate the extent of chromosomal damage in human populations exposed to genotoxic agents (Fenech et al., 1999). Micronucleus formation was shown to be a reliable biomarker for biomonitoring studies of genetic damage (Bonassi et al., 2005). Therefore, the present study was conducted to analyze the cytogenetic damage associated with ionizing radiation exposure using micronucleus assay in hospital workers occupationally exposed to low doses of ionizing radiation.

2. MATERIALS AND METHODS

2.1. Chemicals

RPMI-1640 medium, phytohemagglutinin, and acridine orange were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and cytochalasin B was purchased from Sigma Aldrich Chemical Co. (Bangalore, India).

2.2. Subjects

The volunteers were divided into two groups as described in Chapter 2.

***2.2.1. Exposed group*:** The group comprised of 40 healthy individuals that are occupationally exposed to X-rays for medical diagnostic purposes, working in different hospitals in Aizawl, Mizoram.

***2.2.2. Control group*:** The group comprised of 40 healthy individuals matched for various demographic data, residing in the same urban area. The volunteers did not have any work-related exposure to ionizing radiation or any known genotoxic agent.

After identifying suitable volunteers for the study, each individual was asked to sign a consent form and questionnaire as described in Chapter 2.

2.3. Blood sample collection and lymphocyte culture

The blood samples were collected by venipuncture from each volunteer of both groups in individual sterile heparinized tubes. The lymphocyte culture was carried out according to the method described by Jagetia et al. (2001). Briefly, the blood was allowed to sediment and the buffy coat was collected in individual sterile glass tubes. Usually, 10^6 nucleated cells were inoculated into sterile glass tubes containing RPMI-1640 medium, 10% fetal calf serum and phytohemagglutinin as the mitogen. The cells were allowed to grow for the next 44 hr in a humidified atmosphere of 5% CO_2 in air at 37˚C. Cytochalasin B was added at a final concentration of 5 µg/ml to block cytokinesis and cells were allowed to grow for another 28 hr (Fenech and Morley 1985). Cells were harvested at the end of 72 hr after initiation of the lymphocyte culture by centrifugation. A mild hypotonic solution was added to the cell pellet so as to retain the cell membrane. Cells were then fixed in freshly prepared Carnoy's fixative (methanol: acetic acid, 3:1). The cell suspension was placed onto pre-cleaned coded blinded slides to avoid observer's bias and spread by air blowing. The cells were

stained with acridine orange and scored under a fluorescence microscope (DM 2500, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) by two individuals. Usually, a total of 1000 binucleate cells (BNC) with well-preserved cytoplasm were scored from each individual for the presence of micronuclei (MN) according to the criteria described earlier (Fenech et al., 2003).

2.4. Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). Student "t" test was performed to determine the significance between the exposed and control group for micronuclei frequency. Multiple linear regression analyses were carried out for the prediction of the association of micronucleus frequency with demographic characteristics. Statistical analyses were conducted using Graph Pad Prism ver. 6.0 and SPSS Ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

Occupational exposure to ionizing radiation results in a significant rise in MNBNC (micronucleated binucleate cells) frequency when compared to the control group irrespective of their demographic characteristics (Figure 3.1).

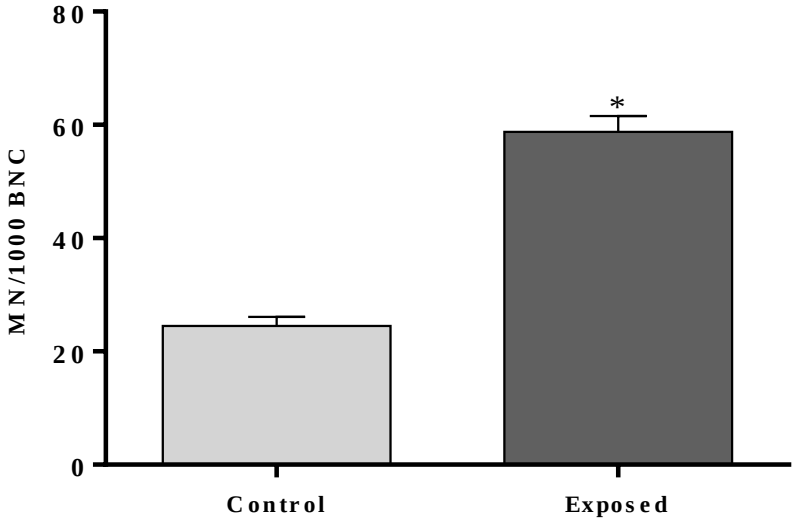


Figure 3.1: Frequency of micronucleated binucleate cells (MNBNC) in exposed and control group. ** indicates significant variations between the group($p<0.05$).*

3.1. Effects of demographic characteristics on micronuclei frequency among the exposed and control groups:

Males and females of the exposed group showed a significantly higher MNBNC when compared with their respective gender of the control group (Figure 3.2 A). Also, the younger members (20-40 years) and elder members (41–60 years) of the exposed group showed significantly higher MNBNC frequency when compared with their respective age group of the control group (Figure 3.2 B). Furthermore, smokers and non-smokers of the exposed group showed significantly higher MNBNC frequency when compared with those belonging to the control group. However, significant variation was not observed in the MNBNC frequency between the smokers and non-smokers of the exposed group (Figure 3.2 C). Occupational exposure to ionizing radiation results in a significant rise in MNBNC frequency regardless of their betelnut chewing, tobacco chewing, alcohol consumption and family history of cancer (Figure

3.2 D- G). Multiple linear regression analyses revealed a significant association of MNBNC frequency with increasing age and betelnut chewing (Table 4).

Among the occupationally exposed group, cumulative dose, duration of employment and patients handled in a day results in an increase in MNBNC frequency (Figure 3.3). Multiple linear regression analyses revealed a significant association of MNBNC frequency with cumulative dose, duration of employment and number of patients handled per day (Table 4).

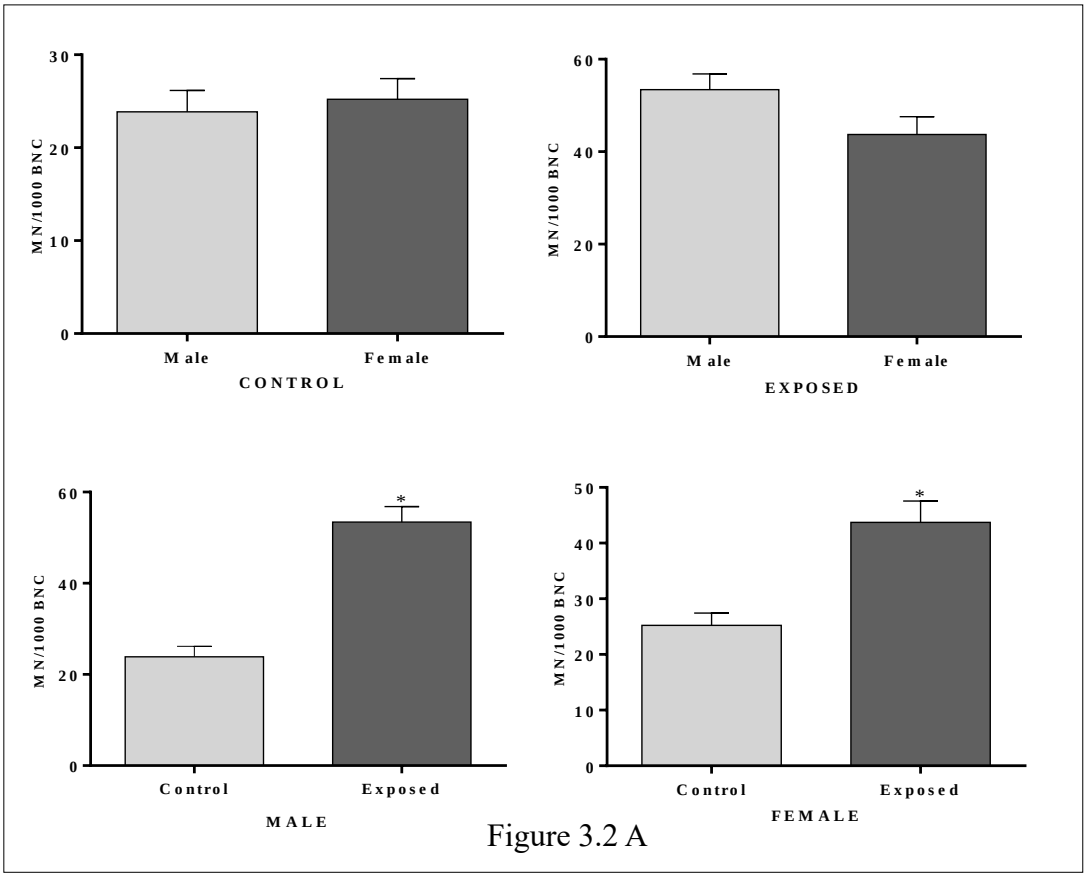


Figure 3.2 A

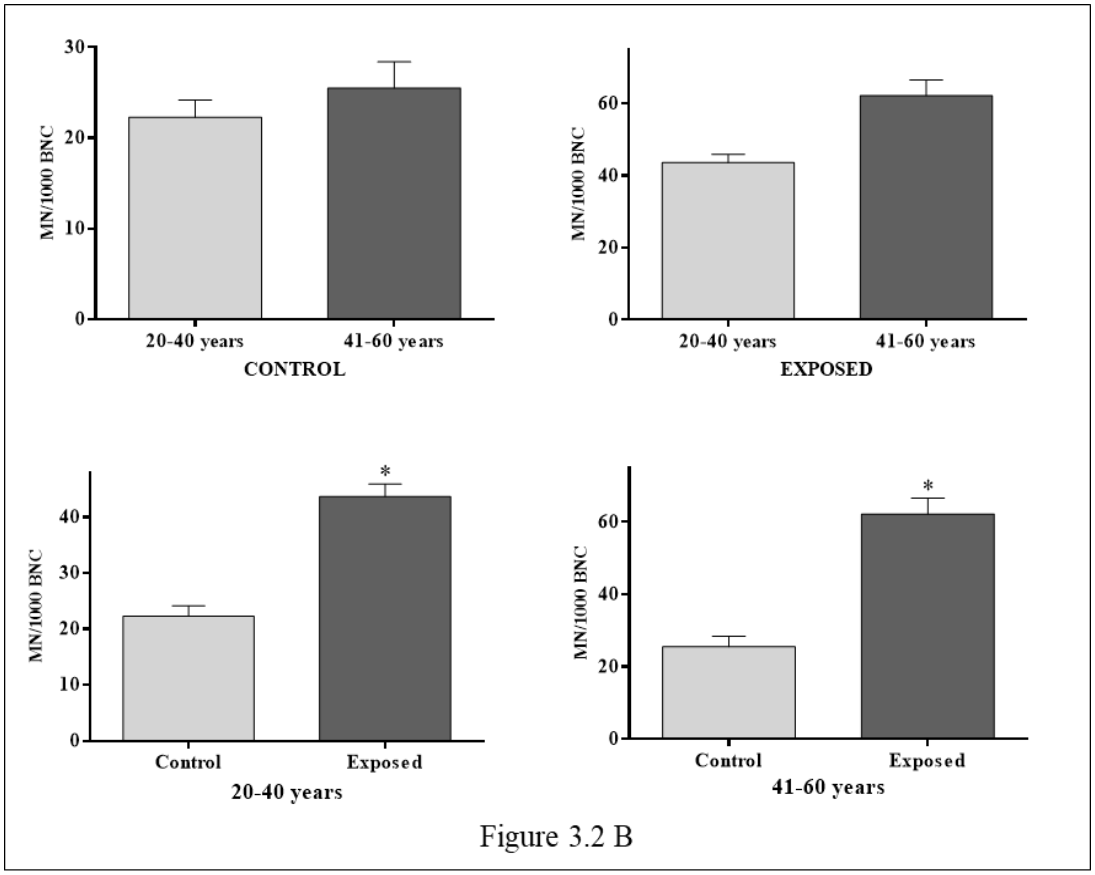


Figure 3.2 B

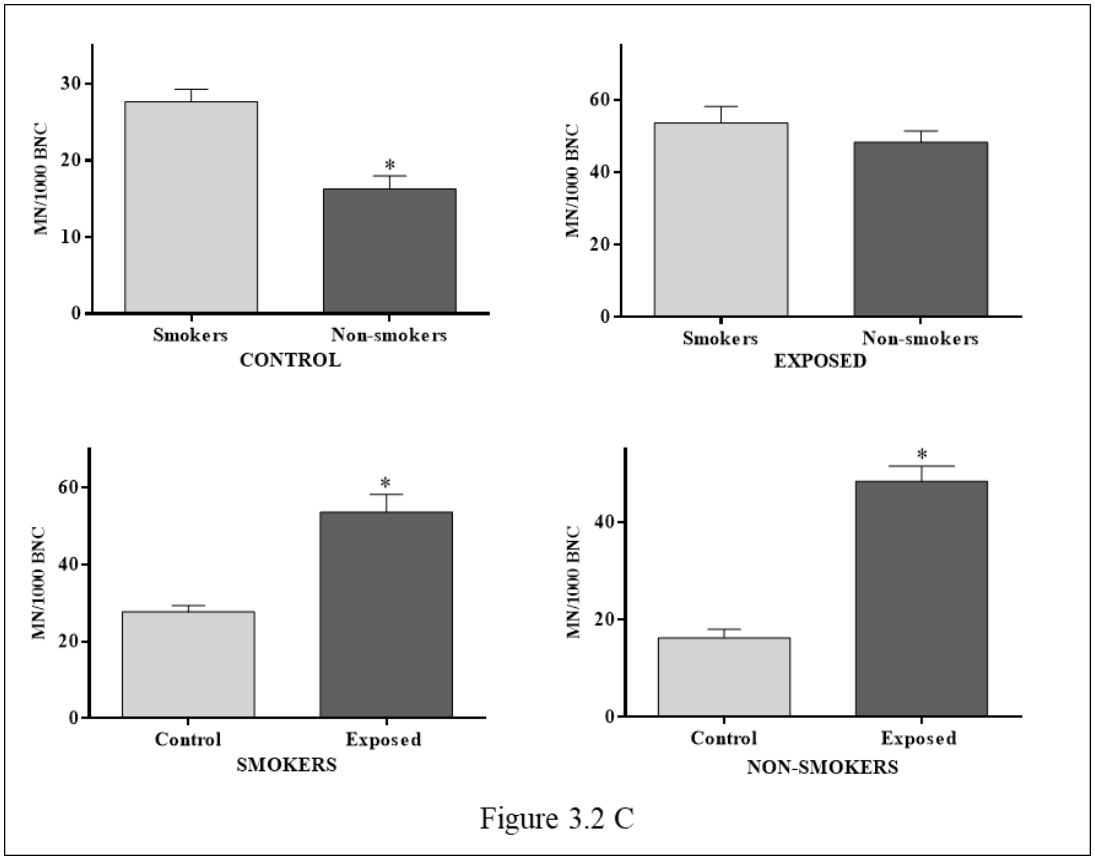


Figure 3.2 C

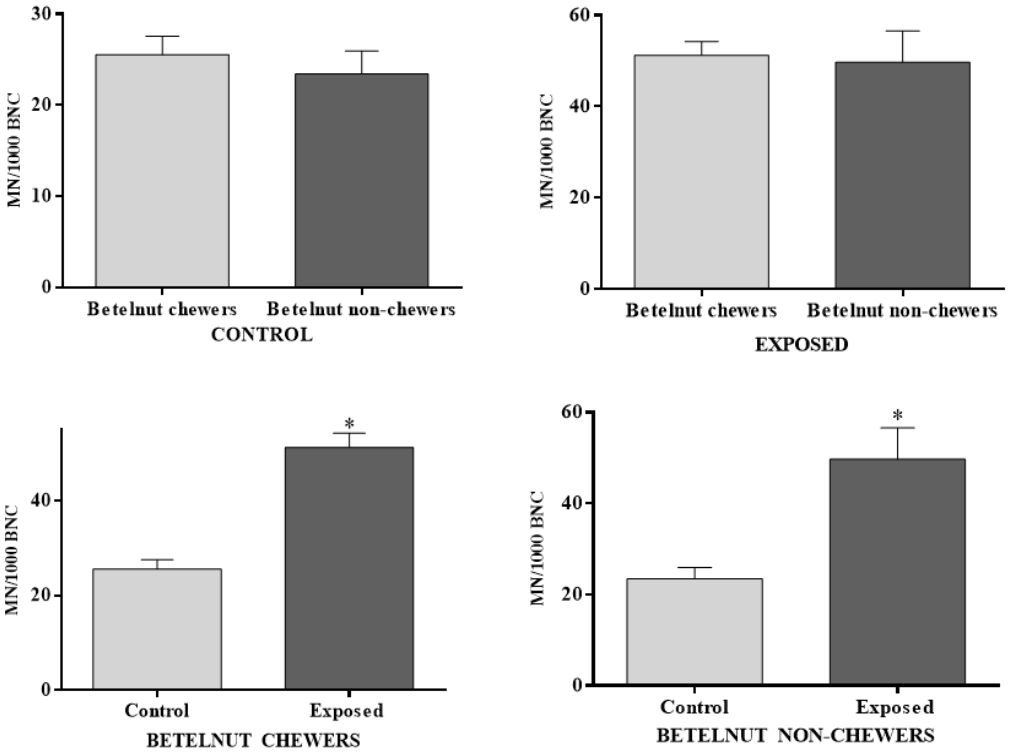


Figure 3.2 D

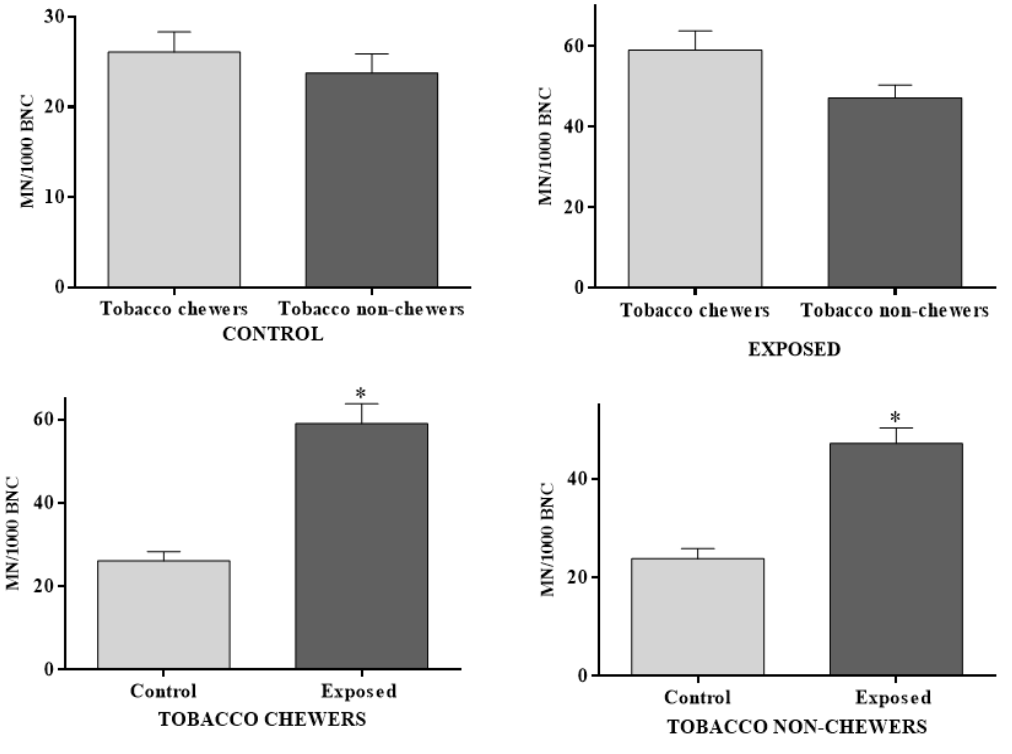


Figure 3.2 E

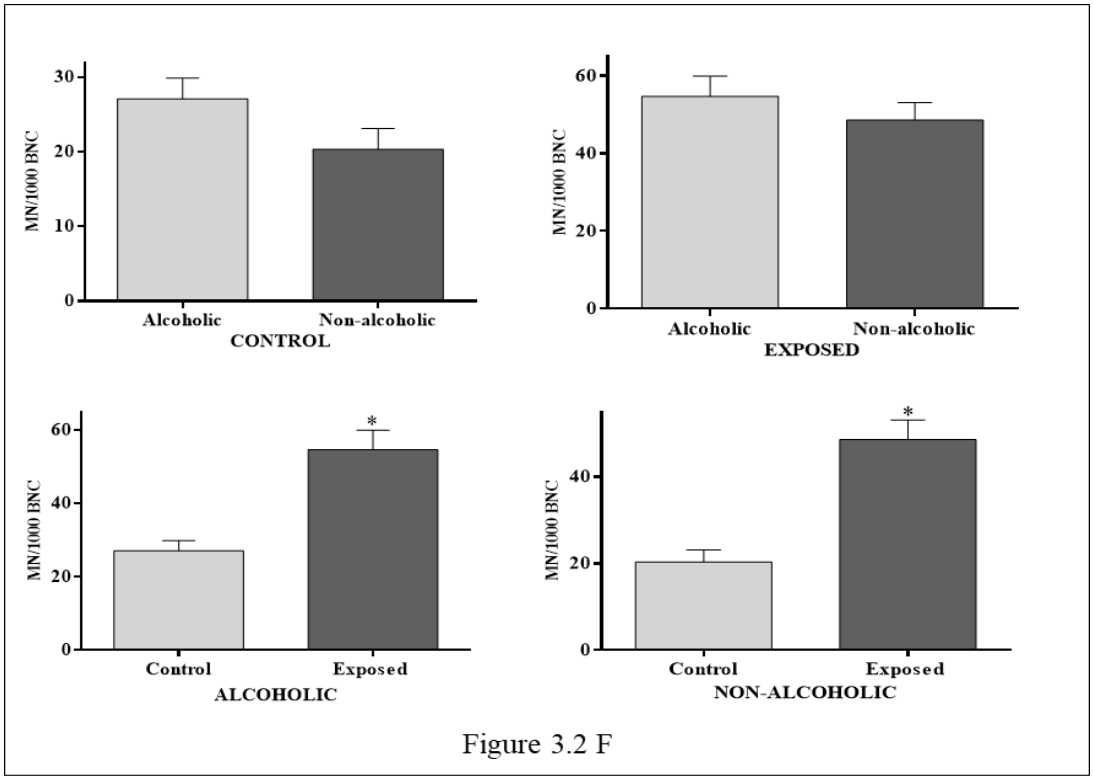


Figure 3.2 F

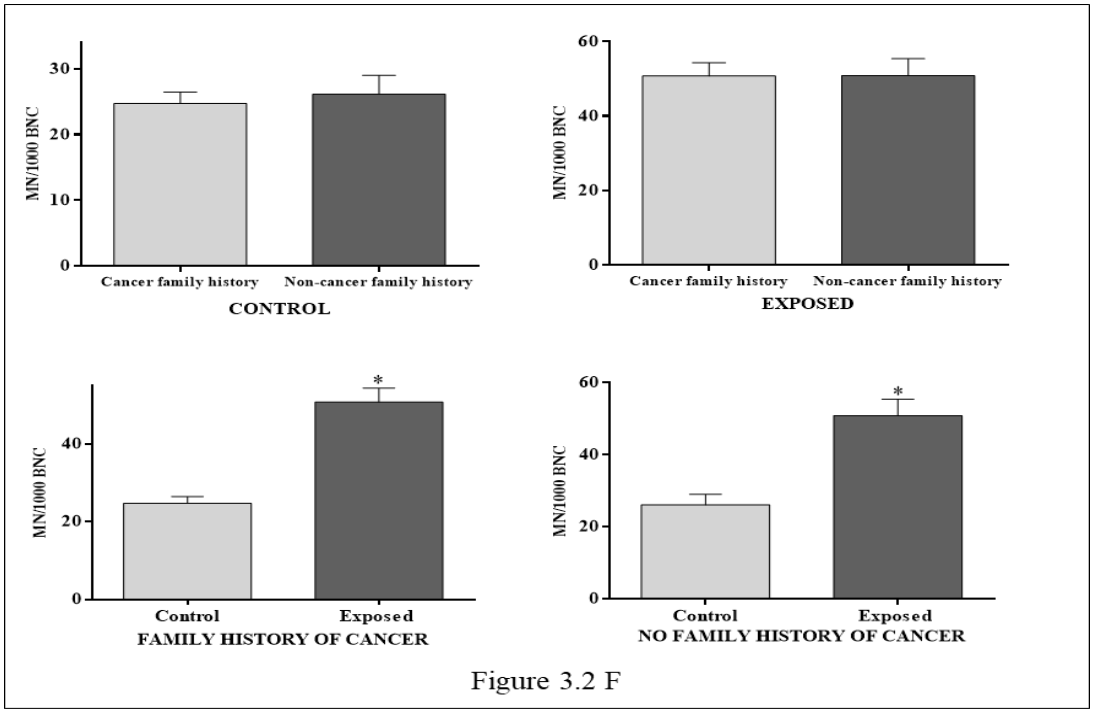


Figure 3.2 F

Figure 3.2: Functions of demographic characteristics on micronuclei frequency in exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. MN: Micronuclei frequency; BNC: Binucleated cells. * *indicates significant variations between the group($p<0.05$).*

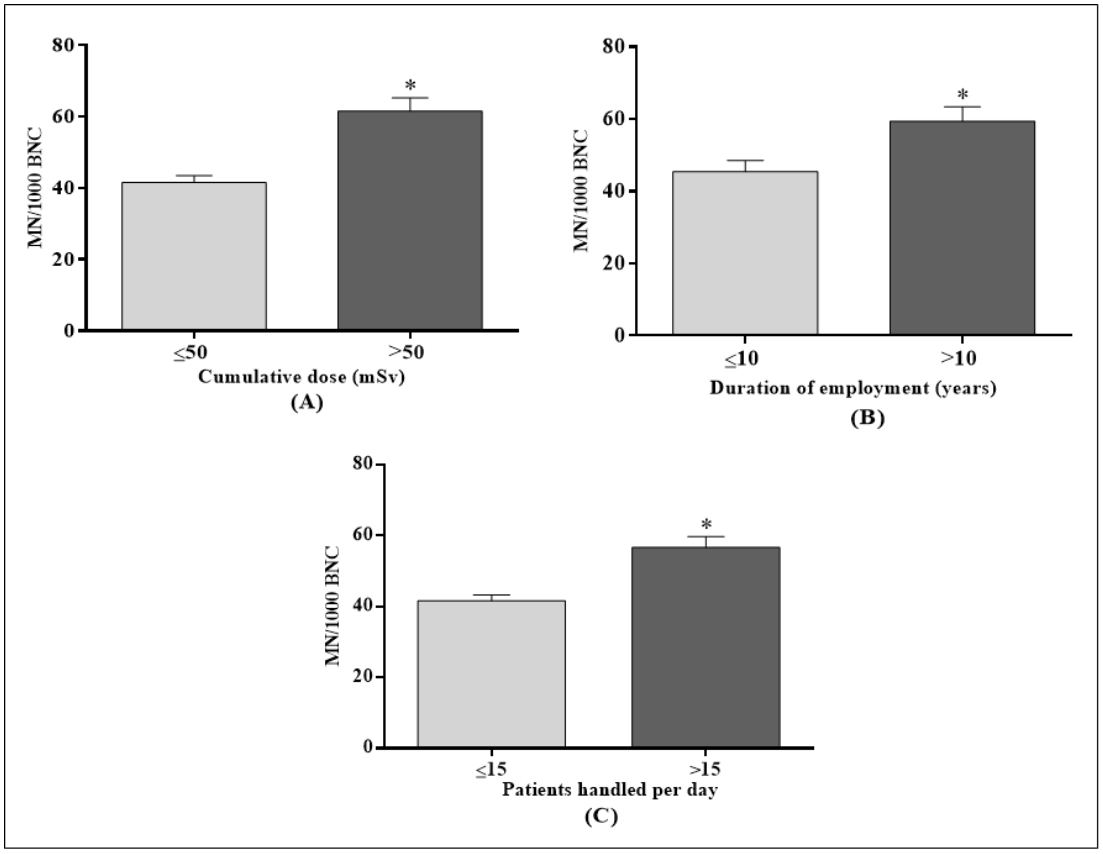


Figure 3.3: Micronuclei frequency among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. Micronuclei frequency; BNC: Binucleated cells.* *indicates significant variations between the group ($p<0.05$).*

Table 4: Influence of demographic characteristics on micronuclei induction in the occupationally exposed and control groups.

Characteristics	Durbin Watson	Beta-value	t-value	p-value
Micronuclei	1.753	-0.08	-0.51	0.61
Gender				
Age		0.29	1.84	**0.04**
Smoking habits		0.10	0.70	0.49
Betelnut chewing		-0.31	-2.24	**0.03**
Tobacco chewing		-0.03	-0.18	0.86
Alcohol consumption		-0.11	-0.69	0.49
Family history of cancer		0.20	1.50	0.14
Time since working (years)		0.42	2.40	**0.02**
No of patients handled per day		0.37	2.15	**0.04**
Cumulative dose (mSv)		1.006	4.622	**0.02**

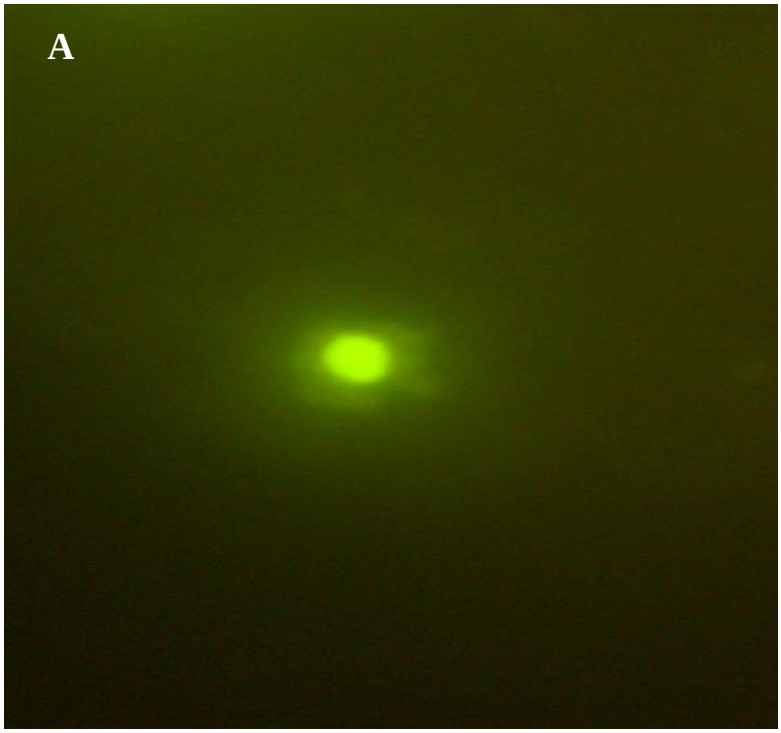
Bold value signify $p<0.05$.

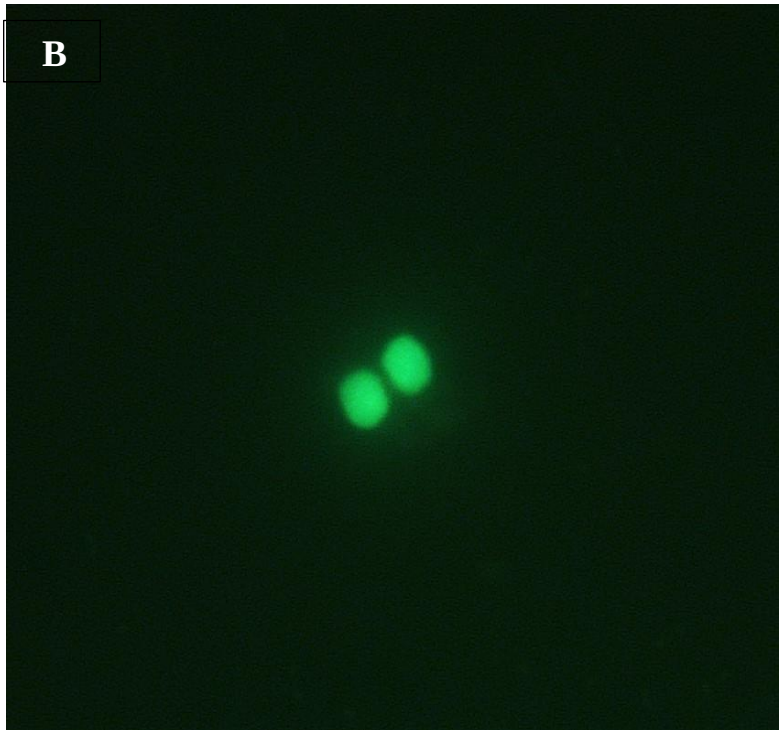
PHOTO PLATES

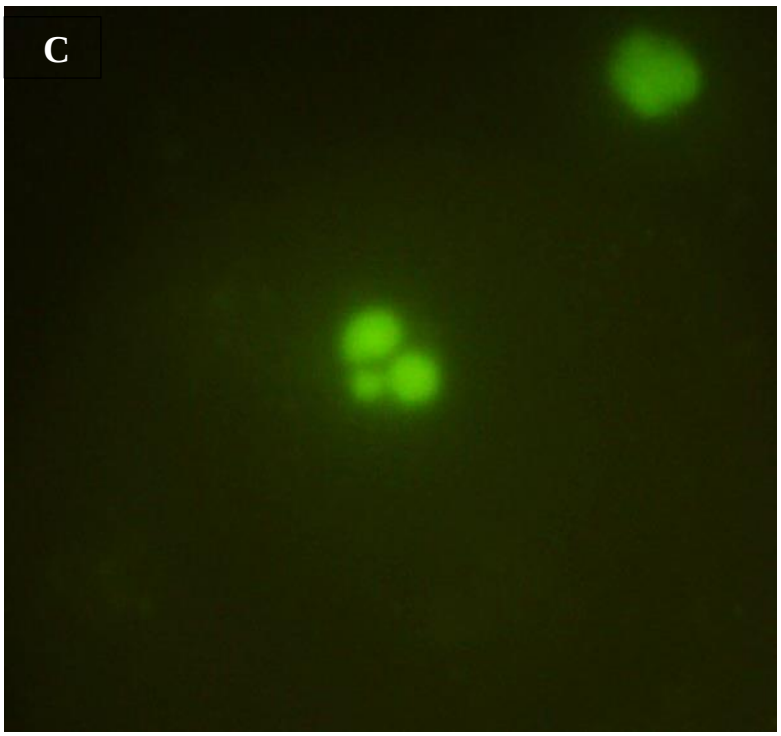
Plate A: Mononucleated lymphocyte.

Plate B: Binucleated lymphocyte.

Plate C: Binucleated lymphocyte with one micronucleus (white arrow).



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4. DISCUSSION

The study of the genotoxic effect of ionizing radiation using MN assay has been a major research area and it has been correlated with cytotoxic effect in various studies (Guo et al., 1998; Ramalho et al., 1998; Jagetia and Venkatesha 2006). It has been recognized for several decades that ionizing radiation can cause chromosome breakage at any stage of mitosis or meiosis. Tissues with a high rate of cell proliferation are more prone to radiation-induced tumor production due to alteration in the fidelity of cellular genome (Elgazzar and Elsaid 2001; Shah et al., 2012; Mavragani et al., 2017). Various occupations are associated with different kinds of hazards depending on the occupation type and working with ionizing radiation is no exception. The individuals working with ionizing radiation in medical radiology may be at a greater disadvantage due to the deleterious and long-term adverse health effects triggered by ionizing radiation including the development of cancer. Furthermore, studies among hospital workers have revealed that exposure to ionizing radiation still presents the hazards of chromosome damage which may subsequently induce cancer (Barquinero et al., 1993; Bonassi et al., 1997; Rozgaj et al., 1999; Sakly et al., 2012; Choi et al., 2013; Wang et al., 2016). We have, therefore, attempted to obtain an insight into the adverse effects of ionizing radiation in the radiology workers of hospitals exposed to X-rays using MN assay in the cultured peripheral blood lymphocytes. The influence of age, gender, and lifestyle including smoking habit, alcohol consumption, and tobacco usage, by radiology workers, has also been determined on MN frequency by multiple linear regression analysis.

MN assay has been used as a quantitative indicator of X-ray induced chromosome damage in several studies, both *in vitro* and *in vivo* (Ramalho et al., 1998; Maffei et al., 2002; Joseph et al., 2004; Ropolo et al., 2012; Koyama et al., 2016). Our results indicate that the mean MNBNC frequency was significantly higher in exposed workers than in controls irrespective of their demographic characteristics. Our findings are in agreement with other studies on occupational workers from China, Korea and Romania population, where a higher MN frequency has been reported after exposure to chronic low doses of ionizing radiation (An and Kim 2002; Mihalache et al., 2007; Sahin et al., 2009; Eken et al., 2010; Zakeri and Hirobe 2010; Sakly et al., 2013; Qian et al., 2016; Gharibdousty et al., 2017; Lusiyanti et al., 2017). The radiological

technicians also showed an increase in chromosome translocations in a US study (Sigurdson et al., 2008). In contrast, some studies did not find any increase in the MN frequency in the exposed group as compared to controls, which may be due to several other confounding factors (Demirel et al., 1997; Thierens et al., 2000).

Our results further indicated a strong association between MNBNC frequency and the duration of working with ionizing radiation (X-rays), number of patients handled by a radiology worker per day and the age of the exposed group. Age as a function of increased MNBNC frequency has been reported in several studies earlier (Fenech and Morley 1985; Migliore et al., 1991; Joseph et al., 2004; Ropolo et al., 2012). Age has been found to increase the frequency of micronuclei in the population chronically exposed to low doses of ionizing radiation (An and Kim 2002). This increase in micronuclei frequency with age of the exposed group can be attributed to the progressive deterioration of the antioxidant and DNA repair systems and resultant attrition of DNA repair with increasing age (Thierens et al., 1996; Fenech et al., 2011). Apart from this fact, the rise in MNBNC frequency may be attributed to prolonged chronic exposure to ionizing radiation as the older subjects may have higher cumulative doses of ionizing radiation due to the longer time spent working with radiation. A similar association between chromosomal aberrations and duration of employment has been reported earlier among hospital workers occupationally exposed to ionizing radiation (Mihalache et al., 2007; Vellingiri et al., 2014). This may be due to chronic exposure resulting in the priming of antioxidant and DNA repair processes leading to reduced DNA damage compared to a single acute exposure, where repair may be overwhelmed due to a sudden burst of oxidative stress. We have observed 30 MN per 1000 BNC among workers who are occupationally exposed to ionizing radiation with a mean cumulative dose of 48.88 mSv, which is similar to the frequency of MN observed in the human peripheral blood lymphocytes (HPBLs) exposed to a single acute dose of 300 mGy. Furthermore, the individual exposed to protracted exposure experience higher oxidative stress due to sustained production of ROS, when compared to a single acute ionizing radiation exposure. The constant oxidative stress due to protracted exposure may have produced greater amount of DNA damage in the occupationally exposed individuals who received almost 1/6 of single dose of 300

mGy in the experimental condition. The low dose protracted exposure has been reported to produce higher damage than the single acute exposure (Beyea 2012).

The present study revealed a higher MNBNC frequency in the males (53.42 ± 3.40) of exposed group than females (43.71 ± 3.87) that was not statistically significant. This is interesting given the effect of gender in radiation sensitivity is ambiguous and there are contrasting earlier reports where females have shown higher number of micronuclei than the males (Fenech et al., 1994; Bonassi et al., 1995). This may be due to various genetic, internal factors such as hormonal effects as well as external factors and epigenetic interactions. For instance, estradiol has been reported to protect against radiation-induced damage in women (Rong et al., 2018). Our results did not show any association between the MNBNC frequency and lifestyles of the participants including alcohol consumption, betel nut chewing and tobacco chewing, as well as the family history of cancer. The frequency of MNBNC was significantly higher among smokers of controls when compared to non-smokers. Certain demographic factors such as gender, diet, smoking, and alcohol consumption have been reported to influence the MN frequency earlier (Fenech et al., 1999; An and Kim 2002). Micronuclei in lymphocytes are caused by breaks in the double-strand DNA and thus considered to be particularly important in biology as their repair is intrinsically more difficult than the other types of DNA lesions (Khanna and Jackson 2001; Medvedeva et al., 2007). The MNBNC frequency in healthy non exposed individuals in the present study was also higher than the range reported in some earlier studies (Demirel et al., 1997; Maffei et al., 2002; Joseph et al., 2004; Ropolo et al., 2012). This may be due to various environmental, lifestyle and dietary factors of Mizos who regularly eat smoked food including pork, consume tobacco, betel nut, and alcohol. Despite this, our results are in conformation with other studies that have reported similar MN frequency in controls (Thierens et al., 2000; Cardoso et al., 2001). The low doses of ionizing radiations have been reported to activate oxidative stress, and NF-κB, which is also activated against DNA damage (Rodrigues-Moreira et al., 2017; Wang et al., 2017; Yim et al., 2017).

CHAPTER VI

ASSESSMENT OF THE ALTERATION IN THE GENE EXPRESSION OF THE OCCUPATIONALLY EXPOSED GROUP USING qPCR TECHNIQUE

ABSTRACT

One of the hazards of working in diagnostic radiology is the possibility of long-term exposure to low-level radiation and any associated biological effects. Increased genomic instability among the occupational X-ray technicians was indicated by our previous study where the DNA damage levels were significantly higher in the exposed group when compared with the controls. Ionizing radiation is known to induce alterations in gene expression that are critical for the cell choice between either resuming normal function or undergoing cell death. Therefore the relative expression of the cell cycle regulatory gene (*GADD45A*), apoptosis regulatory gene (*FDXR*) and genes involved in DNA repairing (*DDB2, POLH* and *C12orf5*) were studied using RT-PCR technique among the occupational X-ray technicians who received a low dose of ionizing radiation on daily basis. Occupational exposure to ionizing radiation results in an up-regulation of the *FDXR* gene, and downregulation of *GADD45A* and *DDB2*. However, no significant differences were observed in the relative expression of *POLH* and *C12orf5* between the exposed and control group. Multiple linear regression analysis showed a significant association between relative expression of *FDXR* gene and cumulative dose. Alterations in gene expression patterns observed in exposed radiation workers reinforce the need for reduction of radiation dose received by a person that causes genetic damage as well as the long-term effects on genomic instability, including cancer.

1. INTRODUCTION

More than 2 million medical workers are exposed to various forms of ionizing radiation every day for health care alone (Linet et al., 2012). According to UNSCEAR, out of 7.35 million workers around the world having an exposure to low doses of ionizing radiations, medical workers represent the largest occupational group accounting for 75% of workers exposed to man-made sources of radiation. The increasing use of ionizing radiation in medical diagnosis and treatment has raised many concerns about its potential long-term impacts on health including genomic instability and cancer (Visweswaran et al., 2019; Basheerudeen et al., 2017; Grosse et al., 2009). Although the acute harmful effects of high doses of ionizing radiation were recognized way back in the 19th century (Vaiserman et al., 2018), the detrimental health effects of radiation at the lower doses remains debatable. Generally, radiation at a cumulated dose of 100 mSv is referred to as low-dose radiation. According to ICRP Publication 26 (ICRP 1977), the recommended dosage limits for routine occupational exposure include a 50 mSv annual effective dose equivalent to limit stochastic effects, 0.3 Sv for the lens of an eye and 0.5 Sv for all other organs and tissues for the protection of tissue responses. Monitoring of occupational radiation workers consists of regular dosimetry checks of the film and periodic medical examinations.

Various cytogenetic studies clearly demonstrated the damaging effects of chronic low doses of irradiation in the chromosome (Hall et al., 2017). Although estimation of absorbed radiation dose for the occupationally exposed group is possible, is it difficult to evaluate the extent of potential long-term health effects for occupationally exposed individuals. Thus, the welfare of this exposed group became a great concern. Therefore, it is necessary to develop new strategies for the reliable assessment of radiation exposure among individuals who are occupationally exposed to various forms of ionizing radiation. Due to some of the serious shortcomings posed by physical and chemical dosimetry (Al-Mohammed et al., 2010), bio-dosimetry has become one of the best methods used in the evaluation of radiation exposure dose in various situations including radiation protection and bio-effects research of radiation. Ionizing radiation induces large-scale changes in gene expression that are critical for the cell choice between either resuming normal function or undergoing cell death

(Jackson 2002; Shiloh 2003) and analysis of gene expression modulation could provide new insights into the complex nature of radiation stress responses. Gene expression is highly sensitive to certain environmental factors and analysis has emerged as a fast and accurate method for the assessment of ionizing radiation exposure to common sources including occupational exposure for dosimetry purposes (O'Brien et al., 2018; Cruz-Garcia et al., 2018; Manning et al., 2017).

Analysis of gene expression profiling demonstrated an up-regulation of genes involved in cell cycle control and DNA repair in response to ionizing radiation exposure in different mammalian cell types (Amundson et al., 2001; Chaudhary et al., 2003; Heinloth et al., 2003; Momota et al., 2003; Sakamoto-Hojo et al., 2003; Yin et al., 2003). Although many of the existing studies revealed altered expression of genes in response to ionizing radiation in a variety of cell systems at relatively high radiation doses, there still remains considerable uncertainty about the impact of chronic low doses of ionizing radiation on gene expression profiles in human populations. In the present study, the relative expression of five DNA damage response genes was screened as potential molecular biomarkers for the exposure doses in the peripheral blood lymphocytes of individuals occupationally exposed to low doses of ionizing radiations using qPCR technique. Our study demonstrates the altered expression of the cell cycle regulatory gene (*GADD45A*), apoptosis regulatory gene (*FDXR*) and genes involved in DNA repairing (*DDB2, POLH* and *C12orf5*).

2. MATERIALS AND METHODS

2.1. Selection of subjects

The volunteers were divided into two groups as described in Chapter 2.

***2.1.1. Exposed group*:** The group comprised of 40 healthy individuals that are occupationally exposed to X-rays for medical diagnostic purposes, working in different hospitals in Aizawl, Mizoram.

***2.1.2. Control group*:** The group comprised of 40 healthy individuals matched for various demographic data, residing in the same urban area. The volunteers did not have any work-related exposure to ionizing radiation or any known genotoxic agent.

After identifying suitable volunteers for the study, each individual were asked to sign a consent form and questionnaire as described in Chapter 2.

2.2. Blood sample collection and lymphocytes isolation

Blood samples were collected from each volunteer of both the groups by venepuncture and are stored in individual sterile heparinized tubes. Approximately, 7 ml of whole blood was transferred into a polypropylene centrifuge tube and are mixed with RBC lysis buffer after which lymphocytes was isolated by centrifugation.

2.3. RNA extraction, cDNA synthesis

Total RNA was extracted using Tri reagent solution (BR Biochem, Life Science Pvt. Ltd, R1022) as per manufacturers protocol. Extracted RNA was quantified using Nanodrop Spectrophotometer (Eppendorf Biophotometer Plus, Hamburg, Germany) and RQ1 DNase kit (Promega, M198A, Madison, WI, USA) was used to remove the genomic contamination. cDNA was synthesized from 2 µg of total RNA using first-strand cDNA synthesis kit (Thermoscientific, K1621; Lithuania, Europe).

2.4. Gene expression analysis using qRT-PCR

Gene-specific primers for DNA repairing genes were designed using Primer 3, Boston, MA, USA. The primer sequences used in qRT-PCR analyses were mentioned in Table 6.1. qPCR was performed using Quant-Studio 5 (ThermoFisher Scientific, Foster City, CA, USA). PCR reaction volume of 7 µl for each gene comprised of 1 µl each of cDNA, gene-specific forward and reverse primers, 3 µl PowerUp™ SYBR™ Green

Master Mix (Thermo Fisher Scientific, A25742, Lithuania, Europe) and 1 µl of nuclease-free water (ThermoFisher Scientific, A19938, Bangalore, India). The cycling condition of qPCR were 1 cycle at 95°C (20 sec), 35 cycles at 95°C (01 sec), 60°C (20 sec) and 95°C (01 sec), additional melt curve plot step included 1 cycle of 60°C (20 s) and 1 cycle of 95°C (01 sec). Melting curves were generated to confirm a single uniform peak. *HPRT* gene was used as a reference gene for determining the relative expression levels of specific target genes. Each sample was run in duplicate along with non-template and negative controls. The relative expression of genes was determined using ΔΔCt method (Livak and Schmittgen 2001).

Table 5: Gene specific primers used for qPCR analysis

Gene	**Forward primer (5' → 3')**	**Reverse primer (5' → 3')**
DDB2	TGAACATGGACGGCAAAGA	TGGCCAGGAACCAATCAC
FDXR	GCTTCTGCCACCATTTCTCC	CTTTAGCAGGTGTTGGGCC
POLH	GGGAAGCCAGTGTTGAAGT	CTTGTACAGCACTGGTCAGAT
GADD45A	TGCTCAGCAAAGCCCTGAGT	GCTTGGCCGCTTCGTACA
C12orf5	AAAGAAGCGGATCAAAAAGAACA	CTGCCAAAGAAGTTTCCAGACA
HPRT1	TCAGGCAGTATAATCCAAAGATGGT	AGTCTGGCTTATATCCAACACTTCG

2.5. Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). Student "t" test was used for comparison of demographic variables between the exposed and control groups. Student "t" test was performed to determine the statistical significance to compare gene expression between the groups. Multiple linear regression analyses were carried out for the prediction of relative expression of radiosensitive genes with the different demographic characteristics. Statistical analyses were conducted using Graph Pad Prism ver. 6.0 and SPSS Ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

The relative expression of the cell cycle regulatory gene (*GADD45A*), apoptosis regulatory gene (*FDXR*) and genes involved in DNA repairing (*DDB2, POLH* and *C12orf5*) were studied using RT-PCR technique. Occupational exposure to ionizing radiation results in an up-regulation of the *FDXR* gene, and downregulation of *GADD45A* and *DDB2* when compared to control group irrespective of their demographic characteristics (Figure 4.1). However, no significant differences were observed in the relative expression of *POLH* and *C12orf5* between the exposed and control group (Figure 4.1).

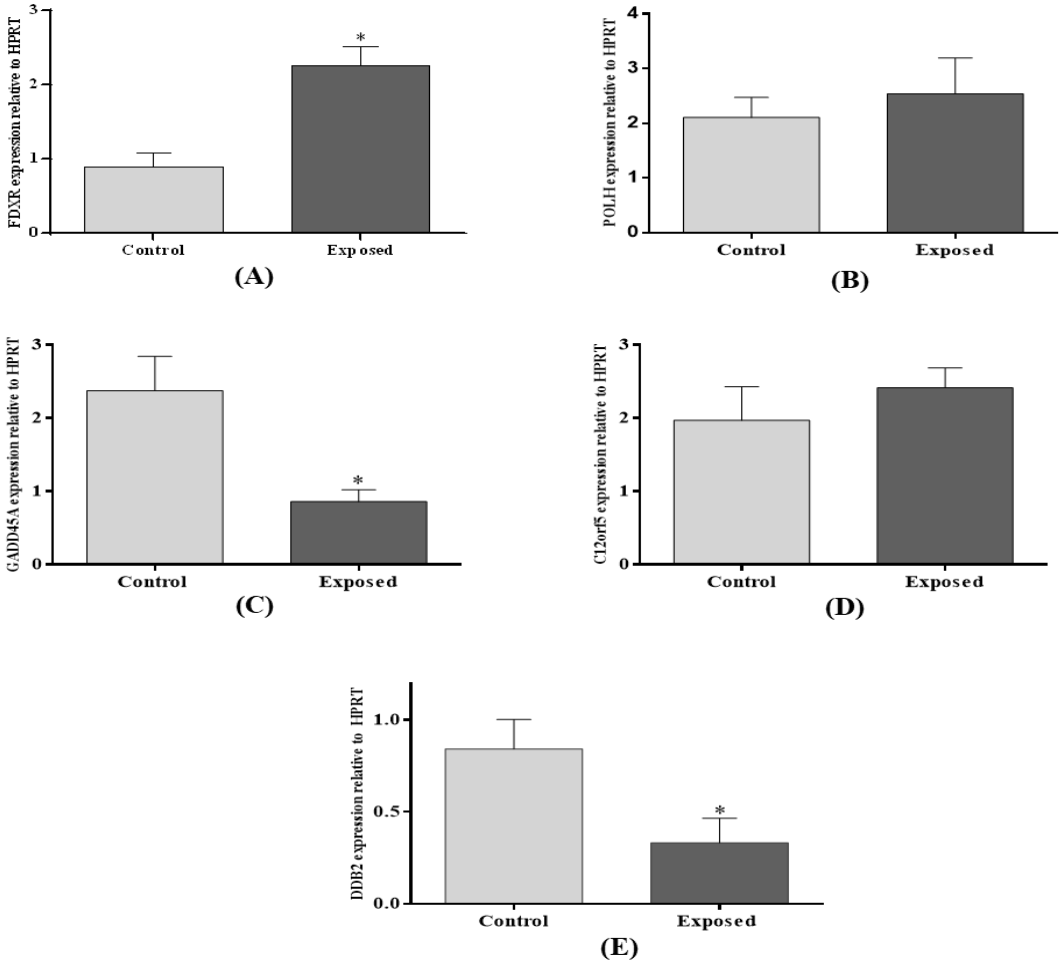


Figure 4.1: Overall comparison of the relative expression of radio-sensitive genes in the exposed and control groups. (A) Ferredoxin reductase (*FDXR*); (B) DNA polymerase eta (*POLH*); (C) Growth arrest and DNA-damage inducible alpha *(GADD45A*); (D) Chromosome 12 open reading frame 5 *(C12orf5*); (E) DNA damage binding protein *(DDB2*). * *indicates significant variations between the groups (p<0.05).*

3.1. Effects of demographic characteristics in the relative expression of Ferredoxin reductase (*FDXR)*among the exposed and control groups:

Gender-wise analysis revealed up-regulation of *FDXR* genes among the males of the exposed group when compared to the males of the control group. However, no significant variation was observed in the relative expression of *FDXR* between males and females of the exposed and control groups (Figure 4.2 A). The expression level of *FDXR* was not differed between younger (20-40 yrs) and elder (41-60 yrs) members within the control and exposed group, but both the younger and elder members of the exposed group showed significantly higher level of *FDXR* expression when compared to the control group (Figure 4.2 B). Non-smokers of the exposed group showed higher level of *FDXR* expression than non-smokers of the control group (Figure 4.2 C). Betelnut chewing also led to an up-regulation of *FDXR* gene expression among the exposed group. Similarly, betelnut chewers and non-chewers of the exposed group showed higher *FDXR* gene expression when compared with the control group (Figure 4.2 D). Occupational exposure to IR also results in up-regulation of *FDXR* gene expression regardless of their tobacco chewing habits (Figure 4.2 E). Among the non-alcoholic volunteers, individuals who are occupationally exposed to IR showed higher level of *FDXR* gene expression. However, alcohol consumption among the exposed and control groups was not associated with the relative expression of *FDXR* (Figure 4.2 F). Family history of cancer among the exposed and control groups did not show significant variation in the relative expression of *FDXR* gene (Figure 4.2 G).

Among the occupationally exposed group, technicians who have cumulative effective dose of >50 mSv and those who have been working with X-rays for >10 years showed higher level of *FDXR* gene expression than those workers who have cumulative effective dose of ≤50 mSv and handled X-rays for ≤10 years during their employment. However, workers handling >15 patients per day did not show significant variation in the *FDXR* relative expression when compared with workers handling ≤15 patients per day (Figure 4.3). Multiple linear regression analysis showed a significant association between relative expression of *FDXR* gene and cumulative dose (Table 6).

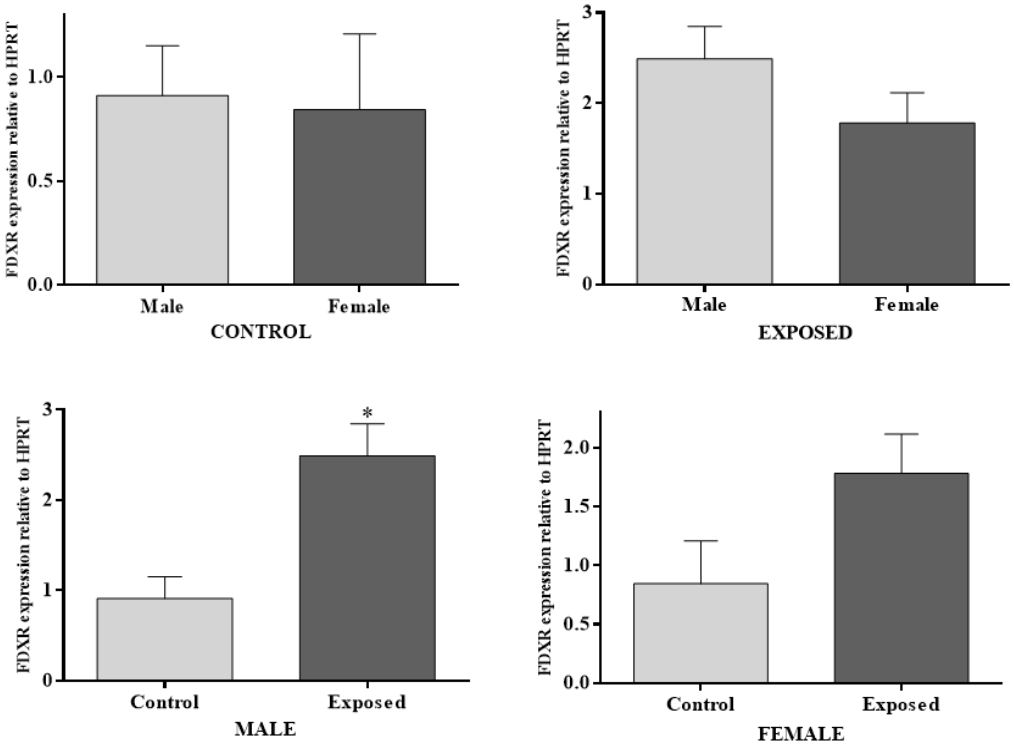


Figure 4.2 A

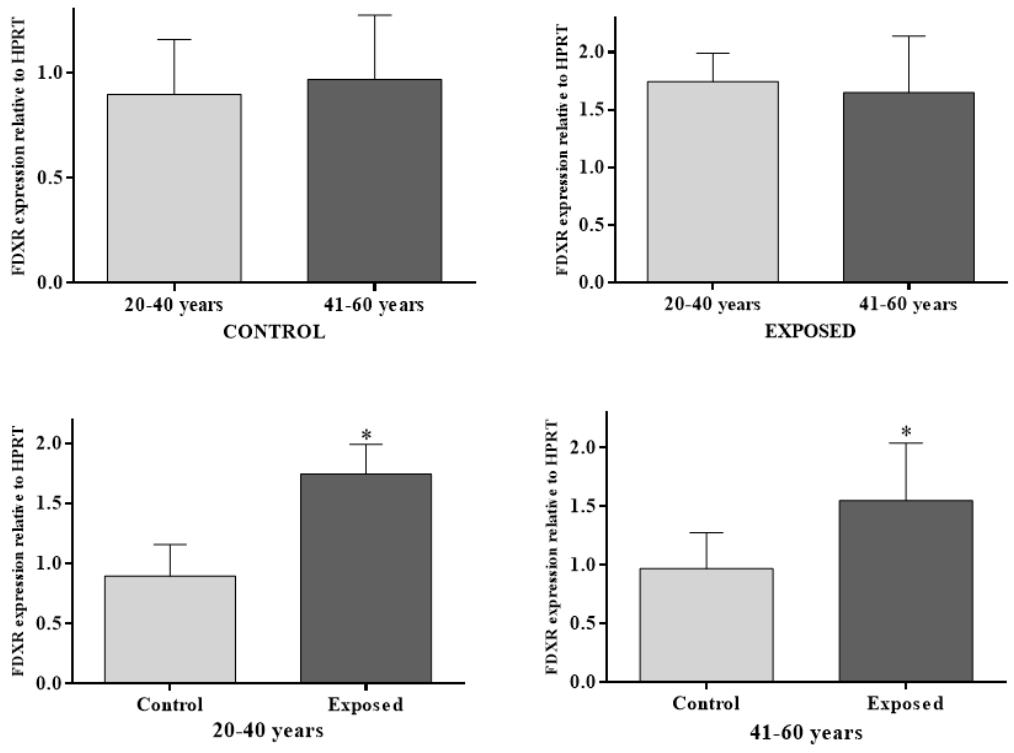


Figure 4.2 B

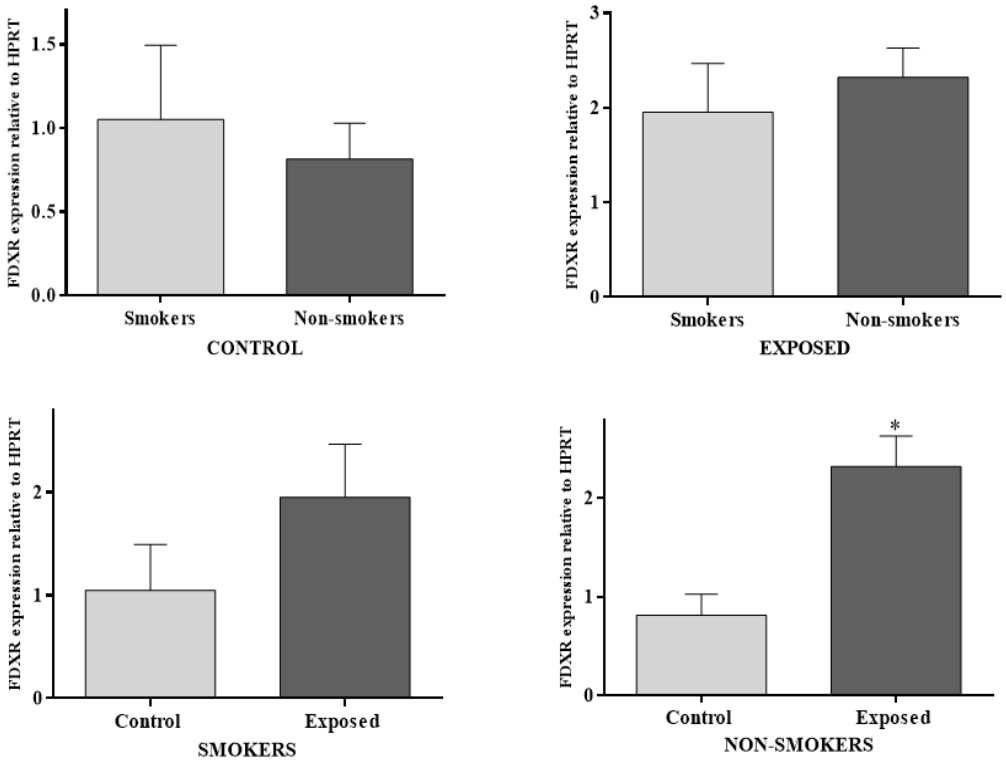


Figure 4.2 C

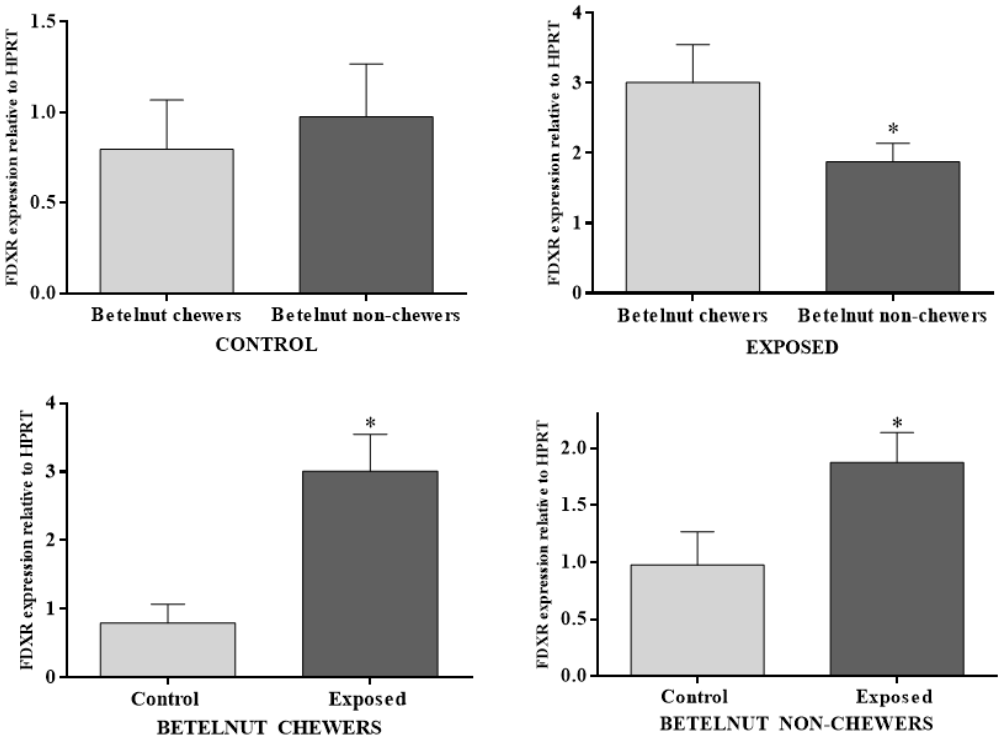


Figure 4.2 D

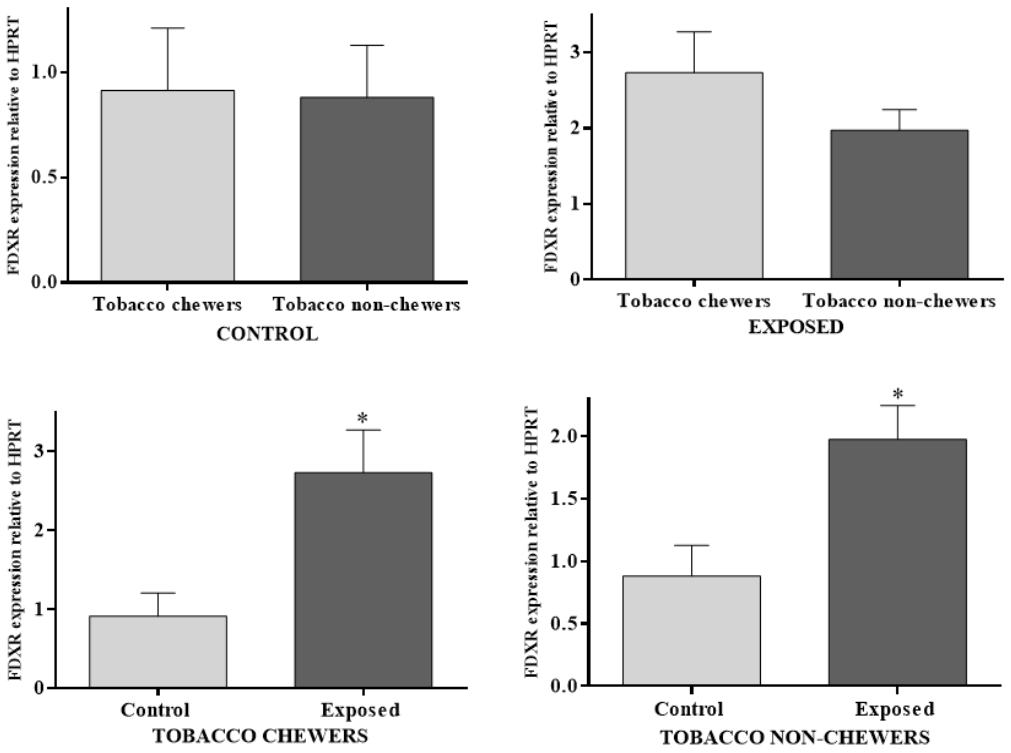


Figure 4.2 E

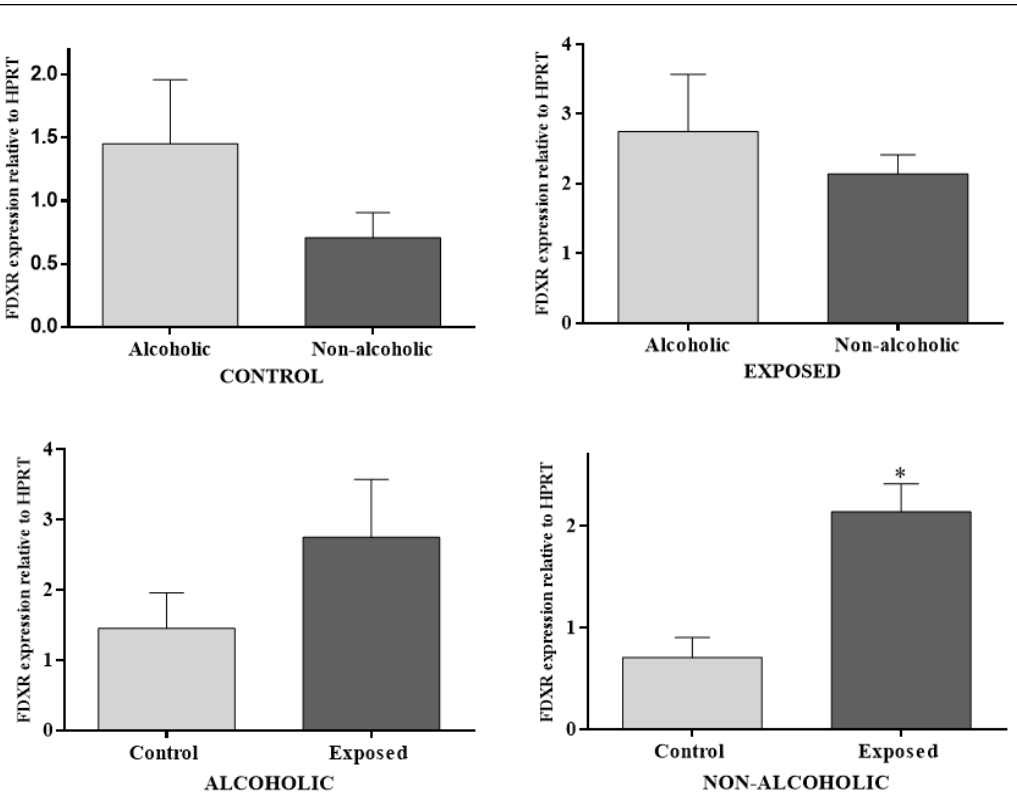


Figure 4.2 F

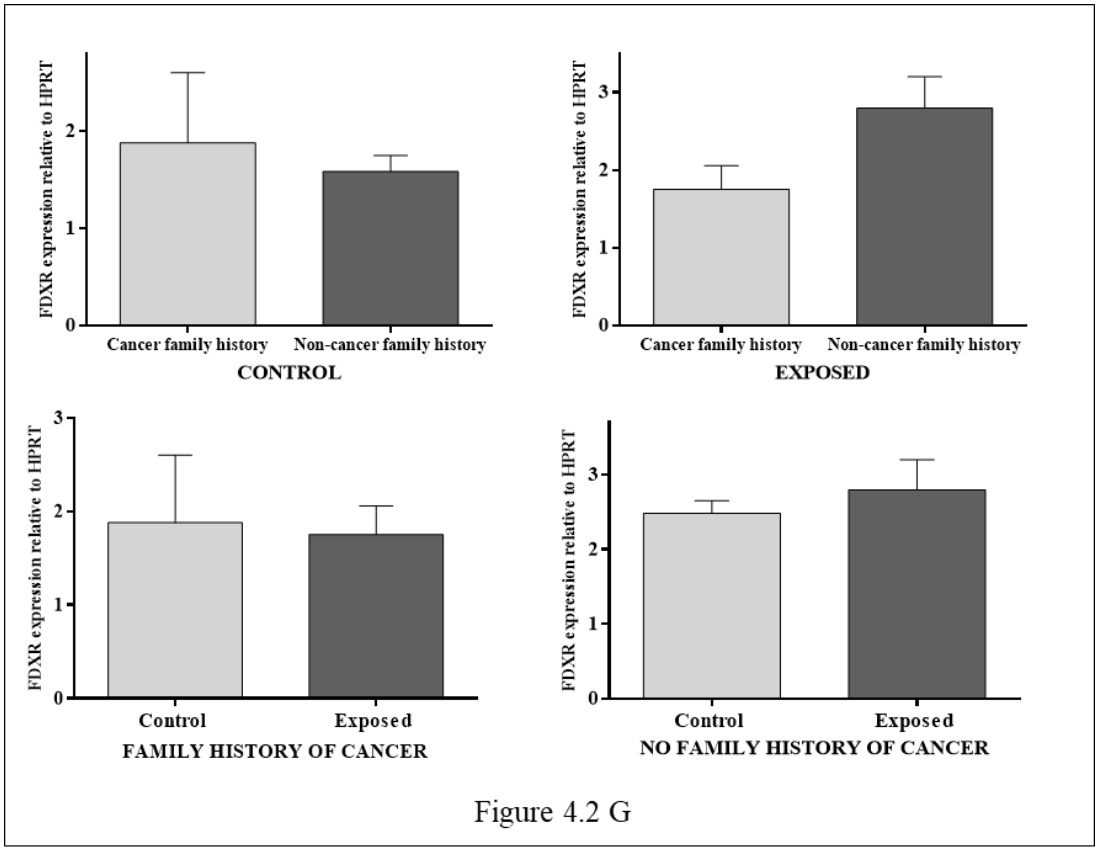


Figure 4.2: Functions of the demographic characteristics on the relative expression of Ferredoxin reductase (*FDXR*) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. **indicates significant variations between the groups ($p<0.05$).*

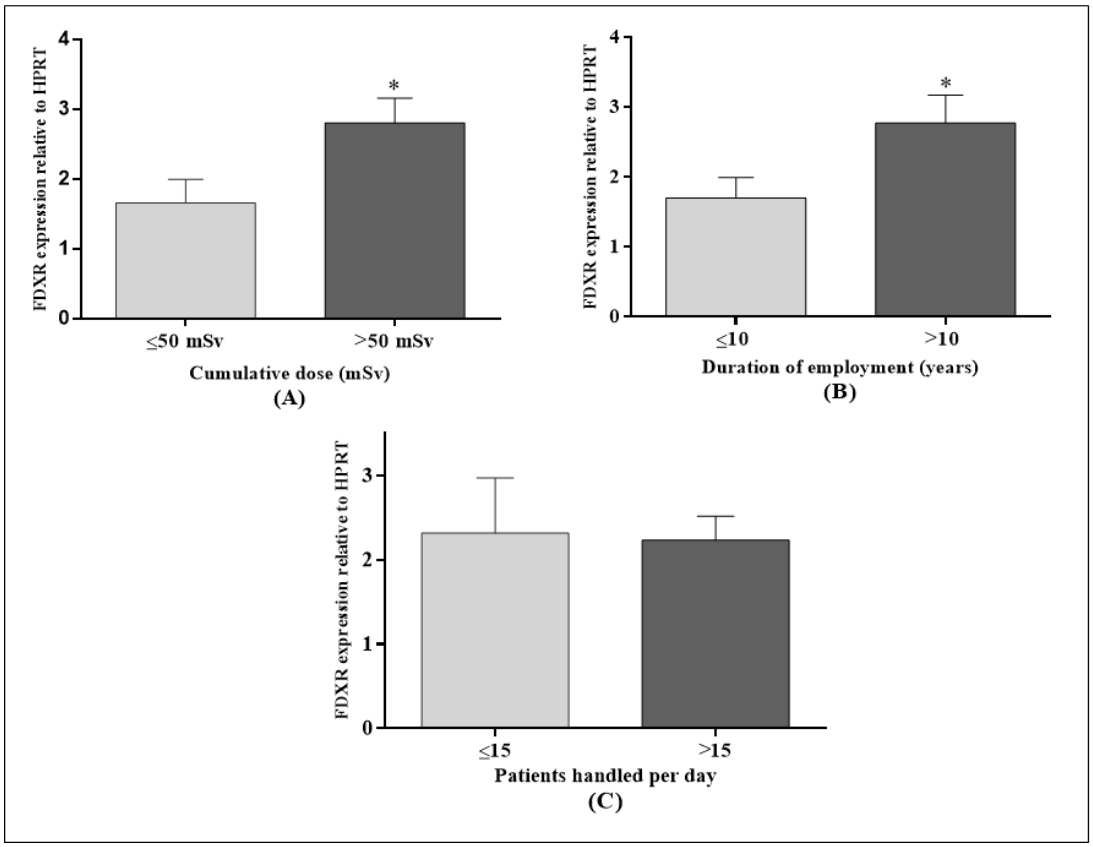


Figure 4.3: Functions of the demographic characteristics on the relative expression of Ferredoxin reductase (*FDXR*) among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day.**indicates significant variations between the groups($p<0.05$).*

3.2. Effects of demographic characteristics in the relative expression of DNA polymerase eta (POLH) among the exposed and control groups:

Among the control group, males showed relatively higher level of *POLH* expression than the females, but no gender bias was observed in the relative expression of the gene among the exposed group. Females of the exposed group showed higher expression of *POLH* but no variation was observed amongst the males of control and exposed groups (Figure 4.4 A). No significant association was observed between relative expression of *POLH* and ages in both the control and exposed groups (Figure 4.4 B). Smokers of the exposed group also showed significantly higher *POLH* expression than the smokers of the control group, but no association was found between smoking habit and relative expression of *POLH* among the control group (Figure 4.4 C). Alcohol user in the

exposed group also exhibited significantly higher level of *POLH* expression than the alcohol user of the control group (Figure 4.4 F). Betelnut chewing, tobacco chewing and family history of cancer did not show significant association with the relative expression of *POLH* gene in both control and exposed groups (Figure 4.4 D, E & G).

Among the occupationally exposed group, technicians who have cumulative effective dose of >50 mSv showed significantly higher level of *POLH* gene expression than those workers having an cumulative effective dose of ≤50 mSv. However, duration of employment and number of patients handled per day did not show significant effect on the relative expression of *POLH* gene (Figure 4.5).

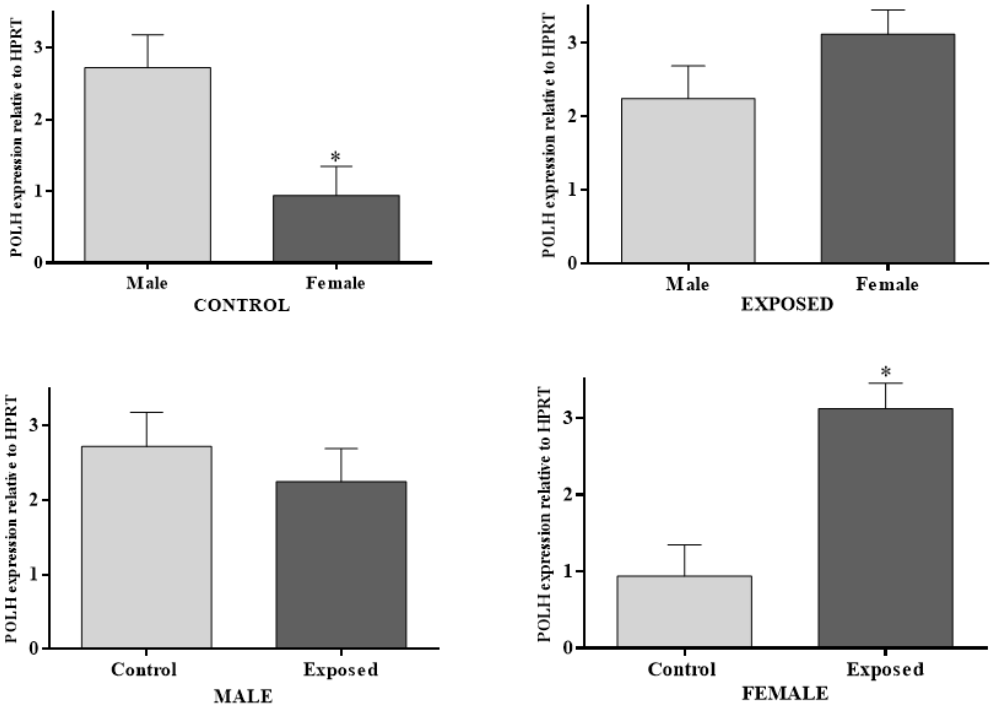


Figure 4.4 A

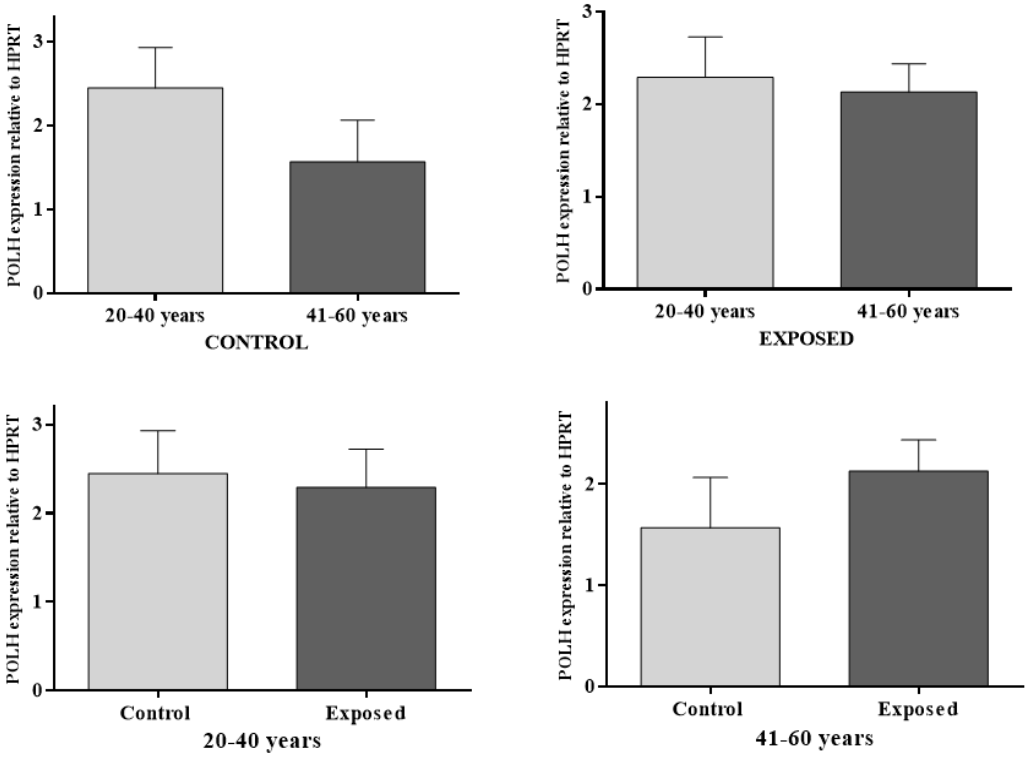


Figure 4.4 B

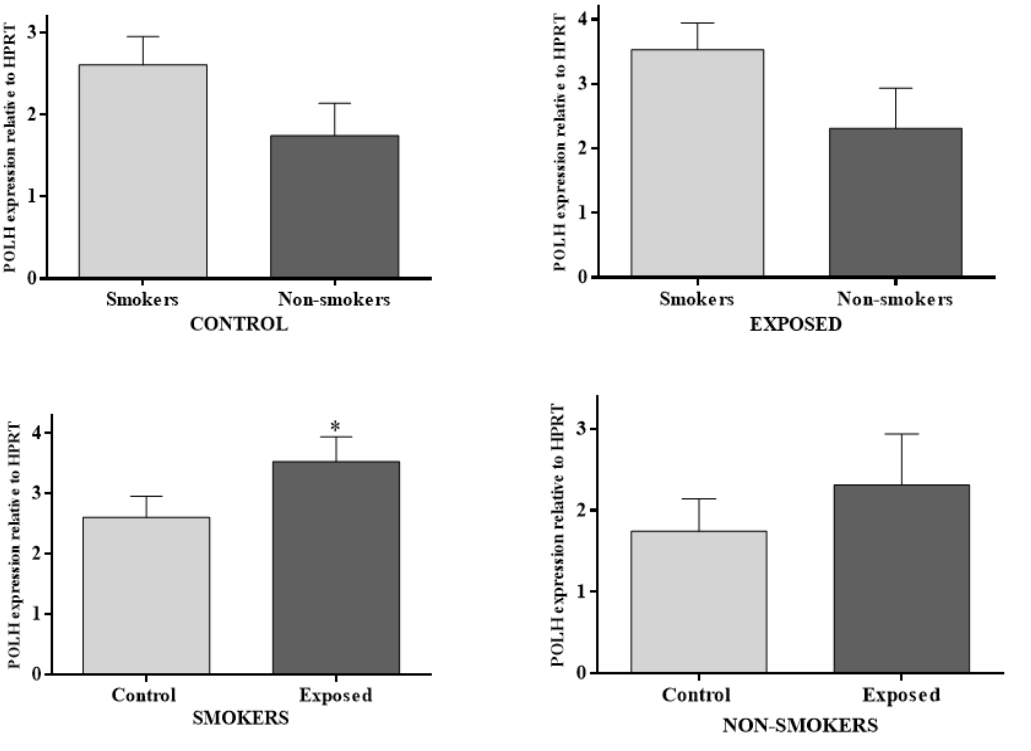


Figure 4.4 C

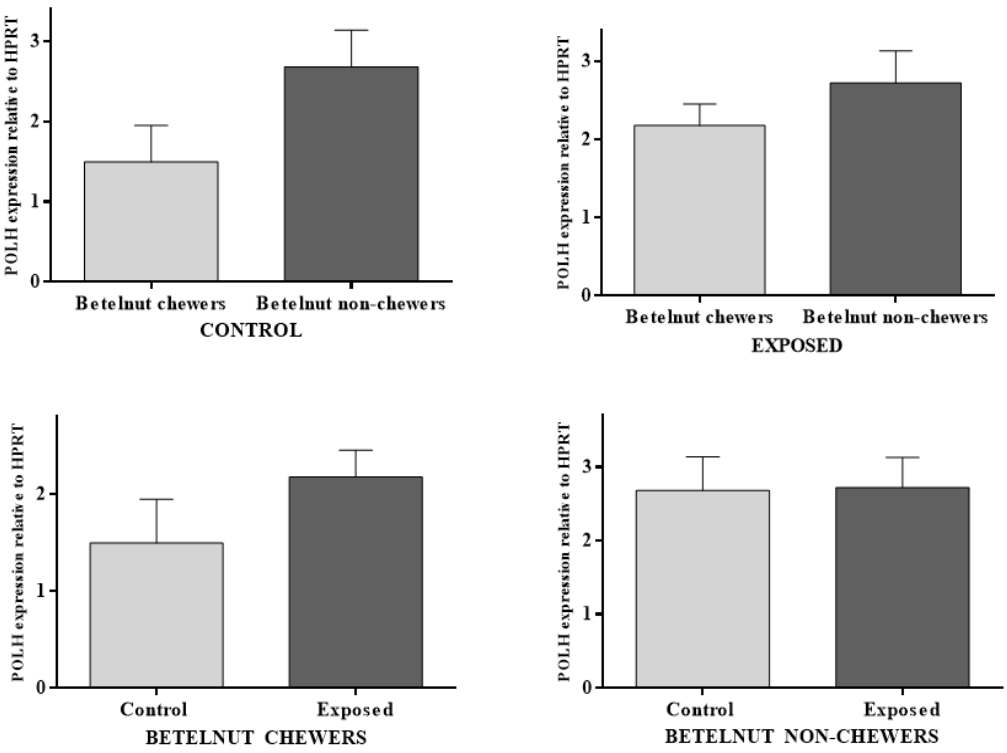


Figure 4.4 D

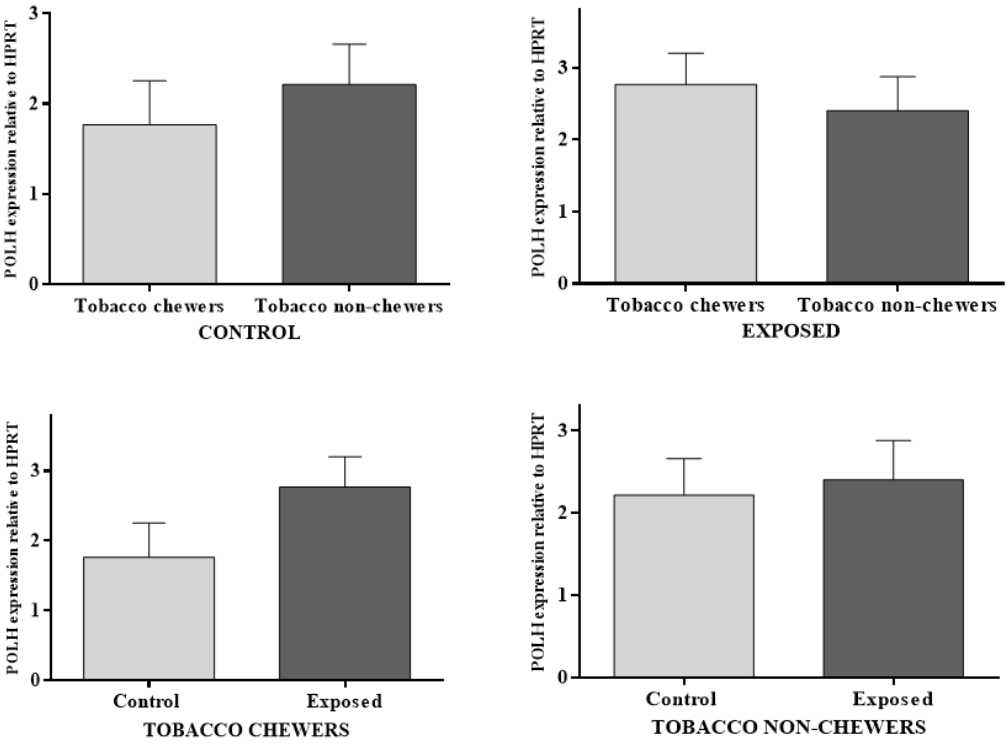


Figure 4.4 E

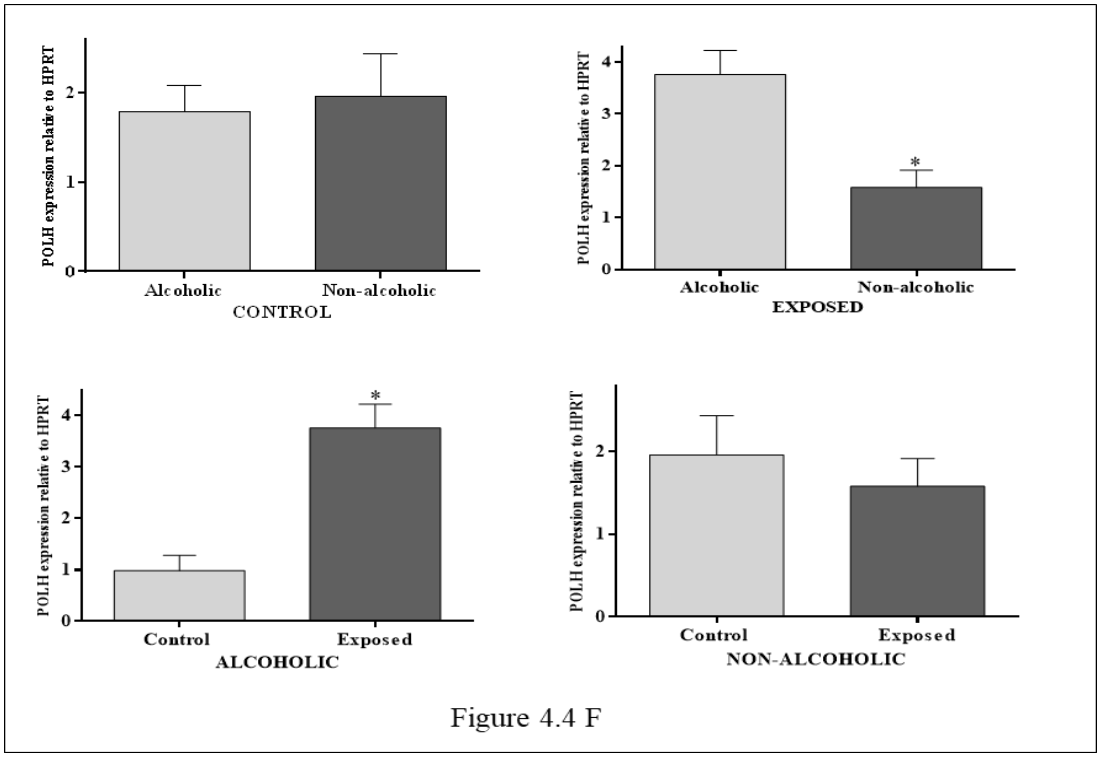


Figure 4.4 F

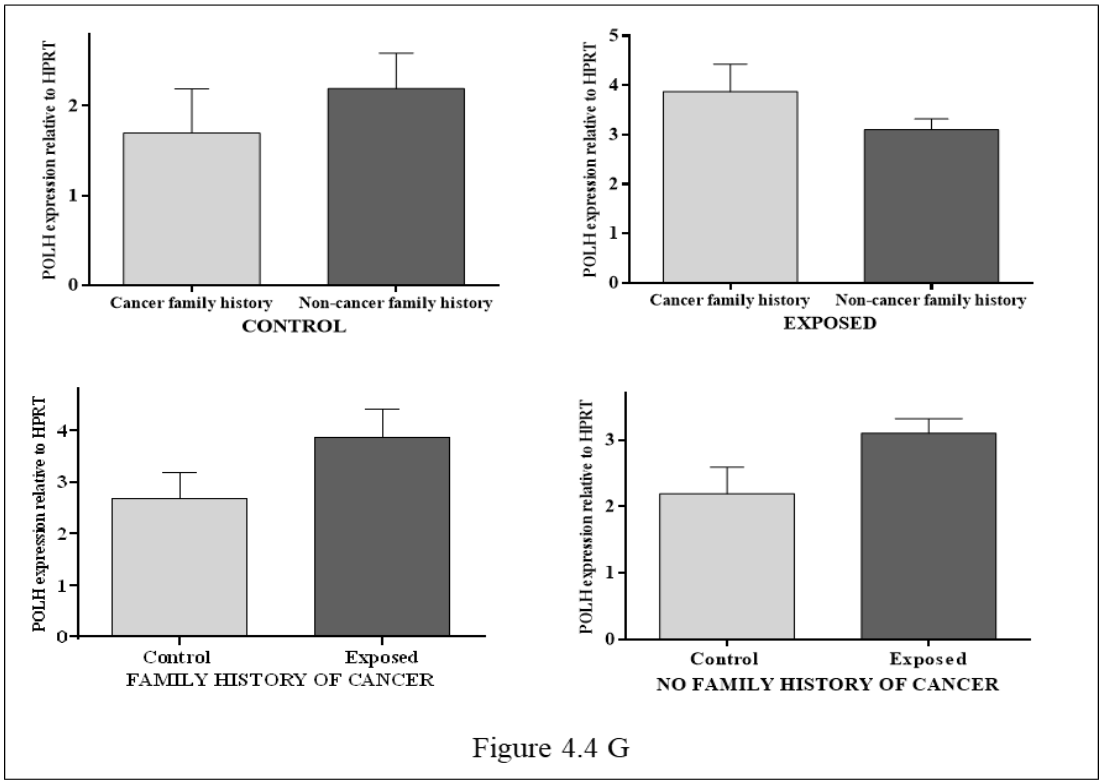


Figure 4.4 G

Figure 4.4: Functions of the demographic characteristics on the relative expression of DNA polymerase eta (*POLH*) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the groups ($p<0.05$).*

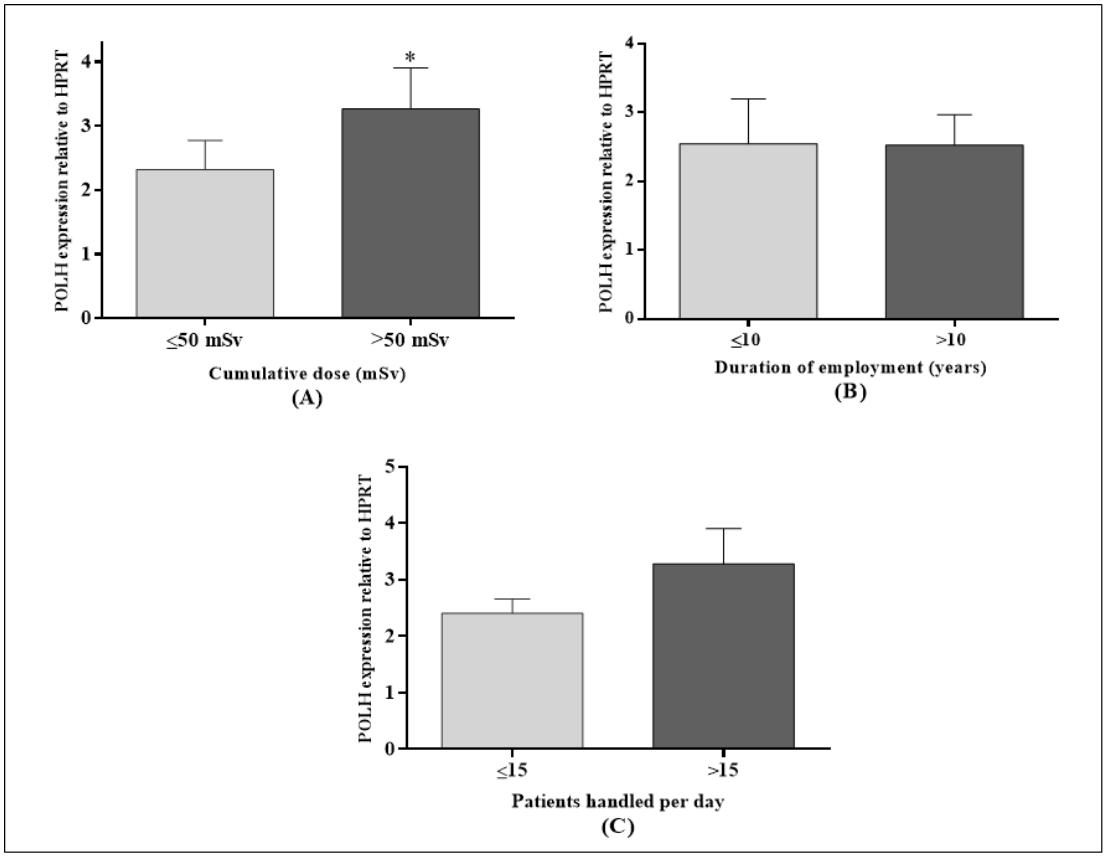


Figure 4.5: Functions of the demographic characteristics on the relative expression of DNA polymerase eta (*POLH*) among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day.* *indicates significant variations between the groups($p<0.05$).*

3.3. Effects of demographic characteristics in the relative expression of Growth arrest and DNA-damage inducible alpha (*GADD45A*) among the exposed and control groups:

The relative expression of *GADD45A* gene was downregulated in males of exposed group when compared with females of exposed group. Also, males and females of exposed group showed significantly lower level of *GADD45A* expression when compared with the respective gender in the control group (Figure 4.6 A). Significant variation was not shown in the relative expression of *GADD45A* gene between the younger members (20-40 years) and the elder members (41-60 years) within each exposed and control groups. However, the younger members and the elder members of the exposed group showed lower expression level of GADD45A when compared

with the respective age group in the control (Figure 4.6 B). Occupational exposure to IR results in downregulation of *GADD45A* gene expression regardless of their smoking habits, betelnut chewing, tobacco and alcohol consumptions (Figure 4.6 C-F). Family history of cancer did not show significant association with the relative expression of *GADD45A* in both exposed and control groups (Figure 4.6 G).

Among the occupationally exposed group, technicians who have a cumulative effective dose of >50 mSv showed significantly lower level of the *GADD45A* gene expression than those workers having a cumulative effective dose of ≤50 mSv. However, duration of employment and number of patients handled per day did not show significant effect on the relative expression of *GADD45A* gene (Figure 4.7).

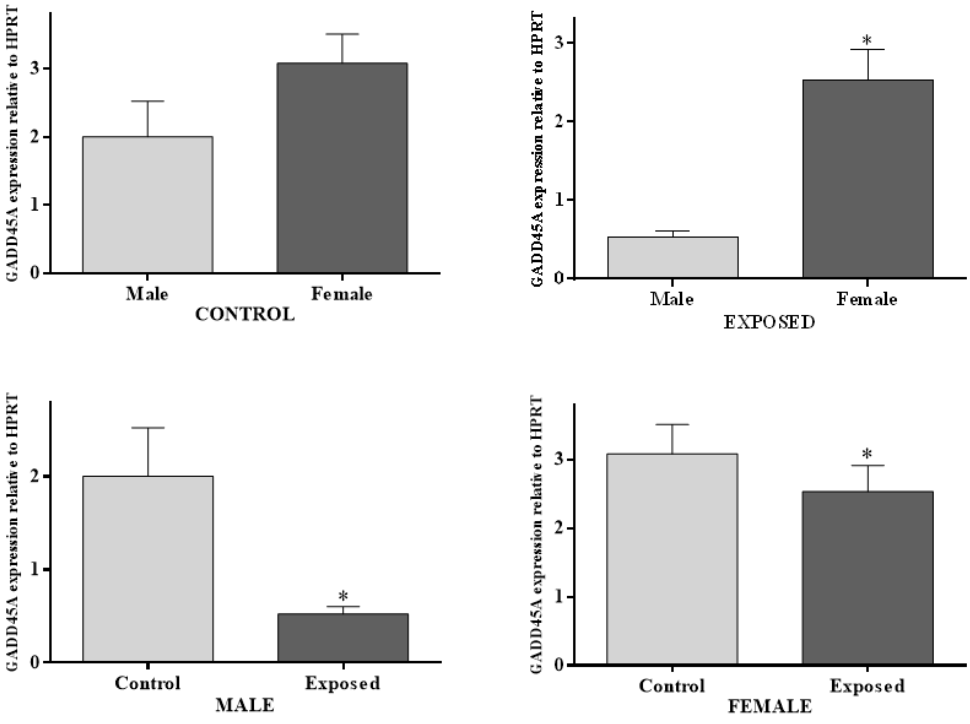


Figure 4.6 A

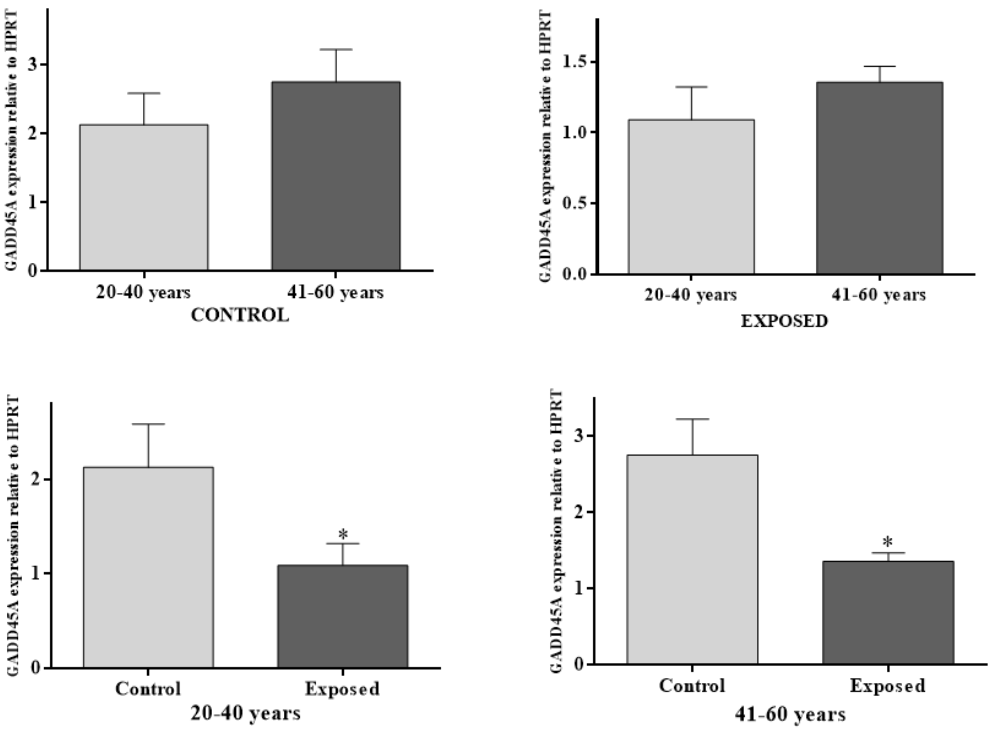


Figure 4.6 B

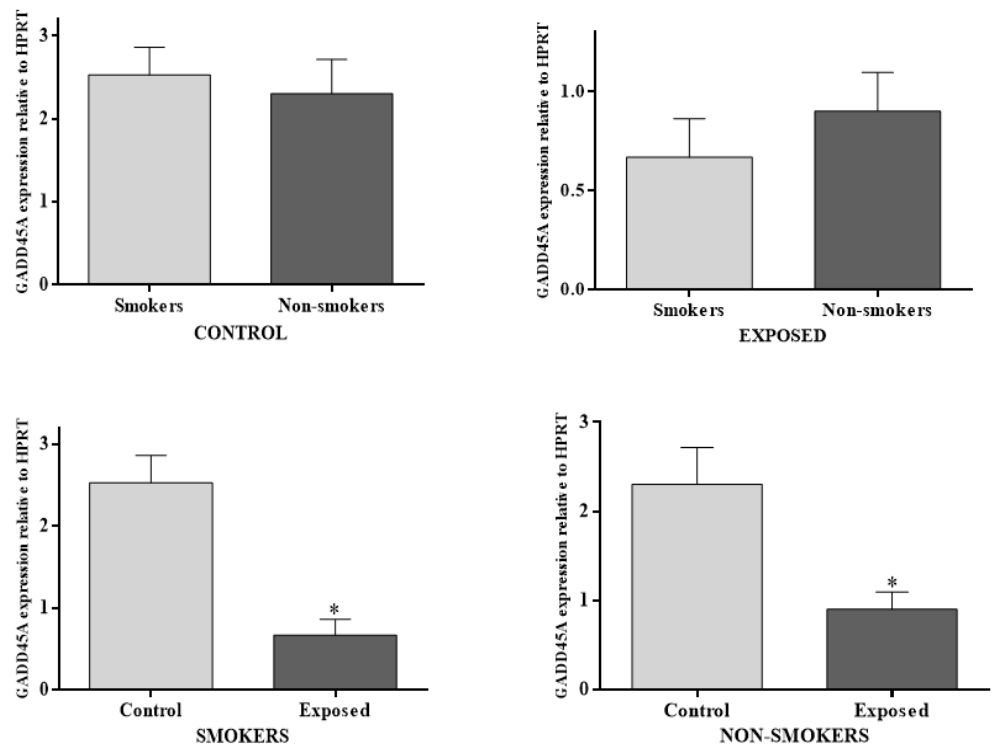


Figure 4.6 C

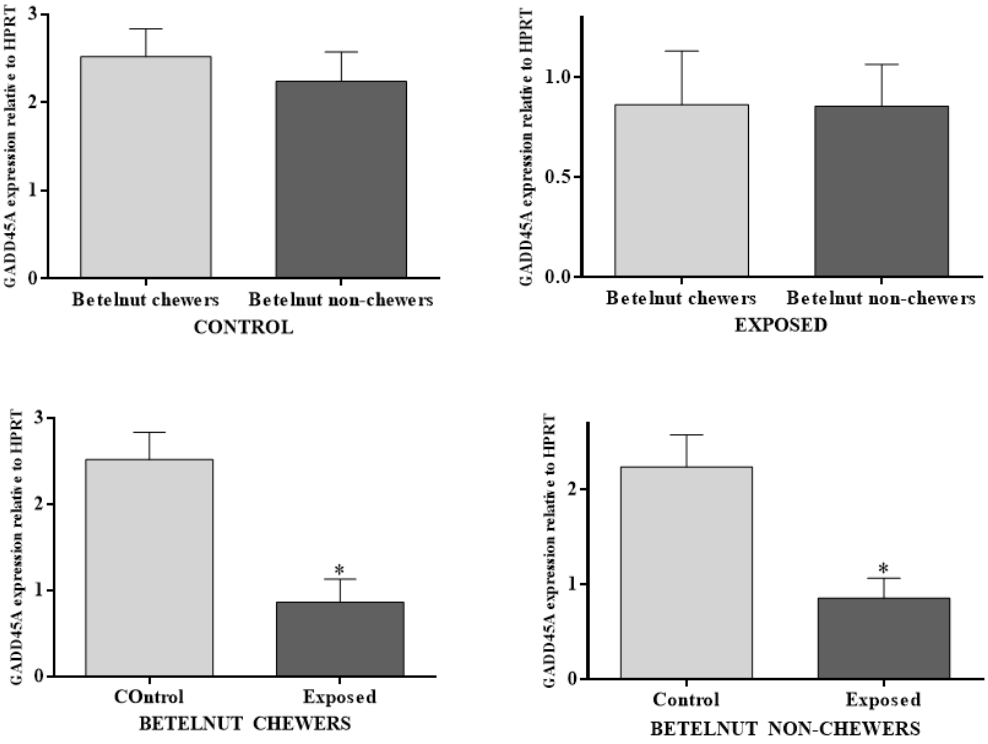


Figure 4.6 D

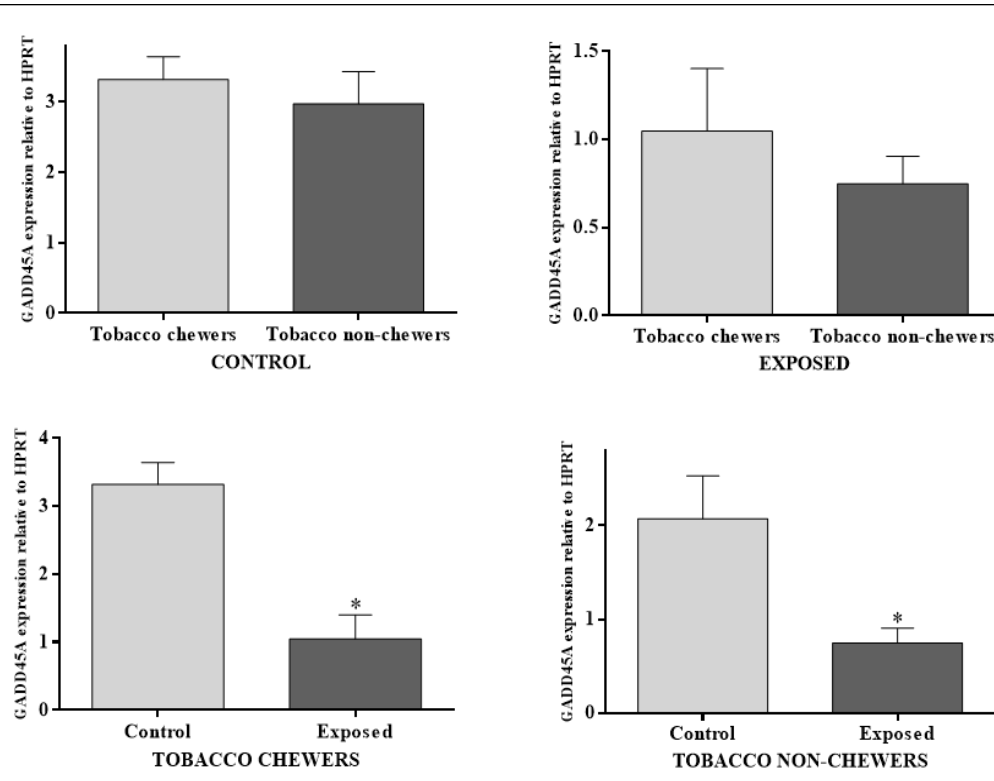


Figure 4.6 E

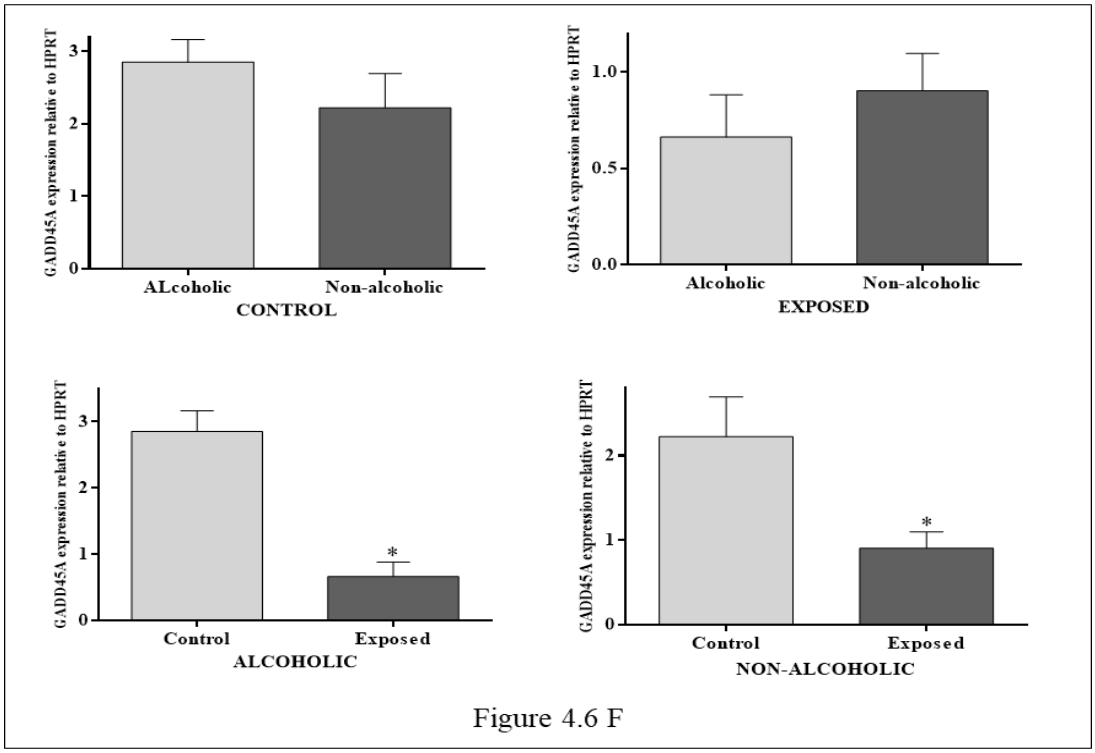


Figure 4.6 F

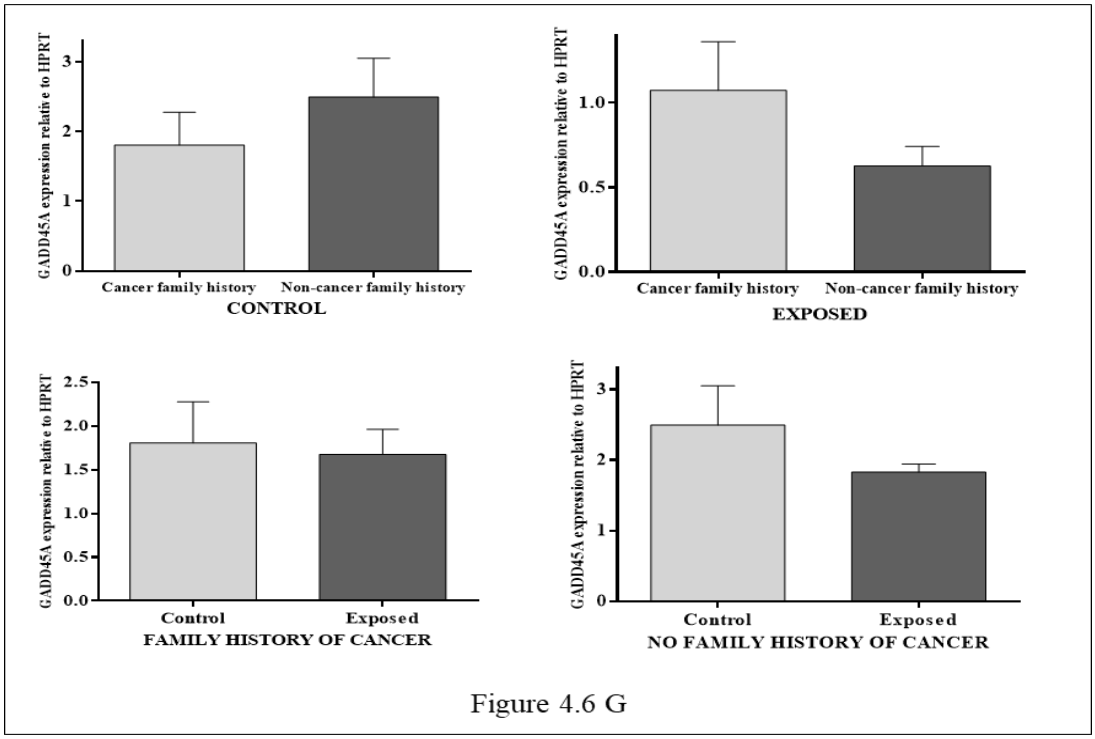


Figure 4.6 G

Figure 4.6: Functions of the demographic characteristics on the relative expression of Growth arrest and DNA-damage inducible alpha (*GADD45A*) in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the groups (p<0.05).*

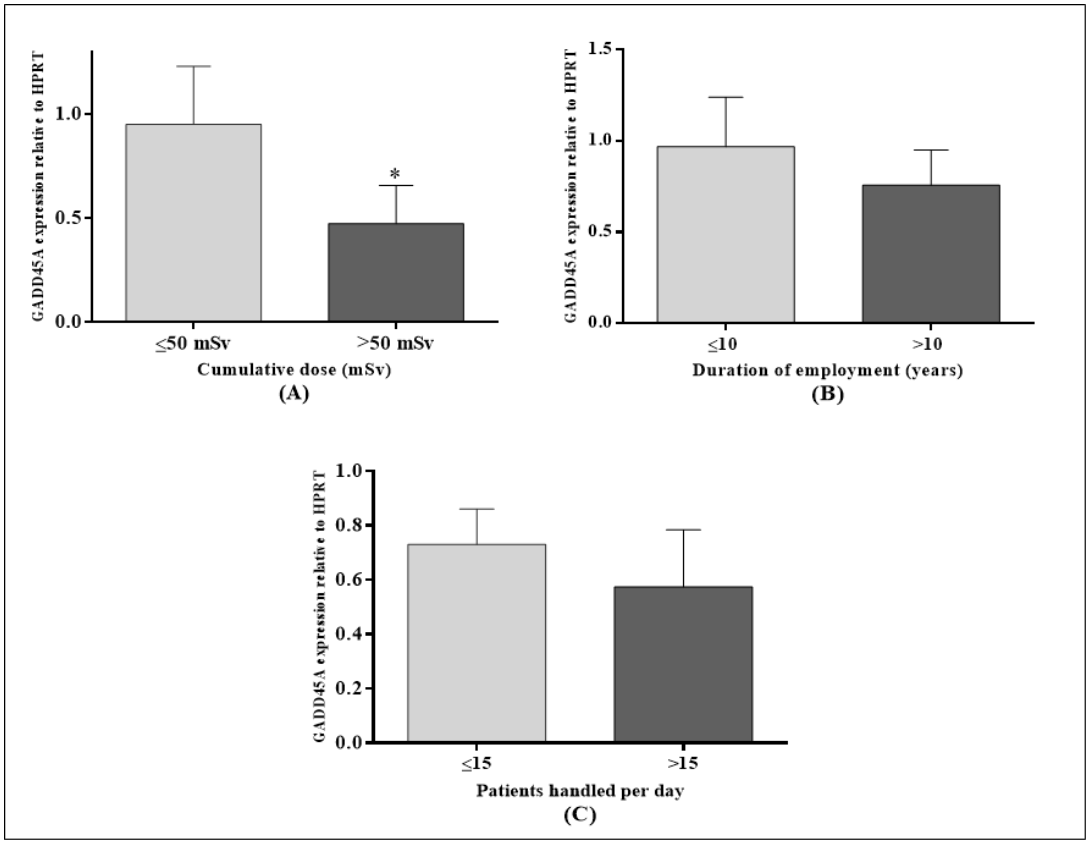


Figure 4.7: Functions of the demographic characteristics on the relative expression of Growth arrest and DNA-damage inducible alpha (*GADD45A*) among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the groups (p<0.05).*

3.4. Effects of demographic characteristics in the relative expression of Chromosome 12 open reading frame 5 (*C12orf5)* among the exposed and control groups:

No demographic characteristics was found to have an effect on the relative expression of *C12orf5* gene in both exposed and control groups (Figure 4.8 A-G). Also, among the exposed group, cumulative dose (mSv), duration of employment (years) and the number of patients handled per day did not show significant effect on the relative expression of the *C12orf5* gene on individuals working with X-rays (Figure 4.9).

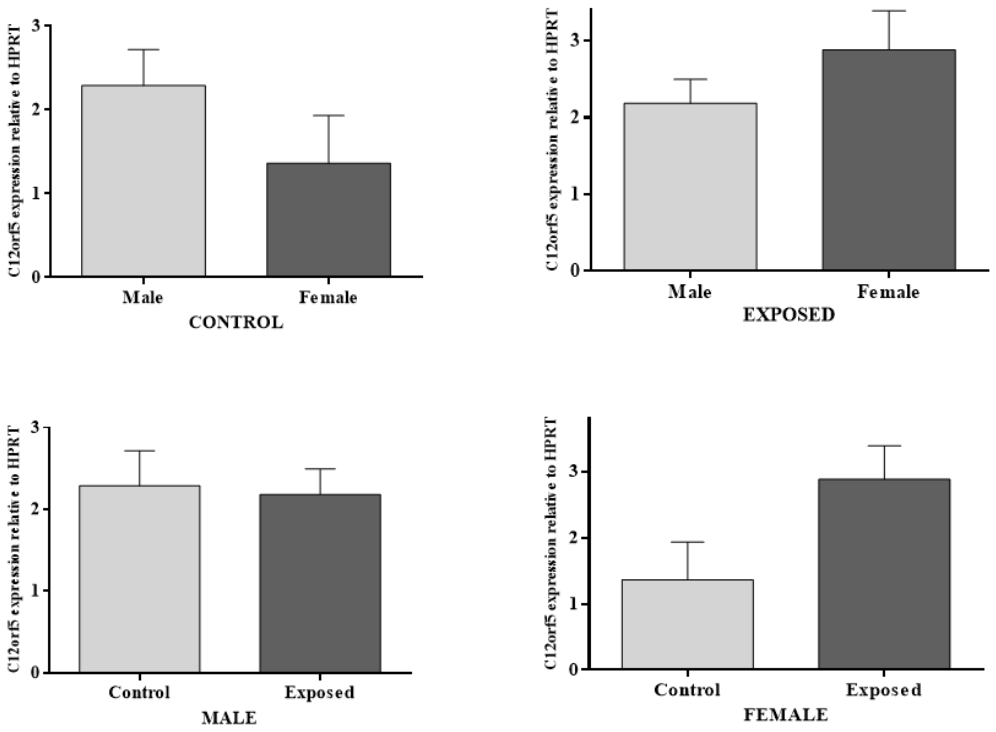


Figure 4.8 A

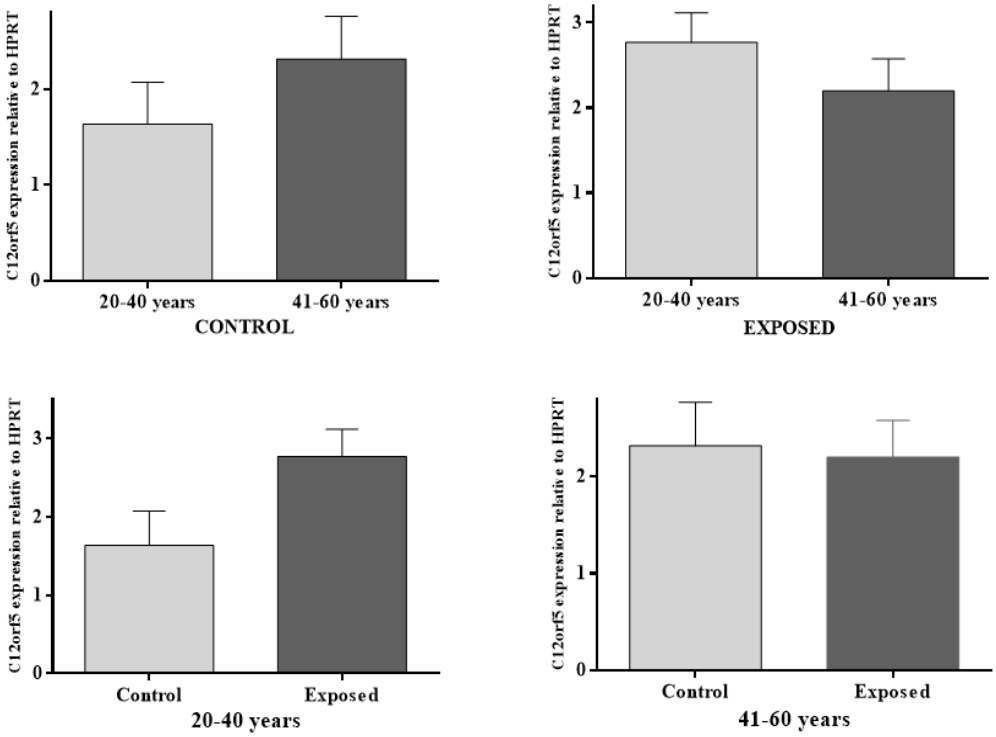


Figure 4.8 B

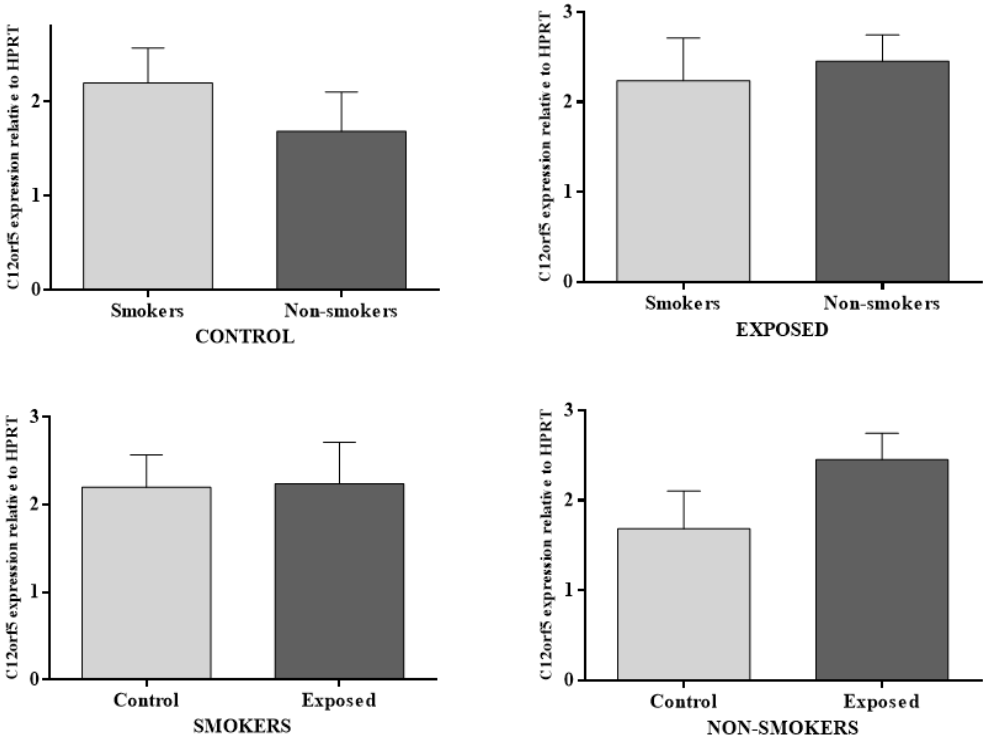


Figure 4.8 C

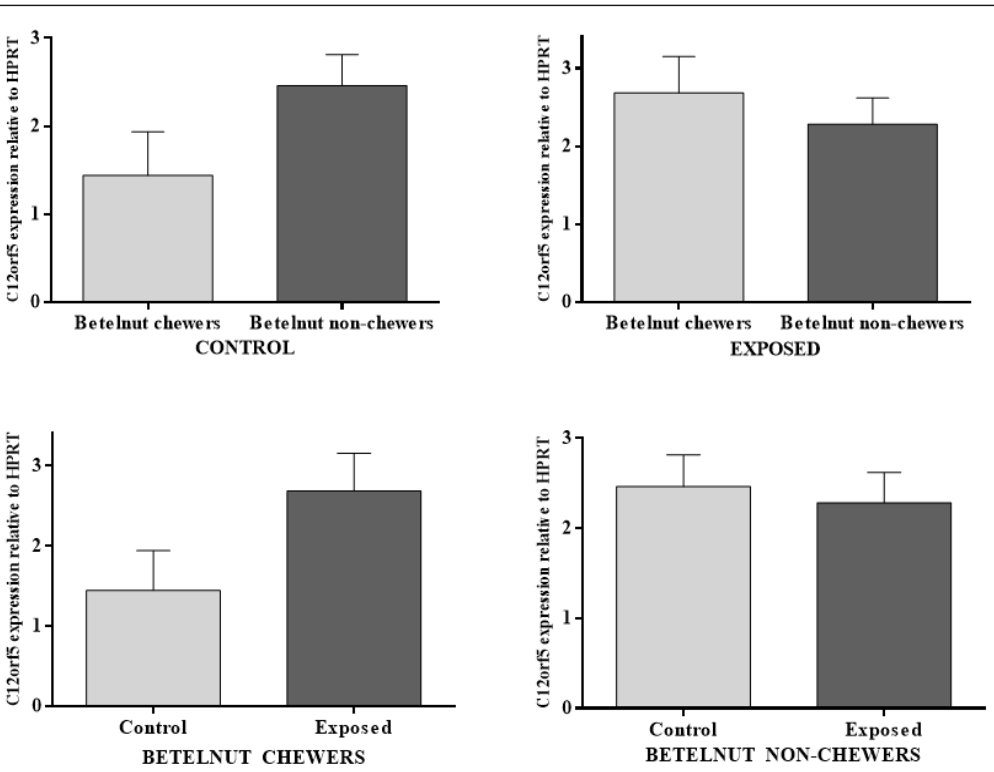


Figure 4.8 D

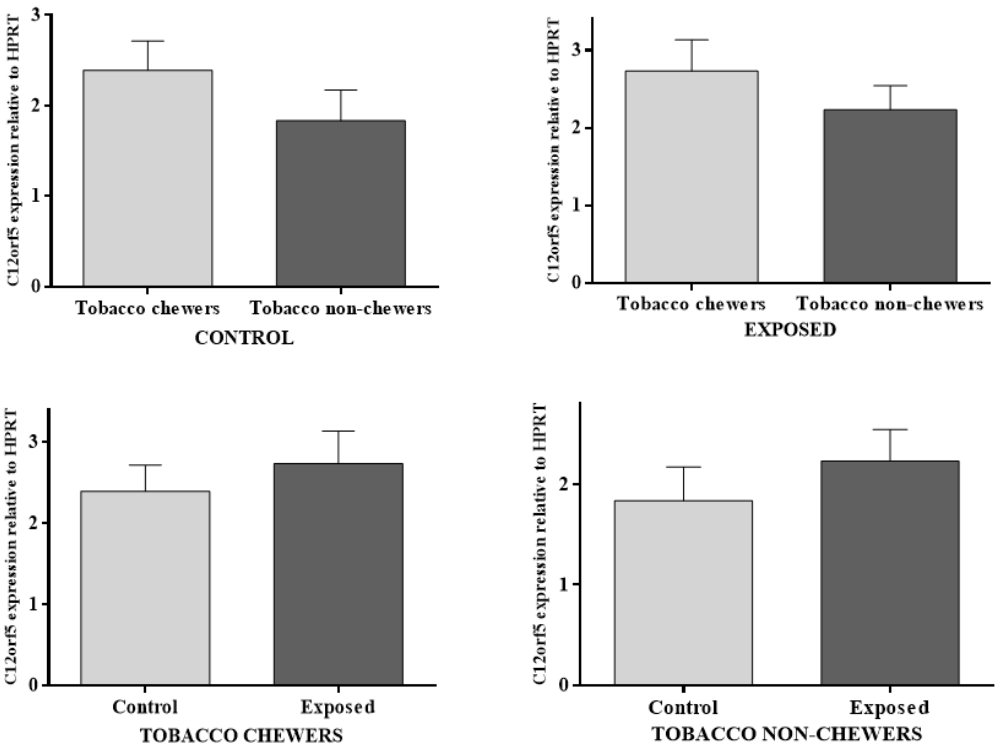


Figure 4.8 E

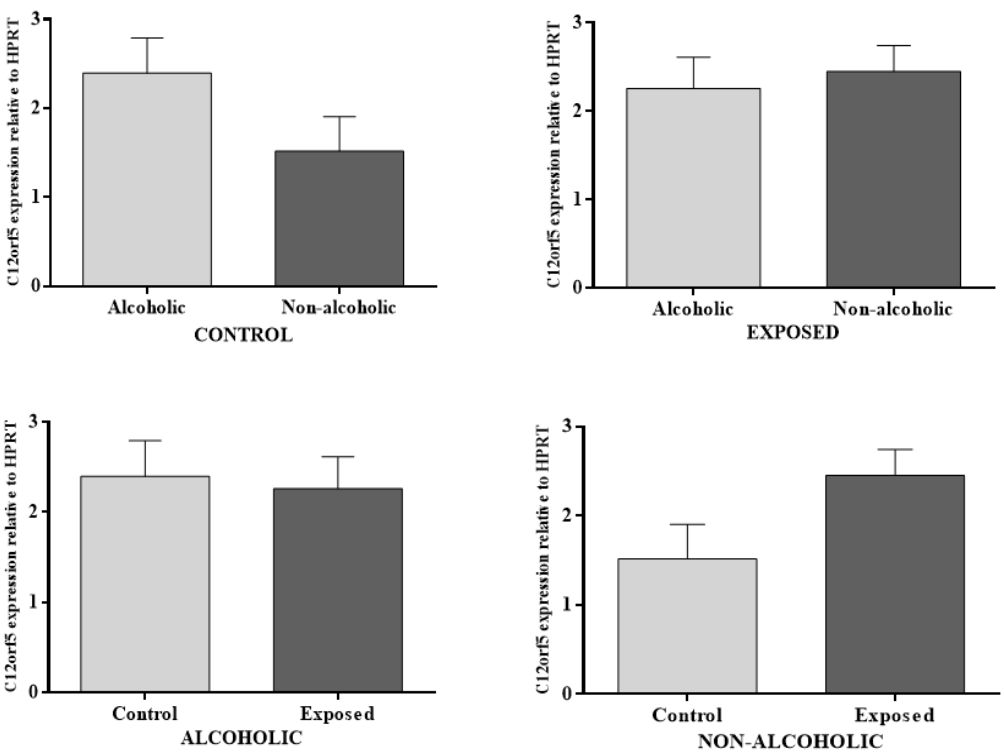


Figure 4.8 F

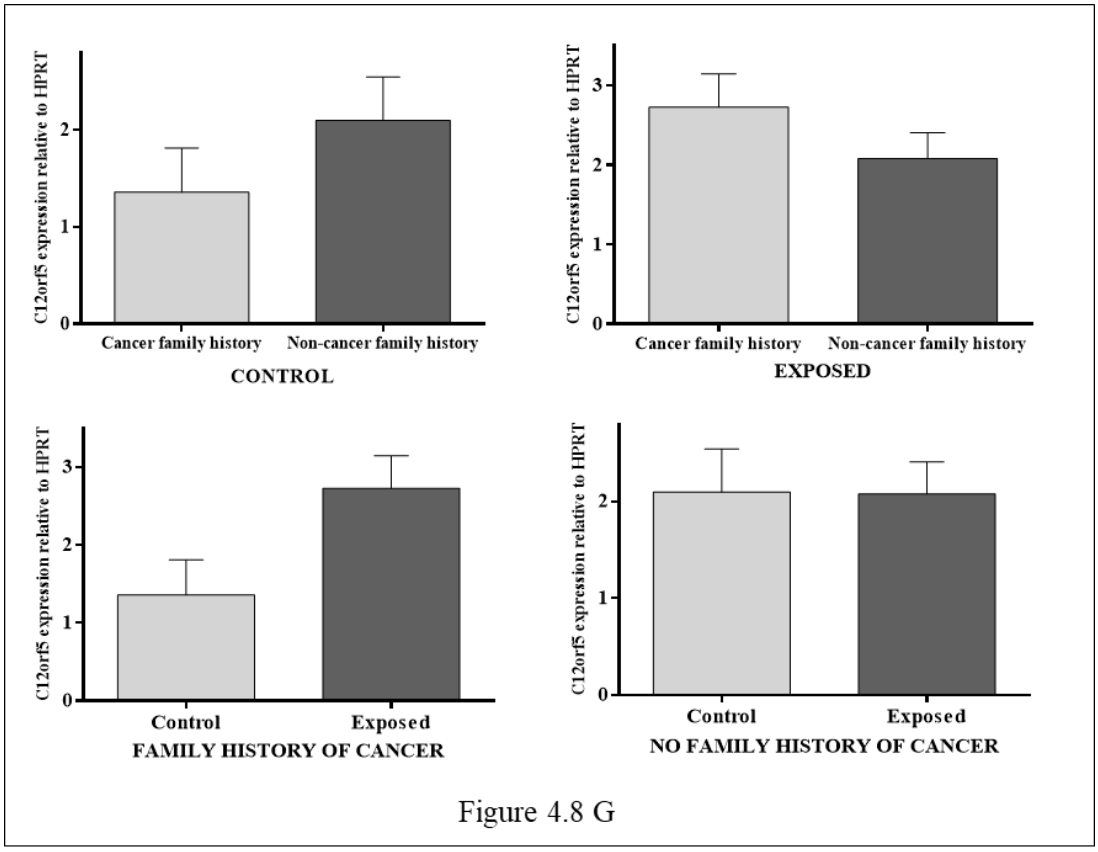


Figure 4.8: Functions of the demographic characteristics on the relative expression of Chromosome 12 open reading frame 5 (*C12orf5)* in the exposed and control groups. (A) Gender; (B) Age; (C) Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. * *indicates significant variations between the groups (p<0.05).*

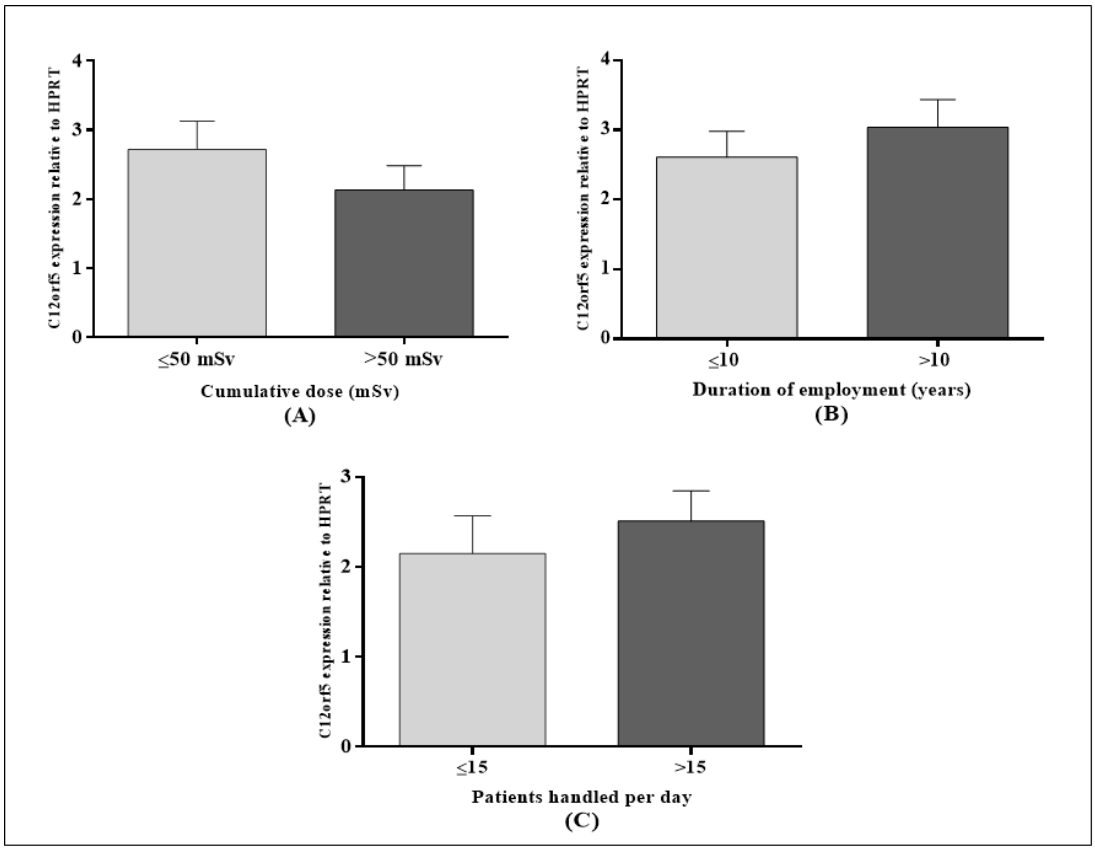


Figure 4.9: Functions of the demographic characteristics in the relative expression of Chromosome 12 open reading frame 5 (*C12orf5)* among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day. * *indicates significant variations between the groups (p<0.05).*

3.5. Effects of demographic characteristics in the relative expression of DNA damage binding protein *(DDB2*) among the exposed and control groups:

Gender-wise analysis revealed that males of exposed and control groups showed downregulation in the relative expression of *DDB2* gene when compared with females of the respective group. Also, females of exposed group showed significantly lower *DDB2* gene expression when compared with females of control group (Figure 4.10 A). Among the betelnut chewers and tobacco chewers, participants belonging to the exposed group showed lower expression of *DDB2* when compared with the control group (Figure 4.10 D & E). However, significant variation was not observed in the relative expression of *DDB2* gene with respect to age, smoking habits, consumption of

alcohol and family history of cancer in both exposed and control groups (Figure 4.10 B, C, F & G).

Cumulative dose, duration of employment, and the number of patients handled per day did not show significant effect on the relative expression of the *DDB2* gene on individuals working with X-ray (Figure 4.11).

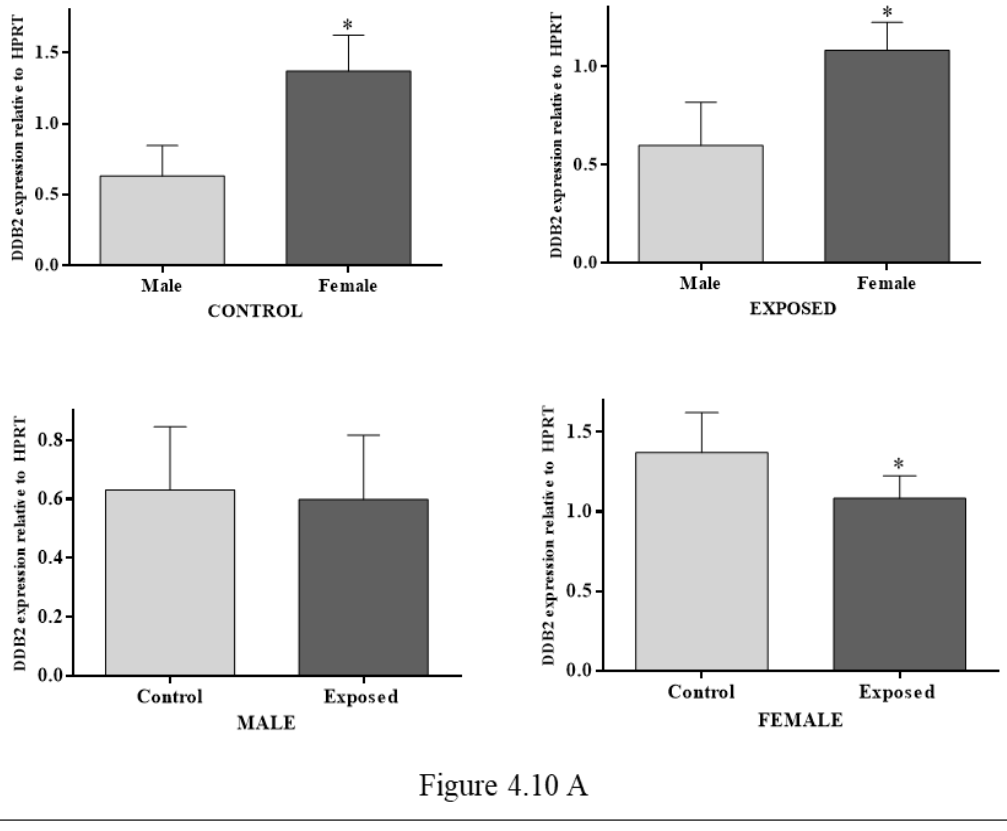


Figure 4.10 A

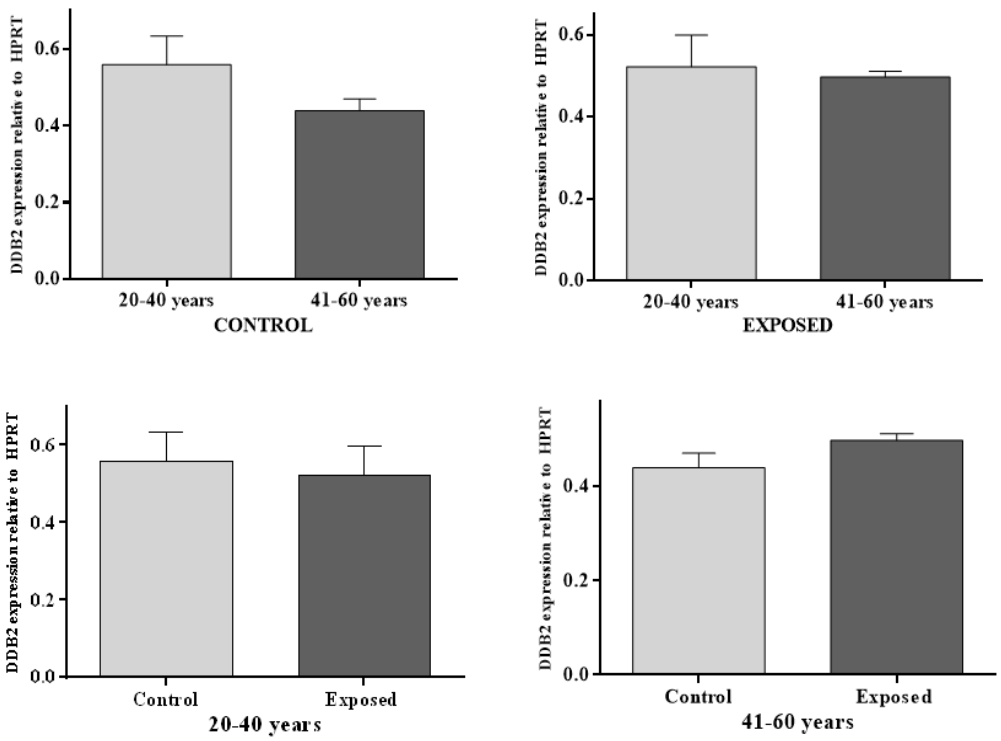


Figure 4.10 B

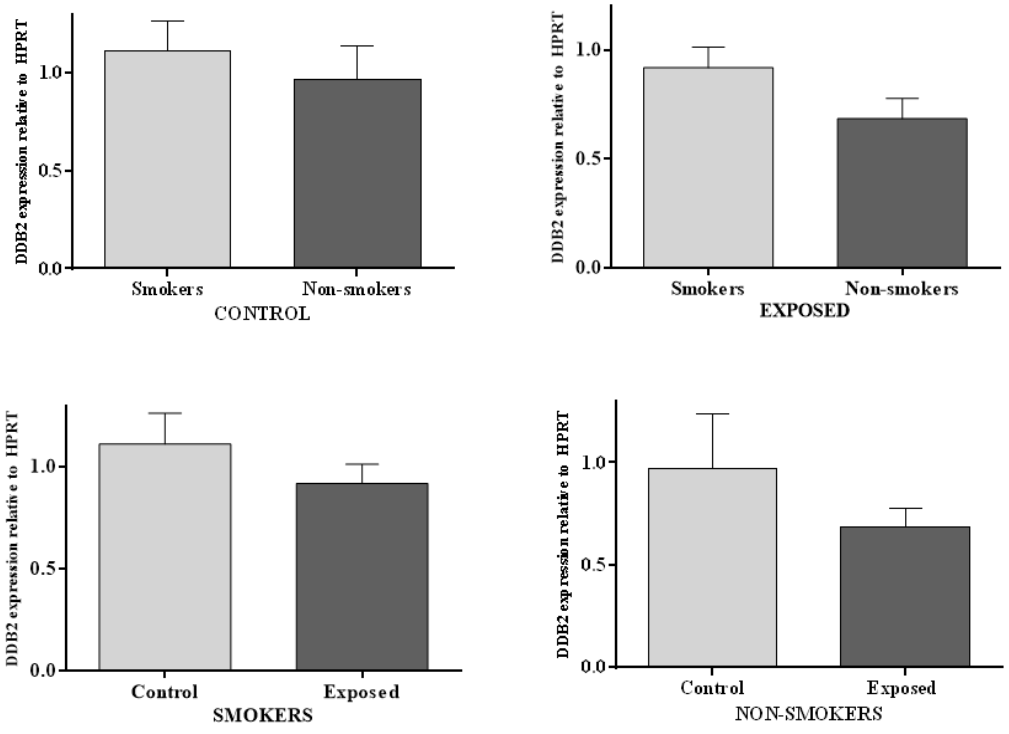


Figure 4.10 C

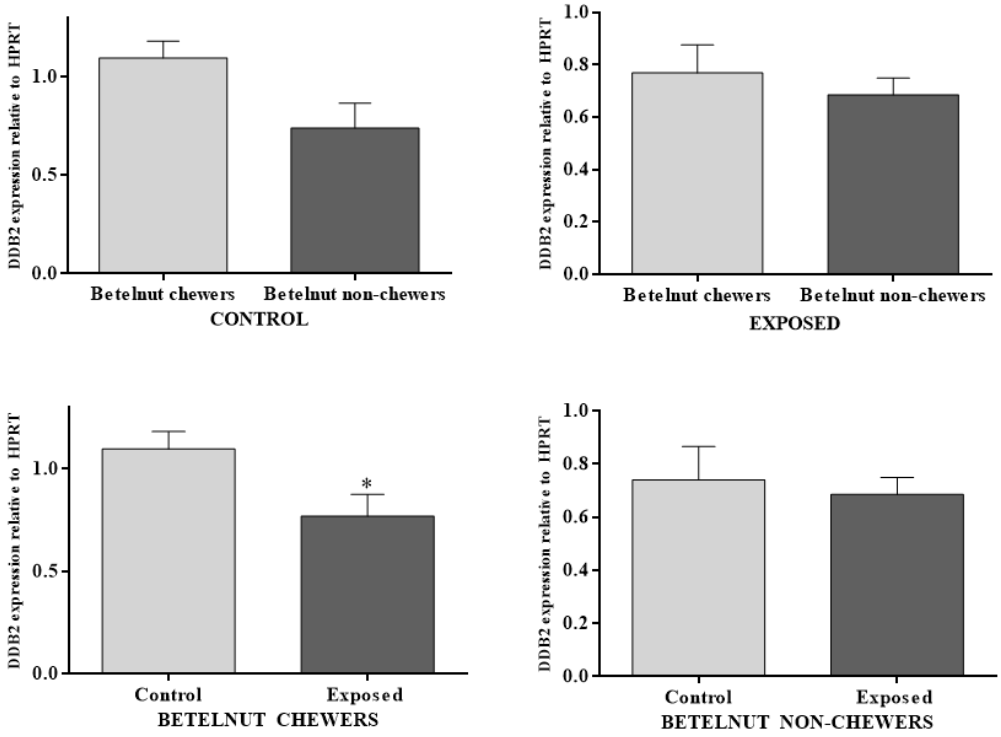


Figure 4.10 D

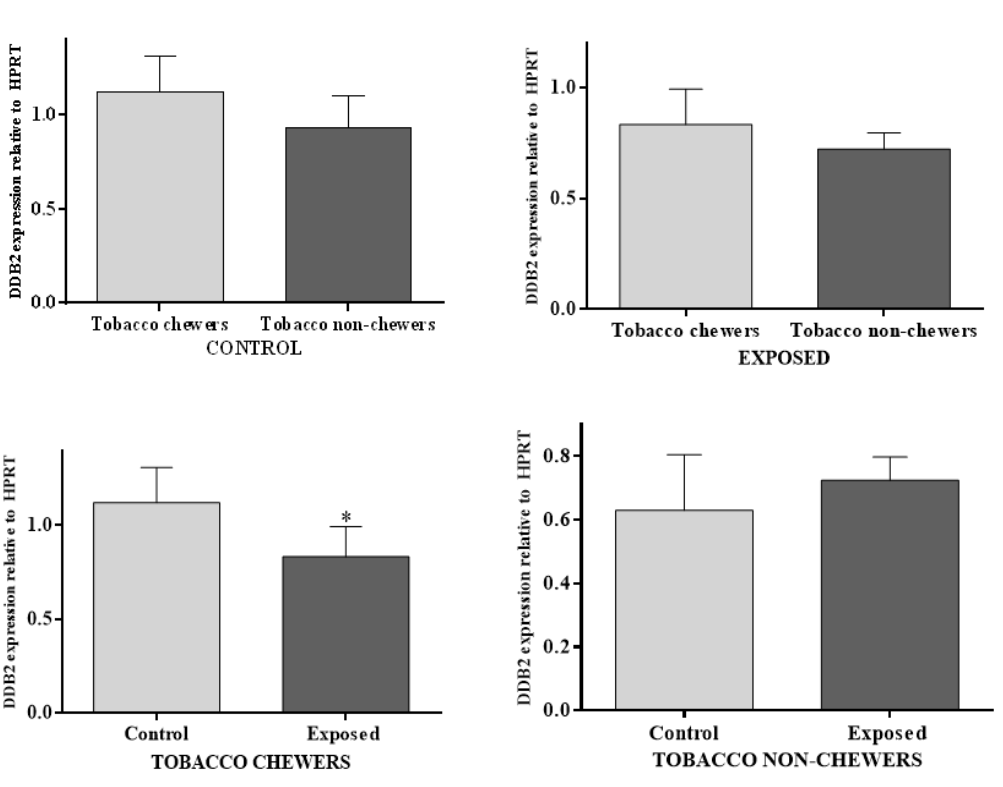


Figure 4.10 E

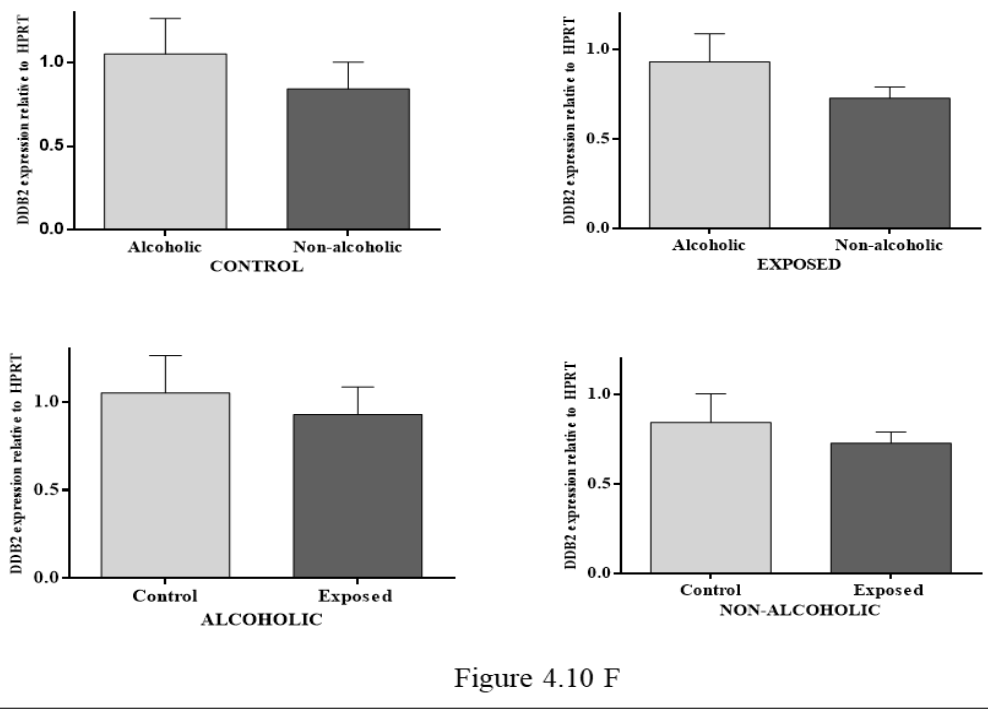


Figure 4.10 F

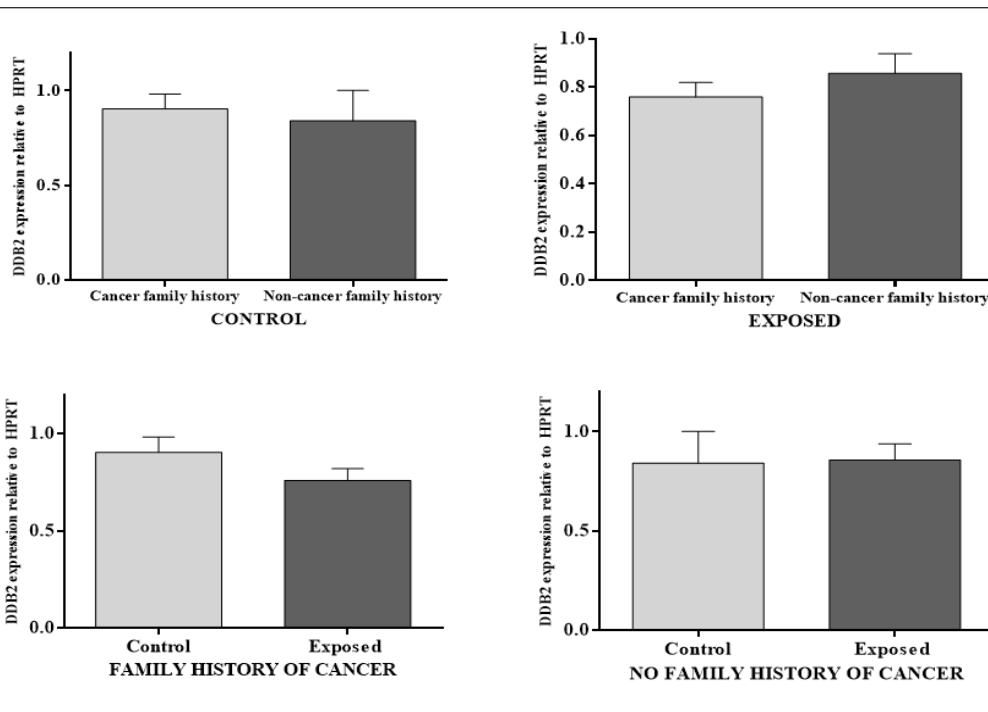


Figure 4.10 G

Figure 4.10: Functions of the demographic characteristics on the relative expression of DNA damage binding protein *(DDB2*) in the exposed and control group. (A) Gender; (B) Age; (C)

Smoking habits; (D) Betelnut chewing; (E) Tobacco chewing; (F) Alcohol consumption; (G) Family history of cancer. ** indicates significant variations between the groups (p<0.05).*

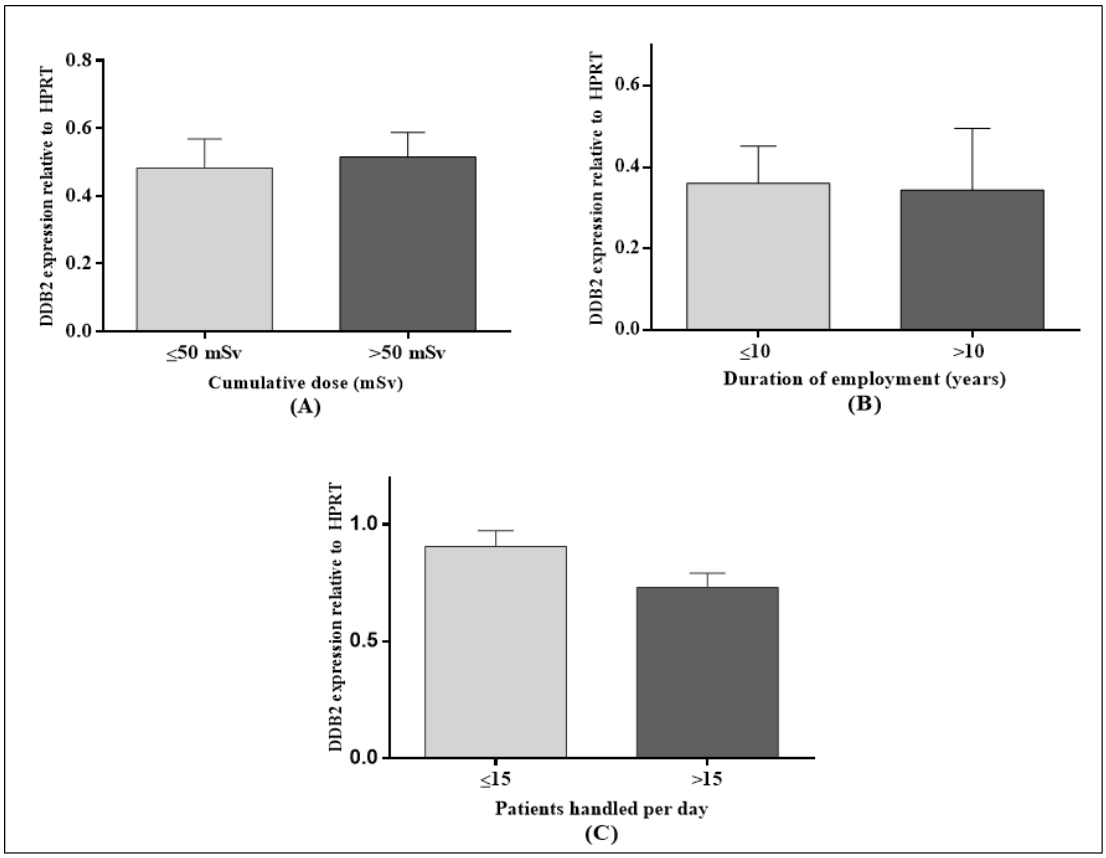


Figure 4.11: Functions of the demographic characteristics in the relative expression of DNA damage binding protein *(DDB2*) among the occupationally exposed group. (A) Cumulative dose (mSv); (B) Duration of employment (years); (C) Patients handled per day.
** indicates significant variations between the groups (p<0.05).*

Table 6: Influence of demographic characteristics on the relative expression of radiation sensitive genes in the occupationally exposed group and control group.

Characteristics	Durbin Watson	Beta-value	t-value	p-value
FDXR				
Gender	1.786	0.382	1.107	0.285
Age		-0.374	-0.911	0.376
Smoking habits		0.110	0.458	0.653
Betelnut chewing		-0.091	-0.341	0.737
Tobacco chewing		0.251	0.775	0.450
Alcohol consumption		-0.149	-0.550	0.590
Family history of cancer		-0.418	-1.596	0.130
Time since working (years)		0.336	0.783	0.445
No. of patients handled per day		0.074	0.275	0.787
Cumulative dose (mSv)		0.126	0.400	**0.045**
POLH				
Gender	2.136	0.348	1.124	0.278
Age		0.686	1.863	0.081
Smoking habits		-0.507	-2.355	0.072
Betelnut chewing		-0.287	-1.202	0.247
Tobacco chewing		0.182	0.626	0.540
Alcohol consumption		-0.185	-0.761	0.458
Family history of cancer		-0.511	-2.179	0.075
Time since working (years)		-0.780	-2.026	0.060
No. of patients handled per day		0.594	2.464	0.575
Cumulative dose (mSv)		0.161	0.572	0.055
GADD45A				
Gender	1.397	0.450	1.357	0.193
Age		-0.272	-0.691	0.500
Smoking habits		-0.015	-0.064	0.949
Betelnut chewing		0.016	0.065	0.949
Tobacco chewing		-0.004	-0.013	0.990
Alcohol consumption		-0.232	-0.895	0.384
Family history of cancer		0.000	-0.002	0.999
Time since working (years)		-0.162	-0.392	0.700
No. of patients handled per day		0.437	1.696	0.109
Cumulative dose (mSv)		0.452	1.497	0.154
C12orf5				
Gender	2.118	0.549	1.969	0.067
Age		-0.178	-0.536	0.599
Smoking habits		0.007	0.034	0.973

Betelnut chewing		-0.501	-2.332	0.053
Tobacco chewing		0.500	1.911	0.074
Alcohol consumption		-0.214	-0.980	0.342
Family history of cancer		-0.442	-2.088	0.053
Time since working (years)		0.257	0.740	0.470
No. of patients handled per day		0.311	1.430	0.172
Cumulative dose (mSv)		-0.020	-0.079	0.938
DDB2				
Gender	2.217	-0.500	-1.003	0.342
Age		-0.208	-0.308	0.765
Smoking habits		0.336	1.198	0.262
Betelnut chewing		-0.075	-0.142	0.890
Tobacco chewing		-0.347	-0.617	0.552
Alcohol consumption		-0.059	-0.151	0.883
Family history of cancer		0.186	0.572	0.581
Time since working (years)		0.136	0.241	0.815
No. of patients handled per day		-0.335	-0.817	0.435
Cumulative dose (mSv)		0.211	0.382	0.711

Bold value signify $p<0.05$.

4. DISCUSSION

Increased genomic instability among the occupational X-ray technicians was clearly indicated by our previous study using Comet assay and MN assay where the DNA damage levels and the MN frequency were significantly higher in the exposed group when compared with the controls. In this chapter, we conducted DNA damage-related gene expression profiling using qPCR technique among the occupational X-ray technicians who received a low dose of ionizing radiation on daily basis. The effect of occupational exposure to low doses of ionizing radiation is a serious concern to a large number of radiation workers (UNSCEAR). Although radiation workers are not directly exposed to radiation, they receive scatter radiation that is extremely variable. Effective dose per year was estimated to be 0.04-0.05 mSv/year for the primary operator, and 0.03-0.04 mSv/year for those assisting (Efstathopoulos et al., 2003). Whereas the effective doses ranged from 0.02-38.0 µSv for DC (diagnostic catheterizations), 0.17-31.2 µSv for PCI (percutaneous coronary interventions), 0.24-9.6 µSv for ablations, and 0.29-17.4 µSv (Kim et al., 2008). Several studies have reported alteration in the gene expression profiles following irradiation at low doses. Alterations in the relative expression of *BAX, PCNA, GADD45, DDB2* and *CDKN1A* was observed in human peripheral blood lymphocytes irradiated to low doses of γ-rays as low as 20 mGy (Franco et al., 2005; Gruel et al., 2008; Knops et al., 2012; Amundson et al., 2000). Moreover, *ex-vivo* studies in X-irradiated skin fibroblasts carried out by Ding et al. (2005) reported qualitative and quantitative differences between gene expression profiles induced by 2 cGy and 4 Gy.

The molecular mechanisms of cellular response to high and low doses of ionizing radiation are well known and linked to the DNA damage response (DDR) (Howe et al., 2021). Therefore, the present study focused on genes central to the DDR such as the ATM/CHK2/p53 pathway signalled by ionizing radiation-induced DNA damage. p53 modulates the main DNA repair processes in eukaryotic cells such as base excision repair (BER), nucleotide excision repair (NER), non-homologous end-joining (NHEJ) and homologous recombination (HR) along with direct roles in the induction of DNA damage-induced cell cycle arrest and apoptosis. DNA damage results into activation of p53 through phosphorylation and the activated p53 induced the expressions of downstream genes that are involve in cell cycle arrest (Zhang et al.,

2011). Quantitative real-time PCR (qRT-PCR) was carried out for the five radiation-sensitive genes against a housekeeping control Hypoxanthine-Guanine phosphoribosyl transferase 1 (*HPRT1*). These included the cell cycle regulators (*GADD45A*), apoptosis regulators (*FDXR*) and genes involved in DNA repairing (*DDB2, POLH* and *C12orf5*). Other factors such as age, gender, smoking, environmental factors, or infection can potentially affect gene expression and these potential confounding factors could change endogenous transcriptional levels (O'Brien et al., 2018). Therefore, in the present study, the influence of age, gender, and lifestyle including betelnut chewing, tobacco usage, smoking habit and alcohol consumption and family history of cancer of radiology workers on the differential expression of the selected genes was also determined by multiple linear regression analysis.

Out of the five genes studied, exposure to ionizing radiation results in an up-regulation of the *FDXR* gene, and downregulation of *GADD45A* and *DDB2* in radiology workers when compared to the control group. However, no significant differences were observed in the relative expression of *POLH* and *C12orf5* between the exposed and control group. On consideration of their demographic characteristics, multiple linear regression analysis revealed an association of altered gene expression between *FDXR* and the cumulative dose of the exposed group. The altered expression of FDXR in the exposed group may indicate the potential effects of low dose irradiation in the relative expression of the gene. *FDXR* is an essential gene, critical for p53-dependent tumour suppression via iron regulatory protein (Zhang et al., 2017). The expression level of *FDXR* has been commonly used as a biomarker of radiation exposure in several *ex vivo* studies (O'Brien et al., 2018; Abend et al., 2016). Our finding is consistent with numerous *ex vivo* studies that reported an upregulation of the relative expression of *FDXR* in blood after low dose radiation exposure (Manning et al., 2017; Badie et al., 2013; Abend et al., 2016). Furthermore, studies carried out by O'Brien et al. (2018) reported that blood exposed *ex vivo* to 150 mGy of ionizing radiation led to up-regulation of *FDXR* gene. Monitoring the transcriptional expression of the gene *FDXR* in human blood is remarkable, especially in the case of partial body exposures and low dose exposure that could cause acute and chronic radiation toxicity (Lucas et al., 2014; Joiner et al., 2011).

DDB2 and *GADD45A* genes plays significant roles in DNA repair/cell cycle control, and the alteration in the relative expression of *DDB2* and *GADD45A* following low dose of irradiation have been reported earlier by various studies (Tusher et al., 2001; Amundson et al., 2000; Rieger et al., 2004; Snyder et al., 2004). However, the majority of the study reported an up-regulation of *DDB2* and *GADD45A* relative expressions which is in contrast to our findings. In the present study, the relative expression of *DDB2* in radiology workers was found to be significantly lower than the control group. Although the majority of the previous study reported upregulation of DDB2 expression after low dose irradiation in both *in vivo* and *in vitro* experiments (Amundson et al., 2004; Meadow et al., 2008; Paul and Amundson 2008), Li et al. (2011) showed the downregulation of *DDB2* expression in mice after 2 Gy whole-body irradiation. The expression of NER genes in radiation workers seems to indicate their participation either in the repairing of DNA strand breaks or in the repairing of oxidative DNA damage. NER gene such as *ERCC5* (*XPG*) gene was also found to be downregulated in the radiation workers exposed to low dose of ionizing radiation (Fachin et al., 2009). *ERCC5* is a human endonuclease that incises 3′ at DNA lesions during the NER pathway. Recently, Stoyanova et al. (2009) reported that *DDB2* could indirectly participate in nucleotide excision repair (NER) by negatively regulating the cellular levels of $p21^{Waf1/Cip1}$ thereby probably activating the apoptotic pathway. Similarly, the downregulation of DDB2 in the present study may allow the cells to undergo apoptosis probably due to severe DNA damage.

In the present study, the relative expression of *GADD45A* in radiology workers was also found to be significantly lower than the control group. Goldberg et al. (2004) did not find a consistent dose-response or time-response pattern for *GADD45A* gene analyzed in irradiated skin fibroblasts. However, expression of *GADD45A* was downregulated at 24 hr after 6 Gy exposure to ionizing radiation in mice, whereas expression at 24 hr after 2 Gy exposure was upregulated in the *ex vivo* human blood culture model (Bessho 1999; Paul et al., 2011; Tucker et al., 2012). Variations observed between our study and others can be due to differences in cell types, experimental conditions and different levels of radiation exposure.

CHAPTER VII

EFFECTS OF LOW-DOSE IRRADIATION (X-RAY) ON CHROMOSOMAL DAMAGE AND ANTIOXIDANT STATUS IN CULTURED HUMAN PERIPHERAL BLOOD

ABSTRACT

The use of X-rays has become a popular medical diagnosis and presents a major source of radiation exposure for the general population. There is an increasing concern over the magnitude of health risks from exposures to man-made radiation such as diagnostic X-rays that involve low doses. In view of this, the study was carried out to evaluate the *in-vitro* effects of low-dose irradiation on chromosomal damage and antioxidant levels in cultured human peripheral blood. Blood samples collected from healthy young volunteers (male and female) in sterile heparinized tubes were irradiated at 97 kVp with 300 mAs using an X-rays machine. *In-vitro* irradiation of whole blood was performed at different doses (0, 25, 50, 100, 200 and 300 mGy) with an average dose rate of 19.6 mGy/sec. After irradiation, the lymphocytes were collected and cultured for 72 hr. Micronuclei (MN) assay was carried out following the standard protocol for the assessment of chromosomal damage. The level of glutathione (GSH), activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD) were estimated from the blood plasma. Malondialdehyde (MDA) content was also estimated to determine oxidative stress after low-dose irradiation. A significant positive correlation (r^2=0.98, p <0.001) was observed between total micronuclei frequency and irradiation dose in human peripheral blood lymphocytes. Irradiation of blood also caused a significant decrease in GSH level and GST activities with an increase in irradiation dose. A significant reduction in SOD activity was observed only at doses ≥100 mGy. Induction of oxidative stress in human blood due to irradiation was clearly evident from enhanced MDA content. This study indicates that exposure to ionizing radiation less than 100 mGy can cause genetic damage and induce oxidative stress. Furthermore, the results suggested that detection of genetic damage using micronucleus (MN) assay is sensitive enough at a lower dose.

1. INTRODUCTION

Ionizing radiation has the ability to directly interact with the cellular components and causes ionization of biomolecules and initiates a sequence of events leading to pathophysiological changes (Lehnert 2007). The increasing use of ionizing radiation in medical diagnosis and treatment has raised concern about its potential long-term effects on human health as prolonged exposure to low-dose radiation has been found to cause adverse health effects including cancer (Gilbert 2009; Richardson et al., 2015). The use of X-rays has become a popular medical diagnosis and presents a major source of radiation exposure for the general population. Due to their high energy content, X-ray exposure can injure normal cells, alter genetic materials and induce multiple changes in biomolecules (Hall 2000; Azzam et al., 2012). Although high doses of ionizing radiation are clearly known to produce deleterious consequences such as cardiovascular diseases and cataracts in human (UNSCEAR 2006; Baselet et al., 2016), the potential risk of low-dose exposure is still a matter of debate (Feinendegen et al., 2011; OECD 2016). The biological effects of ionizing radiation such as X-rays are mainly related to damages they cause to DNA, by inducing single-strand DNA breaks, double-strand DNA breaks, DNA base alterations and DNA–DNA cross-links (Ward 1998; Hall 2009). The potential genotoxicity of ionizing radiation can be determined by micronuclei assay, which is an effective tool to evaluate the genotoxic or clastogenic effects of physical and chemical agents (Fenech et al., 1999). Measurement of micronuclei frequency is extensively used in molecular epidemiology and cytogenetics to evaluate the presence and the extent of chromosomal damage in human population exposed to genotoxic agents (Maffei et al., 2002; Kirsch-Volders et al., 2011; Vellingiri et al., 2014; Zothansiama et al., 2017; Zothansiama et al., 2019).

Ionizing radiation directly interacts with atoms or molecules and generates highly reactive oxygen species (ROS) such as hydroxyl radicals ($^{\bullet}OH$) that inflict oxidative damage in cells (Adams 1986; Le Caër 2011). Previous studies have reported an increase in the production of endogenous ROS due to exposure to a high dose of ionizing radiation (Zhou et al., 2011; Kohanoff and Artacho 2017). Excessive generation of ROS results in oxidative stress and attacks molecules in the membranes and tissues resulting in various diseases such as cardiovascular diseases, lens opacity

of the eyes, lung disease and cancer (Yoshikawa and Naito 2002; Wang et al., 2016). Although cells are equipped with an impressive repertoire of antioxidants to counterbalance oxidative stress by neutralizing ROS, excess generation of ROS may sometimes overwhelm the endogenous antioxidants and cause oxidative cellular damage (Kono and Fridovich 1982; Jagetia and Shetty 2016). Reactive oxygen species, due to their extreme reactivity are not amenable to direct measurement in biological material. Therefore, altered levels of antioxidative enzymes such as glutathione-s-transferase, superoxide dismutase, catalase, glutathione peroxidase, and small-molecule antioxidants such as glutathione are routinely used to assess oxidative stress. Elevated levels of ROS alter lipids by producing lipid peroxides such as malondialdehyde and 4-hydroxynonemal (Burton and Traber 1990). Lipid peroxidation has been reported to be associated with various pathological conditions and is used as a marker of oxidative stress (Gutteridge 1995).

According to the United Nations Scientific Committee on Atomic Radiation, low-dose radiation (LDR) includes radiation < 200 mGy for low linear energy transfer (LET) radiation or 50 mGy for high LET radiation (Liu et al., 2007, UNSCEAR 1994). Although several studies have reported increased levels of chromosomal aberrations in human lymphocytes exposed to low doses of ionizing radiations (Andreassi et al., 2009; Zakeri and Hirobe 2010; Sakly et al., 2012), contradictory results have been reported on the genotoxic effects of low-doses of ionizing radiation by various workers (Demirel et al., 1997; Thierens et al., 2002; Joseph et al., 2004). Thus, there is an increasing concern over the magnitude of health risks from exposures to man-made radiation such as diagnostic X-rays that involve low doses (UNSCEAR 2006). The risk associated with LDR and understanding intracellular responses are of social importance. In view of this, this study was carried out to evaluate the *in-vitro* effects of low-dose radiation on chromosomal damage and antioxidant levels in cultured human peripheral blood.

2. MATERIALS AND METHODS

2.1. Chemicals

RPMI-1640 medium, phytohemagglutinin, acridine orange, bovine serum albumin (BSA), glutathione reduced (GSH), nicotinamide adenosine dinucleotide (NADH), nitroblue tetrazolium (NBT) and n-butanol were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Methanol, acetic acid, FolinCiocalteu reagent, potassium tartrate, trichloroacetic acid (TCA), hydrochloric acid (HCl) and potassium chloride (KCl) were purchased from Merck India (Mumbai, India). Cytochalasin B, thiobarbituric acid (TBA) and phenazine methosulphate (PMS), 1-chloro-2, 4-nitrobenzene (CDNB) were purchased from Sigma Aldrich Chemical Co. (Bangalore, India) and 5, 5'- dithio 2- nitrobenzoic acid (DTNB) was procured from Tokyo Chemical Industry (Tokyo, Japan).

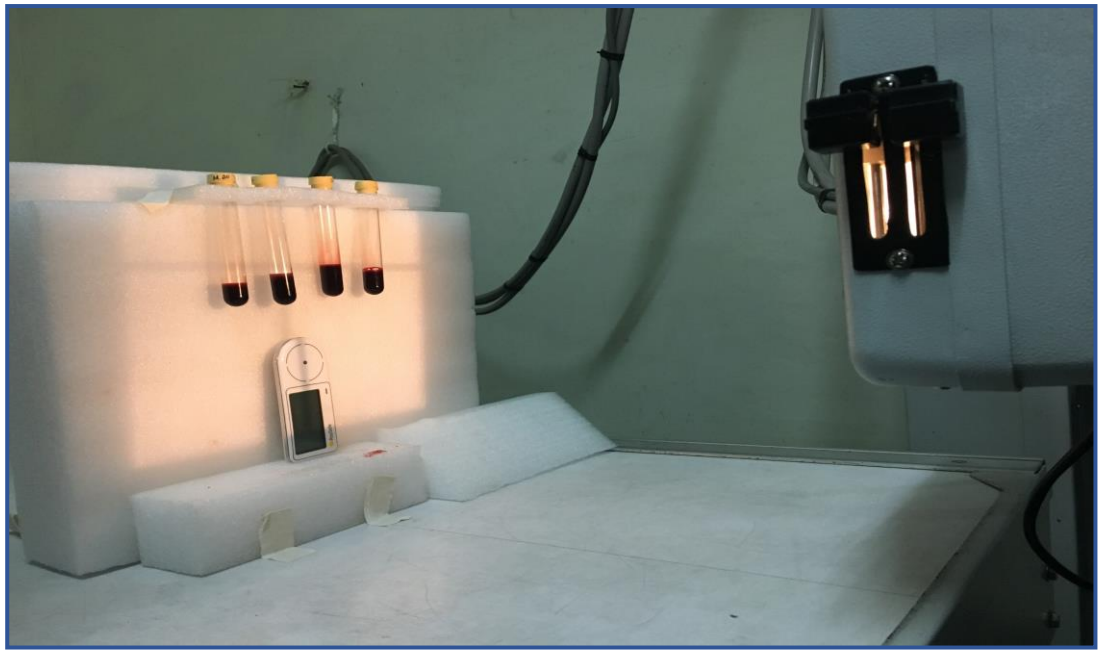
2.2. Collection of blood sample

Blood samples were collected by venipuncture from healthy male and female of 26 years old, non-smoking volunteers, who did not have any previous history of radiation exposure for diagnostic or treatment purposes, tobacco and alcohol use and intake of medicine for the last one year. Blood samples were collected under sterile conditions in heparinized tubes. The study was approved by the Human Ethics Committee, Mizoram University, Aizawl, India vide approval No. MZU/IHEC/2016/001.

2.3. Irradiation of blood

Irradiation of blood was performed in the Department of Radio-diagnosis and Imaging, Civil Hospital, Aizawl, Mizoram. Blood collected from the donors was exposed to a diagnostic X-rays source (Allengers 325 FC, Allengers Medical System Private Ltd., Solan, HP, India) in a polypropylene phantom (dimensions: 30x30 cm^2), placed transversally to the axis of the irradiation source. During the irradiation process, the radiation field was set at 20x20 cm^2, and the distance between the surface of the phantom and the source of radiation was 65 cm. Blood samples were irradiated at 97 kVp with 300 mAs. The average dose rate of exposure was measured using RaySafe. An average dose rate of 19.6 mGy/sec was given for 1.3, 2.6, 5.1,

10.2 and 15.3 sec for the total exposure doses of 25, 50, 100, 200 and 300 mGy, respectively, at an interval of 1 min. All samples were irradiated at room temperature. After exposure, the blood samples were allowed to sediment and the buffy coat was collected in a sterile tube and immediately stored at 4˚C for further use.



Irradiation of blood performed at Department of Radio-diagnosis and Imaging, Civil Hospital, Aizawl, Mizoram.

2.4. Lymphocyte culture and micronucleus assay

Lymphocytes were cultured following the method of Fenech and Morley (1985). Briefly, the blood was allowed to sediment and the buffy coat was collected in individual sterile glass tubes. After counting the viable cells with a haemocytometer, 1 x 10^6 nucleated cells were inoculated into sterile glass tubes containing RPMI-1640 medium supplemented with 10% fetal calf serum and phytohemagglutinin as a mitogen. The cells were allowed to grow for the next 44 hr in a humidified atmosphere of 5% CO_2 at 37˚C. Cytochalasin B was added at a final concentration of 5 µg/ml to block cytokinesis and cells were allowed to grow for another 28 hr. Cells were harvested at the end of 72 hr after initiating lymphocyte culture by centrifugation. A mild hypotonic solution was added to the cell pellet so as to retain the cell membrane. Cells were then fixed with freshly prepared Carnoy's fixative (methanol: acetic acid, 3:1). Cell suspension was placed in pre-cleaned coded slides to avoid observer's bias and spread by air blowing. The cells were stained with acridine orange and scored under a fluorescence microscope (DM 2500, Leica MikrosystemeVertrieb GmbH, Wetzlar, Germany). A total of 1000 binucleated cells (BNC) with well-preserved cytoplasm were scored from each individual for the presence of micronuclei (MN) according to the criteria described by Fenech et al. (2003).

2.5. Estimation of biochemical parameters

Antioxidants were estimated in the plasma of each individual. Total protein contents were determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.5.1. Glutathione (GSH)

GSH content was measured using the method described earlier (Moron et al., 1979). Briefly, 900 µl of 0.02 M sodium phosphate buffer (pH 8.0) and 20 µl of 10 mM 5,5´-dithio-2-nitroben-zoic acid (DTNB) were mixed with 80 µl of plasma and incubated for 2 min at room temperature. The absorbance of the sample was read against blank at 412 nm on a UV-Visible spectrophotometer (SW 3.5.1.0.

Biospectrometer, Eppendorf India Ltd., Chennai), and GSH concentration was calculated from the standard curve and expressed in µmol/mg protein.

2.5.2. Glutathione-s-transferase (GST)

GST activity was estimated by the method of Beutler (1984). Briefly, 850 µl of phosphate buffer was mixed with 50µl of 20 mM 1-chloro-2, 4-nitrobenzene (CDNB), incubated for 10 min at 37˚C followed by the addition of 50 µl each of plasma and 20 mM GSH. The blank consisted of all the reagents and distil water was added instead of plasma. The absorbance of blank and test was measured at 360 nm and enzyme activity was expressed in Unit/mg protein.

2.4.3. Superoxide dismutase (SOD)

SOD activity was measured by the method of Fried (1975). Briefly, 100 µl each of 186 µM phenazine methosulphate and plasma were mixed with 200 µl of 780 µM nicotinamide adenosine dinucleotide (NADH) and 300 µl of 3 mM nitrobluetetrazolium. After incubation for 90 sec at 30°C, 1 ml of acetic acid and 4 ml of n-butanol were added to stop the reaction. The blank consisted of all the reagents, and distil water was added instead of plasma. The absorbance of test and blank was measured at 560 nm and the enzyme activity was expressed in Unit/mg protein (1U = 50% inhibition of NBT reduction).

2.5.4. Lipid peroxidation (LPO)

Lipid peroxidation was estimated by the method of Beuege and Aust (1978) and expressed in terms of malondialdehyde content. Briefly, plasma was added to a mixture containing 10% trichloroacetic acid, 0.8% thiobarbaturic acid and 0.02 N HCl in a1:2 ratio. The mixture was boiled for 10 min in a boiling water bath, cooled immediately and centrifuged at 1000 rpm for 10 min. The supernatant was collected and its absorbance was read at 535 nm against the blank. The blank contained all the reagents and distil water was added instead of plasma. The malondialdehyde (MDA) concentration of the sample was calculated using an extinction coefficient of 1.56 X 10^{-6} M^{-1} cm^{-1}.

2.6. Statistical analyses

The results are expressed as mean ± standard error of the mean (SEM). One-way ANOVA followed by Tukey's post-hoc test was used for multiple comparisons. SPSS Ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA) and Graph Pad Prism ver. 6.0 were used for statistical analyses. A p-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Effects of low dose irradiation in chromosomal damage

The frequency of micronuclei (MN) in human peripheral blood lymphocytes exposed to low doses of X-ray was significantly higher than control (0 mGy), except at the lowest dose (25 mGy) where no significant ($p < 0.05$) change was observed when compared with control (Figure 5.1). A significant positive correlation ($r^2=0.98$, $p < 0.001$) was also observed between total MN frequency and the doses of X-ray exposure in human peripheral blood lymphocytes. Moreover, the formation of two micronuclei was observed only in 200 and 300 mGy irradiated lymphocytes.

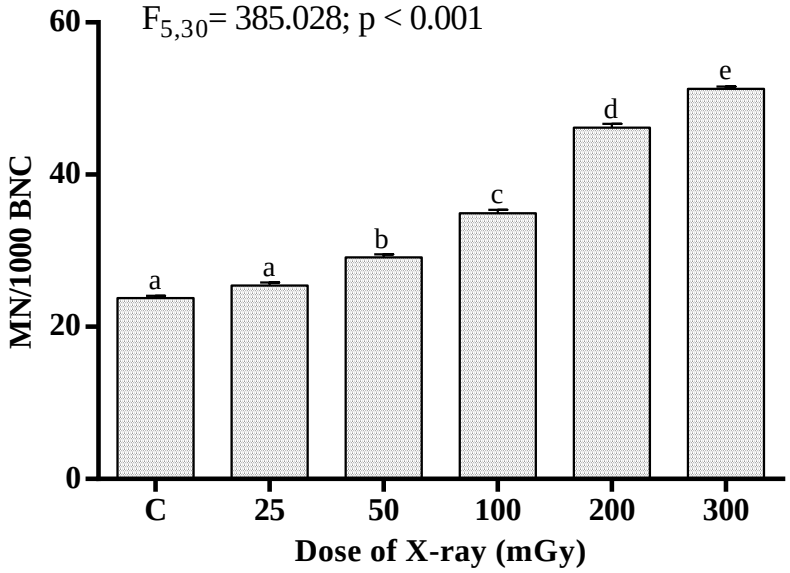
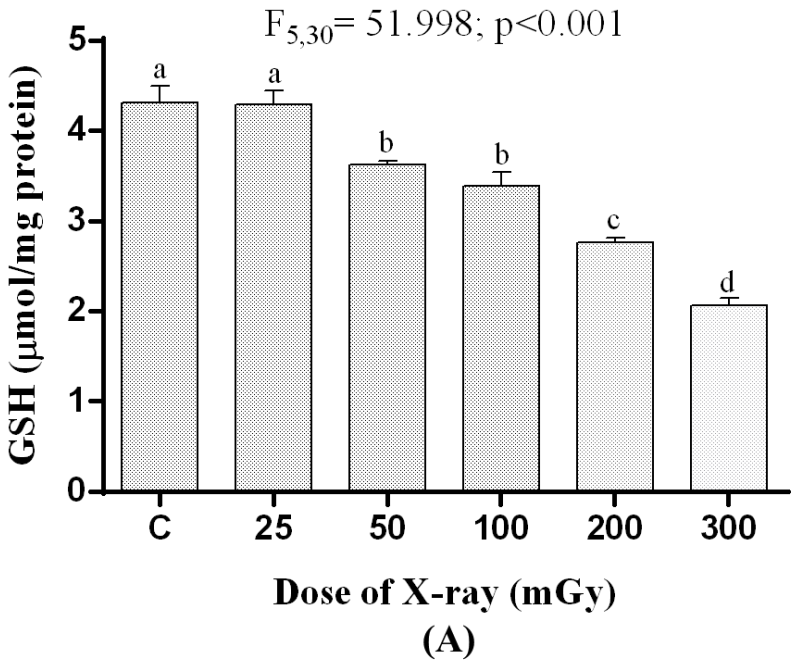
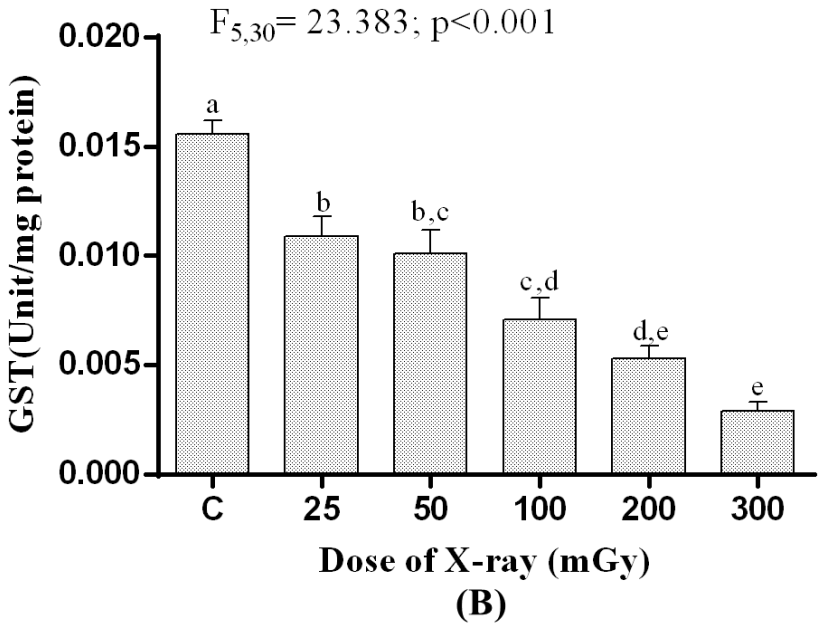


Figure 5.1: Effect of different doses of X-rays on the micronuclei formation in the cultured human peripheral blood lymphocyte of healthy donors. C: Control group; MN: Micronuclei frequency; BNC: Binucleated cells. *Means not sharing the same letter are significantly different (p<0.05).*

3.2. Effects of low dose irradiation in the antioxidant status

X-ray irradiation at low doses also resulted in dose-dependent alleviation of GSH content in human peripheral blood. However, no significant variation was observed between control and lowest dose (25 mGy) in the GSH content (Figure 5.2 A). *In-vitro* irradiation of human blood also caused a considerable change in GST activities. Increased dose of X-ray significantly reduced the GST activities. Even the lowest dose (25 mGy) of radiation led to a significant reduction in the activity of GST when compared with control (Figure 5.2 B). The activities of SOD did not show significant variation up to 50 mGy exposure. A significant reduction in SOD activity was observed only from the doses ≥ 100 mGy. However, no significant change in SOD activity was observed in lymphocytes exposed to doses between 200 mGy and 300 mGy (Figure 5.2 C). Irradiation-induced oxidative stress in human blood was clearly evident by elevated MDA content which is a common indicator of lipid peroxidation (Figure 5.3).





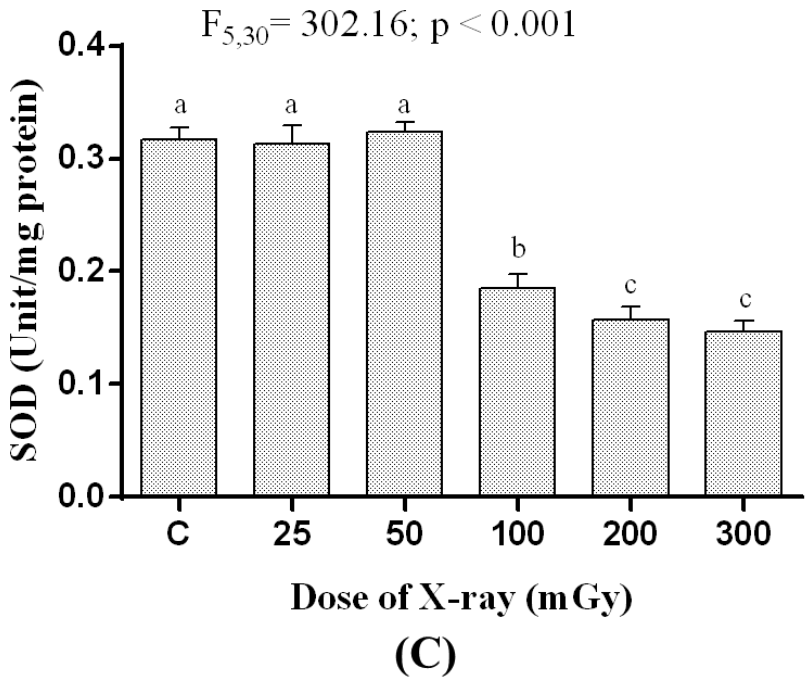


Figure 5.2: Effect of different doses of X-rays on (A) Glutathione (GSH) level (µmol/mg protein); (B) Glutathione-s-transferase (GST) activity (Unit/mg protein) and (C) Superoxide dismutase (SOD) activity (Unit/mg protein) in human peripheral blood lymphocytes of healthy donors. *Means not sharing the same letter are significantly different (p<0.05).*

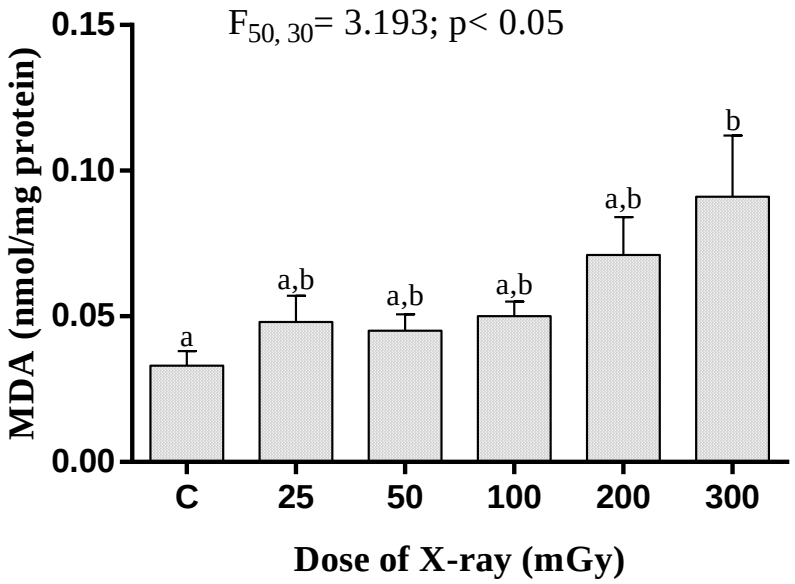


Figure 5.3: Effect of different doses of X-ray on Malondialdehyde (MDA) (nmol/mg protein) content in human peripheral blood lymphocytes of healthy donors. *Means not sharing the same letter are significantly different (p<0.05).*

4. DISCUSSION

The study of the genotoxic effect of ionizing radiation using micronucleus assay has been a major research area and it has been correlated with cytotoxic effect in various studies (Ramalho et al., 1998; Jagetia and Venkatesha 2006; Ropolo et al., 2012; Koyama et al., 2016). Micronuclei assay is an easy and less cumbersome technique for detecting chromosomal damage in human lymphocytes. Despite extensive use of MN assay as a quantitative indicator of X-rays induced chromosome damage in both *in-vitro* and *in-vivo* studies (Ramalho et al., 1988; Maffei et al., 2002; Joseph et al., 2004; Ropolo et al., 2012; Koyama et al., 2016), reports on the effects of fractionated X-rays irradiation on MN formation are limited. Furthermore, the potential effects of very low doses of radiation on the biological system need to be examined as radiation exposure received by human population are of low doses, which are emitted by natural sources such as cosmic rays, radionuclides present in the earth crust and diagnostic X-rays (UNSCEAR 2006). DNA double strand breaks (DSBs) are the major cytotoxic damage caused by ionizing radiation and are potent inducers of cell death (Morgan et al., 1996). In this study, human peripheral blood lymphocytes exposed to 0 to 300 mGy of X-rays increased the frequency of micronuclei in a linear manner. Cells of human embryo, newborn and children exposed to 0.02 to 2 Gy irradiation have been reported to induce micronuclei in a dose-dependent manner (Koyama et al., 2016). Similar results have been reported for human lymphocytes exposed to X-rays and γ-rays in earlier studies (Jagetia et al., 2003; Miszczyk et al., 2015). However, linear quadratic response for chromosomal damage after low-dose irradiation has been also reported (Lusiyanti et al., 2016). Pearce et al. (2012) reported that the risk of developing leukaemia might increase three times among children exposed to CT scans with a cumulative dose of about 50 mGy. In contrast, some studies reported insignificant change in the expression of radiation responsive genes in irradiated human peripheral white blood cells when compared to data from patients receiving total body irradiation (Amundson et al., 2004, Filiano et al., 2011, Paul et al., 2011). Cervelli et al. (2014) also reported insignificant differences in cell viability or DNA repair in vascular endothelial cells exposed to single or fractionated low-dose irradiation. Previous study also documented that the cultured human

lymphocytes can acquire resistance to chromosomal aberrations induced by subsequently high dose radiation when cells are pre-exposed to low-dose radiation known as adaptive response (Olivieri et al., 1984). Nevertheless, the present study confirms the ability of low doses of X-rays to induce MN formation and the ability of MN assay to estimate DNA damage at a low radiation dose of 50 mGy.

The majority of the energy of ionizing radiation such as X-rays deposited in cells results in the ejection of electrons from water which subsequently causes the formation of several ROS including the highly reactive hydroxyl radicals (OH) that inflict instant oxidative damage in various biomolecules (Adams, 1986; Le Caër, 2011). Ionizing radiations produces these free radicals within micro-seconds, however, their effects persist long after their production due to the cascade of events triggered by these free radicals at molecular and cellular level. This finally leads to an increase in the oxidative stress (OS) and instability of genome in the exposed cells (Einor et al., 2016). Ionizing radiation in the presence of molecular oxygen converts hydroxyl, superoxide, and organic radicals into hydrogen peroxide and organic peroxides which are longer lived ROS that continue to damage the cellular genome and other important biomolecules. Moreover, hydrogen peroxide reacts with redox-active metal ions, such as Fe^{2+} and Cu^{2+}, via Fenton`s reactions and generates the most deleterious OH radical and thus intensifying cellular oxidative stress leading to patho-physiology (Biaglow et al., 1992). In this study, human blood lymphocytes exposed to low doses of X-rays showed significantly higher malondialdehyde content indicating increased lipid peroxidation and reduced GSH levels and antioxidant enzyme activities of GST and SOD. Human lymphocytes irradiated with 3 Gy γ-radiation significantly reduced GST, CAT and SOD activities as compared to control (Bravard et al., 1999). Irradiation of mice resulted in a dose-dependent decline in the GSH concentration and activities of GPx; SOD and CAT with concomitant increase in the lipid peroxidation in various tissues including liver, intestine and skin indicating oxidative cellular damage (Jagetia and Reddy 2005; Jagetia and Rajanikant 2015; Jagetia and Shetty 2016). An increase in lipid peroxidation and reduction in CAT activity was observed in intestinal cells of mice exposed to 2 Gy of γ-rays and 1.6 Gy of ^{56}Fe radiation (Datta et al., 2012). A number of studies have revealed biochemical changes associated with a prolonged exposure

to ionizing radiation and their relationship to the antioxidant system (Achudume et al., 2010; Atasoy et al., 2013). The decrease in the GST and SOD activities in human blood lymphocytes exposed to low doses of X-rays may be due to the persistence of oxidative stress. In addition to the rapid burst of ROS observed immediately following irradiation, cells can exhibit a prolonged increase in ROS (Yoo et al., 2000), which may lead to oxidative cellular damage such as DNA lesions including double-strand breaks.

SUMMARY

Our study on the occupationally exposed radiological technicians has shown that protracted low dose exposure to ionizing radiation is an inevitable occupational hazard leading to the persistence of oxidative stress in the radiological technicians depending on the time spent with X-rays, cumulative dose received and the number of patients handled daily. The increased oxidative stress of radiological workers raises the risk of carcinogenesis after chronic low dose exposures. In light of these findings, the risk of cancer induction associated with protracted low-dose irradiation may need revision. The present study also confirmed radiations-imposed DNA damage in radiology workers during their occupational exposure. Although the previous studies have reported that majority of DNA lesions are repaired in a few hours and days following irradiation, DNA damage accumulates with time and the repair capacity decreases with chronic exposure. Furthermore, alterations in gene expression patterns were observed in chronically exposed radiation workers.

This study recommends a reduction of the exposure duration in any radiation area because the radiation dose received by a person is closely related to the time spent in the radiation area. Although monitoring of workers exposed to ionizing radiation consists of regular dosimetry by means of wearable radiation badges and periodic health examinations, personal dosimetry may underestimate the real exposure due to improper wearing of dosimeters or even radiation workers forgetting to wear them sometimes. Therefore, the findings from the present study suggest that the occupational radiology workers receiving chronic low-dose irradiation should be subjected to periodic biological dosimetry to assess genotoxic effects. It is also important to increase awareness about the deleterious effects of low doses of ionizing radiation among radiology workers, highlighting the necessity of best practice in applying radiation protection principles against medical radiation exposure. The regulatory bodies ought to establish and enforce measures for the protection of workers in existing exposure situations with regular maintenance of equipment and quality control programs.

Furthermore, our *in vitro* experiment reaffirms that exposure to low dose of ionizing radiation less than 100 mGy can cause genetic damage and induce oxidative

stress. It also reaffirms the ability of micronucleus assay to estimate DNA damage at a radiation dose as low as 50 mGy indicating its sensitivity to detect DNA damage.

REFERENCES

Abend M., Badie C., Quintens R., Kriehuber R., Manning G., Macaeva E., Njima M., Oskamp D., Strunz S., Moertl S., Doucha-Senf S., Dahlke S., Menzel, M. Port J. Examining Radiation-Induced *In Vivo* and *In Vitro* Gene Expression Changes of the Peripheral Blood in Different Laboratories for Biodosimetry Purposes: First RENEB Gene Expression Study, Radiat Res. 185 (2016) 109–123.

Achudume A., Onibere B., Aina F., Tchokossa P. Induction of oxidative stress in male rats subchronically exposed to electromagnetic fields at non-thermal intensities, JEMAA. 02 (2010) 482–487.

Adams G.E.D. Radiation carcinogenesis. In: Franks LM, Teich N, editors. Introduction to the cellular and molecular biology of cancer, Oxford, Oxford University Press. (1986) 154–175.

Aebi H. Catalase *in vitro*, Meth Enzymol. 105 (1984) 121–126.

Ahmad I.M., Temme J.B., Abdalla M.Y., Zimmerman M.C. Redox status in workers occupationally exposed to long-term low levels of ionizing radiation: A pilot study, Redox Report. 21 (2016) 139–145.

Aleksandra M., Sonja I., Vesna S., Slobodan J. A dose estimation for persons occupationally exposed to ionizing radiation in Montenegro. Arch. Oncol. 16 (2008) 1-2.

Al-Mohammed HI., Mahyoub FH., Moftah BA. Comparative study on skin dose measurement using MOSFET and TLD for pediatric patients with acute lymphatic leukemia. Medical Science Monitor: Int. Med. J. Exp. Clin. Res. 16 (2010) 325-329.

Amundson S.A., Bittner M., Meltzer P., Trent J., Fornace Jr. A.J. Biological indicators for the identification of ionizing radiation exposure in humans, Expert Rev Mol Diagn. 1 (2001) 211–219.

Amundson S.A., Do K.T., Shahab S., Bittner M., Meltzer P., Trent J., Fornace Jr. A.J. Identification of potential mRNA biomarkers in peripheral blood lymphocytes for human exposure to ionizing radiation, Radiat Res. 154 (2000) 342–346.

Amundson S.A., Grace M.B., McLeland C.B., Epperly M.W., Yeager A., Zhan Q., Greenberger J.S., Fornace Jr. A.J. Human *In-vivo* radiation-induced biomarkers: Gene Expression changes in radiotherapy patients, Cancer Res. 64 (2004) 6368–6371.

An M.Y., Kim T.H. Frequencies of micronuclei in peripheral lymphocytes in Korean populations after chronic low-dose radiation exposure, J Vet Sci. 3 (2002) 213–218.

Andreassi M.G., Cioppa A., Botto N., Joksic G., Manfredi S., Federici C., Ostojic M., Rubino P., Picano E. Somatic DNA damage in interventional cardiologists: a casecontrol study, FASEB J. 19 (2005) 998–999.

Andreassi M.G., Foffa I., Manfredi S., Botto N., Cioppa A., Picano E. Genetic polymorphisms in XRCC1, OGG1, APE1 and XRCC3 DNA repair genes, ionizing radiation exposure and chromosomal DNA damage in interventional cardiologists, Mutat Res. 666 (2009) 57–63.

Aoyama T. Radiation risk of Japanese and Chinese low-level dose repeatedly irradiated population, Int J Occup Environ Hlth. 11 (1989) 432–442.

Atasoy H.I., Gunal M.Y., Atasoy P., Elgun S., Bugdayci G. Immuno-histopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices, J. Pediatr. Urol. 9 (2013) 223–229.

Azzam E.I., Jay-Gerin J.P., Pain D. Ionizing radiation–induced metabolic oxidative stress and prolonged cell injury, Cancer. Lett. 327 (2012) 48–60.

Badie C., Kabacik S., Balagurunathan Y., Bernard N., Brengues M. Laboratory intercomparison of gene expression assays, Radiat Res. 180 (2013) 138–148.

Barquinero J.F., Barrios L., Caballin M.R., Miro R., Ribas M., Subias A., Egozcue J. Cytogenetic analysis of lymphocytes from hospital workers occupationally exposed to low levels of ionizing radiation, Mutat Res. 286 (1993) 275–279.

Baselet B., Rombouts C., Benotmane A.M., Baatout S., Aerts A. Cardiovascular diseases related to ionizing radiation: the risk of low-dose exposure, Int J Mol Med. 38 (2016) 1623–1641.

Basheerudeen S.A.S., Kanagaraj K., Jose M.T. Entrance surface dose and induced DNA damage in blood lymphocytes of patients exposed to low-dose and low-dose-rate X-irradiation during diagnostic and therapeutic interventional radiology procedures, Mutat Res. 818 (2017) 1-6.

Bedford J. S., Mitchell J. B. Dose-rate effects in synchronous mammalian cells in culture, Radiat. Res. 54 (1973) 316-327.

Behjati S., Gundem G., Wedge D.C., Roberts N.D., Tarpey P.S., Cooke S.L. Mutational signatures of ionizing radiation in second malignancies, Nat Commun. 7 (2016) 12605.

BEIR VII. Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation. Health Risks From Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2. National Academy of Sciences, National Research Council. The National Academies Press, Washington, 2006.

Berrington A., Gonzalez A De., Darby S. Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries, Lancet. 363 (2004) 345-51.

Bessho T. Nucleotide excision repair 39 endonuclease XPG stimulates the activity of base excision repairenzyme thymine glycol DNA glycosylase, Nucleic Acids Res. 27 (1999) 979–983.

Betti C., Davini T., Gianessi L., Loprieno N., Barale R. Microgel electrophoresis assay (comet test) and SCE analysis in human lymphocytes from 100 normal subjects, Mutat. Res. 307 (1994) 323–333.

Beuege J.A., Aust S.D. Microsomal lipid peroxidation, Methods Enzymol, 30 (1978) 302–310.

Beutler E. Red cell metabolism: A manual of biochemical methods. 3rd Edition, New York, Grune and Stratton Inc. (1984).

Beyea J. The scientific jigsaw puzzle: fitting the pieces of the low-level radiation debate, Bull Atomic Sci. 68 (2012) 13–28.

Biaglow J.E., Mitchell J.B., Held K. The importance of peroxide and superoxide in the X-ray response, Int J Radiat Oncol Biol Phys. 22 (1992) 665-669.

Blair I.A. DNA adducts with lipid peroxidation products, J Biol Chem. 283 (2008) 15545–15549.

Blaisdell J.O., Wallace S.S. Abortive base-excision repair of radiation induced cluster DNA lesions in *Escherichia coli*, Proc. Natl. acad. Sci. USA. 98 (2001) 7426-7430.

Bonassi S., Bolognesi C., Abbondandolo A., Barale R., Bigatti P., Camurri L. Influence of sex on cytogenetic end points: evidence from a large human sample and review of the literature, Cancer Epidemiol Biomark Prev. 4 (1995) 671–679.

Bonassi S., Forni A., Bigatti P., Canevarollo N., De Ferrari M., Lando C., Padovani P., Bevegni M., Stella M., Vecchio D. Chromosome aberrations in hospital workers: evidence from surveillance studies in Italy, Am J Ind Med. 3 (1997) 353–360.

Bonassi S., Ugolini D., Kirsch-Volders M., Stromberg U., Vermeulen R., Tucker J.D. Human population studies with cytogenetic biomarkers: review of the literature and future prospectives, Environ. Mol. Mutagenesis. 54 (2005) 258–270.

Boyaci N., Yalcin R., Cengel A., Erdem O., Dörtlemez O., Dörtlemez H. Evaluation of DNA damage in lymphocytes of cardiologists exposed to radiation during cardiac catheterization by the COMET ASSAY, Jpn Heart J. 45 (2004) 845-53.

Bravard A., Luccioni C., Moustacchi E., Rigaud O. Contribution of antioxidant enzymes to the adaptive response to ionizing radiation of human lymphoblasts, Int. J. Radiat. Biol. 75 (1999) 639-645.

Broerse J.J., Barendsen G. W., Van Kersen G. R. Survival of cultured human cells after irradiation with fast neutrons of different energies in hypoxic and oxygenated conditions, Int. J. Radiat. Biol. 13 ((1968) 559-572.

Budworth H., Dianova II., Podust V.N., Dianov G.L. Repair of clustered DNA lesions: Sequence specific inhibition of long patch base excision repair by 8-oxoguanine, J. Biol. Chem. 277 (2002) 21300-21305.

Burkart W. Combined effect of radiation and other agents: Is there a synergism trap? J. Environ. Pathol. Toxicol. Oncol. 20 (2001) 53-58.

Cardoso R.S., Takahashi-Hyodo S., Peitl P., Ghilardi-Neto T., Sakamoto-Hojo E.T. Evaluation of chromosomal aberrations, micronuclei, and sister chromatid exchanges in hospital workers chronically exposed to ionizing radiation, Teratog, Carcinog Mutagen. 21 (2001) 431–439.

Carter S.B. Effects of cytochalasins on mammalian cells, Nature. 213 (1967) 261–264.

Cervelli T., Panetta D., Navarra T., Andreassi M.G., Basta G., Galli A., Salvadori P.A., Picano E., Turco S.D. Effects of single and fractionated low-dose irradiation on vascular endothelial cells, Atherosclerosis. 235 (2014) 510-8.

Chaubey C., Bhilwade H. N., Rajagopalan R., Bannur S. V. Gamma Ray Induced DNA Damage in Human and Mouse Leukocytes Measured by SCGE-Pro: a Software Developed for Automated Image Analysis in Data Processing for Comet Assay, Mutat. Res. 490 (2001) 187–197.

Chaudhary M.A., Chodosh L.A., McKenna W.G., Muschel R.J. Gene expression profile of human cells irradiated in G1 and G2 phases of cell cycle, Cancer Lett. 195 (2003) 221–233.

Choi K.H., Ha M., Lee W.J., Hwang S.S., Jeong M., Jin Y.W., Kim H.J., Lee K.Y., Lee J.E., Kang J.W. Cancer risk in diagnostic radiation workers in Korea from 1996–2002, Int J Environ Res Public Health. 10 (2013) 314–327.

Christmann M., Tomicic M.T., Roos W.P., Kaina B. Mechanisms of human DNA repair; an update, Toxicology. 193 (2003) 3-34.

Church D.F., Pryor W.A. Free-radical chemistry of cigarette smoke and its toxicological implications, Environmental Health Perspectives. 64 (1985) 111-126.

Clutton S.M., Townsend K.M., Walker C., Ansell J.D., Wright E.G. Radiation-induced genomic instability and persisting oxidative stress in primary bone marrow cultures, Carcinogenesis. 17 (1996) 1633–1639.

Collins A.R. The comet assay for DNA damage and repair: principles, applications, and limitations, Mol Biotechnol. 26 (3) (2004) 249-261.

Collins A.R., Rašlová K., Somorovská M., Petrovská H., Ondrusová A., Vohnout B., Fábry R., Dusinská M. DNA damage in diabetes: correlation with a clinical marker. Free Radic. Biol. Med. 25 (1997) 373–377.

Connor N. What is stochastic effect- Ionizing Radiation- Definition. (2019). https://www.radiation-dosimetry.org/what-is-stochastic-effect-ionizing-radiation-definition/

Covens P., Berus D., Buls N., Clerinx P., Vanhavere F. Personal dose monitoring in hospitals: Global assessment, critical applications and future needs, Radiation Prot. Dosimetry. 124 (3) (2007) 250–259.

Cruz-Garcia L., O'Brien G., Donovan E., Gothard L., Boyle S., Laval A., Testard I., Ponge L., Wozniak G., Miszczyk L. Influence of confounding factors on radiation dose estimation using in vivo validated transcriptional biomarkers. Health Phys. 115(2018) 90–101.

Dariusz L. The Grand Challenge: use of a new approach in developing policies in the area of radiation and health, Frontiers in Public Health. 2 (50) (2014) 1–4.

Datta K., Suman S., Kallakury B.V.S., Fornace A.J. Exposure to heavy ion radiation induces persistent oxida-tive stress in mouse intestine, PloS One. 7 (2012) e42224.

De Bont R., van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data, Mutagenesis. 19 (2004) 169–185.

Deckbar D., Jeggo P.A., Lobrich M. Understanding the limitations of radiation-induced cell cycle checkpoints, Crit. Rev. Biochem. Mol. Biol. 46 **(**2011) 271-83.

Demirel S., Durakbasi G., Zamani A.G., Aynur. X-ray induced micronucleus frequency assay on radiology technicians' lymphocytes, Turk J Med Res. 15 (1997) 56-59.

Dhawan A., Mathur N., Seth P.K. The effect of smoking and eating habits on DNA damage in Indian population as measured in the Comet assay, Mutat. Res. 474 (2001) 121–128.

Ding L.H., Shingyoji M., Chen F., Hwang J.J., Burma S., Lee C. Gene expression profiles of normal human fibroblasts after exposure to ionizing radiation: a comparative study of low and high doses, Radiat. Res. 164 (2005) 17–26.

Do K.H. General principles of radiation protection in fields of diagnostic medical exposure, J. Korean Med. Sci. 31 (2016) S6–S9. doi: 10.3346/jkms.2016.31.S1.S6.

Edwards J.C., Chapman D., Cramp W.A., Yatvin M.B. The effects of ionizing radiation on biomembrane structure and function, Prog. Biophys. Mol. Biol. 43 (1984) 71-93.

Efstathopoulos E.P., Makrygiannis S.S., Kottou S., Karvouni E., Giazitzoglou E., Korovesis S., Tzanalaridou E., Raptou P.D., Katritsis D.G. Medical personnel and patient dosimetry during coronary angiography and intervention, Phys. Med. Biol. 48 (2003) 3059–3068.

Einor D., Bonisoli-Alquati A., Costantini D., Mousseau T.A., Møller A.P. Ionizing radiation, antioxidant response and oxidative damage: a meta-analysis, Sci Total Environ. 548 (2016) 463–471.

Eken A., Aydın A., Erdem O., Akay C., Sanal H.T., Soykut B., Sayal A., Somuncu I. Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation, Toxicol Ind Health. 26 (2010) 273–280.

Eken A., Aydin A., Erdem O., Akay C., Sayal A., Somuncu I. Induced antioxidant activity in hospital staff occupationally exposed to ionizing radiation, Int J Radiat Biol. 88 (2012) 648–653.

Elgazzar A.H., Elsaid M. Biological effects of ionizing radiation. In Elgazzar AH, editor. The pathophysiologic basis of nuclear medicine, Berlin: Springer-Verlag; (2001) 369–370.

Elkind M., Sutton-Gilbert M., Moses H., Alescio W. B., Swain R. B. Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic), Radiat. Res. 25 ((1965) 359-376.

Evans H.J. Molecular mechanisms in the induction of chromosome aberrations, in: D. Scott, B.A. Bridges, F.H. Sobels (Eds.), Progress in Genetic Toxicology, Elsevier North Holland Biomedical Press, (1977), 57–74.

Evans H.J., Buckton K.E., Hamilton G.E., Carothers A. Radiation-induced chromosome aberrations in nuclear dockyard workers, Nature. 277 (1979) 531–535.

Fachin A.L., Mello S.S., Sandrin-garcia P., Junta C.M., Ghilardi-netto T., Donadi E.A., Da silva passos G.A., Sakamoto-hojo E.T. Gene expression profiles in radiation workers occupationally exposed to ionizing radiation, J. Radiat. Res. 50 (2009) 61–71.

Fairbairn D.W., Olive P.L., O'Niell K.L. A comet assay: a comprehensive review. Mutat. Res. 33 (1995) 37-59.

Fazel R., Krumholz H.M., Wang Y., Ross J.S., Chen J., Ting H.H., Shah N.D., Nasir K., Einstein A.J., Nallamothu B.K. Exposure to low-dose ionizing radiation from medical imaging procedures, N Engl J Med. 361 (2009) 849–857.

Feinendegen L.E., Brooks A.L., Morgan W.F. Biological consequences and health risks of low-level exposure to ionizing radiation: commentary on the workshop, Health Phys. 100 (2011) 247–259

Fenech M., Bonassi S. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes, Mutagenesis. 26 (1) (2011) 43–49.

Fenech M., Chang W.P., Kirsch-Volders M., Holland N., Bonassi S., Zeiger E. HUMN project: detailed description of the scoring criteria for the cytokinesis block

micronucleus assay using isolated human lymphocyte cultures, Mutat Res. 53 (2003) 65–75.

Fenech M., Holland N., Chang W.P., Zeiger E., Bonassi S. The Human micronuclei project-an international collaborative study on the use of the micronuclei technique for measuring DNA damage in humans, Mutat. Res. 428 (1999) 271–283.

Fenech M., Kirsch-Volders M., Natarajan A.T., Surralles J., Crott J.W., Parry J., Norppa H., Eastmond D.A., Tucker J.D., Thomas P. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells, Mutagenesis. 26 (2011) 125–132.

Fenech M., Morley A.A. Cytokinesis-block micronucleus method in human lymphocytes: effect of *in vivo* ageing and low dose X irradiation, Mutat. Res. 161 (1986) 193–198.

Fenech M., Morley A.A. Measurement of micronuclei in lymphocytes, Mutat Res. 147 (1985) 29–36.

Fenech M., Morley A.A. The effect of donor age on spontaneous and induced micronuclei, Mutat. Res. 148 (1985) 99–105.

Fenech M., Neville S., Rinaldi J. Sex is an important variable affecting spontaneous micronucleus frequency in cytokinesis-blocked lymphocytes, Mutat Res. 313 (1994) 203–207.

Filiano A.N., Fathallah-Shaykh H.M., Fiveash J., Gage J., Cantor A., Kharbanda S., Johnson M.R. Gene expression analysis in radiotherapy patients and C57BL/6 mice as a measure of exposure to ionizing radiation, Radiat. Res. 176 (2011) 49-61.

Franco N., Lamartine J., Frouin V., Le Minter P., Petat C., Leplat J.J. Low dose exposure to gamma rays induces specific gene regulations in normal human keratinocytes, Radiat. Res. 163 (2005) 623–635.

Frenzili G., Betti C., Davini T. Evaluation of DNA damage in leukocytes of ex-smokers by single cell gel electrophoresis, Mutat Res. 375 (1997) 117-23.

Fried R. Enzymatic and non-enzymatic assay of superoxide dismutase, Biochimie. 57 (1975) 657–660.

Fuchs J., Hengstler J.G., Jung D., Hiltl G., Konietzko J., Oesch F. DNA damage in nurses handling antineoplastic agents. Mutat. Res. 342 (1995) 17-23.

G.W. Burton, M.G. Traber, Vitamin E: antioxidant activity, biokinetics, and bioavailability, Annu. Rev. Nutr. 10 (1990) 357-82.

G.Z. Guo, K. Sasai, N. Oya, T. Takagi, K. Shibuya, M. Hiraoka, Simultaneous evaluation of radiation-induced apoptosis and micronuclei in five cell lines, Int J Radiat Biol. 73 (1998) 297–302.

Garaj-Vrhovac V., Kopjar N. Application of the alkaline comet assay in human biomonitoring for genotoxicity: A study on Croatian medical personnel handling antineoplastic drugs. Mutagenesis. 16 (2002) 71-8.

Garaj-Vrhovac V., Kopjar N. The comet assay- a new technique for detection of DNA damage in genetic toxicology studies and human biomonitoring. Period. Biol. 100 (1998) 361-366.

Gaschler M.M., Stockwell B.R. Lipid peroxidation in cell death, Biochem Biophys Res Commun. 482 (2017) 419–425.

Gharibdousty T.S., Zakeri F., Changizi V., Rajabpour M.R., Farshidpour M.R. Low levels of ionizing radiation exposure and cytogenetics effects in radiopharmacy, Biosc Biotech Res Comm. 10 (2017) 56–62.

Gilbert E.S. Ionizing Radiation and Cancer Risks: What have we learned from epidemiology? Int. J. Radiat. Biol. 85 (2009) 467–482.

Goldberg Z., Schwietert C. W., Lehnert B., Stern R., Nami I. Effects of low-dose ionizing radiation on gene expression in human skin biopsies, Int J Radiat Oncol Biol Phys. 58 (2004) 567–574.

Gonzalez A.B., Darby S. Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries, Lancet. 363 (2004) 345-51.

Grant E.J., Cologne J.B., Sharp G.B., Eguchi H., Stevens R.G., Izumi S. Bioavailable serum estradiol may alter radiation risk of postmenopausal breast cancer: a nested case-control study, Int J Radiat Biol. 94(2) (2018) 97–105.

Grosse Y., Baan R., Straif K., Secretan B., El Ghissassi F., Bouvard V., Benbrahim-Tallaa L., Guha N., Galichet L., Cogliano V. A Review of Human Carcinogens–Part A: Pharmaceuticals, Lancet Oncol. 10 (2009) 13–14.

Gruel G., Voisin P., Vaurijoux A., Roch-Lefevre S., Grégoire E., Maltere P. Broad modulation of gene expression in CD4+ lymphocyte subpopulations in response to low doses of ionizing radiation, Radiat. Res. 170 (2008) 335–344.

Guo G.S., Zhang F.M., Gao R.J., Delsite R., Feng Z.H., Powell S. N. DNA repair and synthetic lethality, Int. J. Oral. Sci. 3 **(**2011) 176-9.

Gutteridge J.M.C. Lipid peroxidation and antioxidants as biomarkers of tissue damage, Clin. Chem. 41 (1995) 1819–1828.

Hall E.J. Radiation biology for pediatric radiologists, Pediatr. Radiol. 39 (2009) S57–64.

Hall E.J. Radiation, the two-edged sword: cancer risks at high and low doses, Cancer. J. 6 (2000) 343–50.

Halliwell B., Gutteridge J.M.C. Free radicals in biology and medicine, Free Radic Biol Med. 1 (1985) 331–332.

Hamada N., Fujimichi Y. Classification of radiation effects for dose limitation purposes: history, current situation and future prospects, J Radiat Res. 55(4) (2014) 629-40.

Han W., Yu K.N. Response of cells to ionizing radiation. Advances in biomedical sciences and engineering. China (Ed.: S.C. Tjong): Bentham Science Publishers Ltd., (2010) 204–262.

Han W., Yu K.N. Response of cells to ionizing radiation. In: Tjong SC, editor, Advances in biomedical sciences and engineering. China: Bentham Science Publishers Ltd. (2010) 204–262.

Hang B. Formation and repair of tobacco carcinogen-derived bulky DNA adducts, J. Nucleic Acids. 709521 (2010) 29. doi:10.4061/2010/709521.

Hartmann A., Plappert U., Raddatz K., Grunert-Fuchs M., Speit G. Does physical activity induce DNA damage? Mutagenesis. 9 (1994) 269–272.

Heinloth A.N., Shackelford R.E., Innes C.L., Bennett L., Li L., Amin R.P., Sieber S.O., Flores K.G., Bushel P.R., Paules R.S. Identification of distinct and common gene expression changes after oxidative stress and gamma and ultraviolet radiation, Mol Carcinog. 37 (2003) 65–82.

Hellman B., Vaghef H., Bostrom B. The concept of tail moment and tail inertia in the single gel electrophoresis assay. Mutat. Res. 336 (1995) 123-131.

Hellman B., Vaghef H., Friis L., Endling C. Alkaline single cell gel electrophoresis of DNA fragments in biomonitoring for genotoxicity: an introductory study on healthy human volunteers. Int. Arch. Occup. Environ. Health. 69 (1997) 185-192.

Hellman B., Vaghef H., Friis L., Endling C., Alkaline single cell gel electrophoresis and human biomonitoring for genotoxicity: a study on subjects with residential exposure to radon. Mutat. Res. 442 (1999) 121-132.

Herman C., Johnson T.E. Introduction to Health Physics; 4th edition, United States: The McGraw-Hill Companies (2009).

Ho W.Y., Wong K., Leung Y., Cheng K. Radiation Doses to Staff in a Nuclear Medicine Department. Radiation Doses to Nuclear Medicine Staff J HK, Coll Radiol. 5 (2002) 24–28.

Hong J.Y., Han K., Jung J.H., Kim J.S. Association of exposure to diagnostic low dose ionizing radiation with risk of cancer among youths in South Korea, JAMA Netw Open. 2(9) (2019) e1910584.

Howard A., Pelc S. R. Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage, Heredity. 6. (1953) 261.

Howe O., White L., Cullen D., O'Brien G., Shields L., Bryant J., Noone E., Bradshaw S., Finn M., Dunne M. A 4-Gene Signature of CDKN1, FDXR, SESN1 and PCNA Radiation Biomarkers for Prediction of Patient Radiosensitivity, Int. J. Mol. Sci. 22 (2021) 10607.

International Agency for Research on Cancer (IARC). Monographs on the evaluation of carcinogenic risks to humans, working group on the evaluation of carcinogenic risks to humans of ionizing radiation, X-ray and gamma radiation and neutrons, IARC. 75 (2000) 1–448.

International Commission on Radiological Protection (ICRP), Recommendations of the International Commission on Radiological Protection. ICRP Publication 26. Ann. ICRP 1 (1977) 1-3.

International Commission on Radiological Protection. The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103, Elsevier, Oxford. Annals of the ICRP 37 (2007) 2-4.

Ishikawa H., Yamamoto H., Tian Y., Kawano M., Yamauchi T., Yokoyama K. Effects of ALDH2 gene polymorphisms and alcohol drinking behaviour on micronuclei frequency in non-smokers, Mutat. Res. 541 (2003) 71–80.

Jackson S. P. Sensing and repairing DNA double-strand breaks. Carcinogenesis. 23 (2002) 687–696.

Jagetia G.C., Jayakrishnan A., Fernandes D., Vidyasagar M.S. Evaluation of micronuclei frequency in the cultured peripheral blood lymphocytes of cancer patients before and after radiation treatment, Mutat. Res. 491 (2001) 9–16.

Jagetia G.C., Rajanikant G.K. Curcumin stimulates the antioxidant mechanisms in mouse skin exposed to fractionated c-irradiation, Antioxidants (Basel). 4 (2015) 25–41.

Jagetia G.C., Reddy T.K. Modulation of radiation induced alteration in the antioxidant status of mice by naringin, Life Sci. 77 (2005) 780–794.

Jagetia G.C., Shetty P.C. Augmentation of antioxidant status in the liver of Swiss albino mice treated with jamun *Syzygium cumini*, Skeels extract before whole body exposure to different doses of γ-radiation, J. Adv. Res. Biotech. 1(1) (2016) 13.

Jagetia G.C., Venkatesh P., Baliga M.S. Evaluation of the radioprotective effect of *Aegle marmelos* (L.) Correa in cultured human peripheral blood lymphocytes exposed to different doses of ɣ-radiation: a micronucleus study, Mutagenesis. 18 (2003) 387–393.

Jagetia G.C., Venkatesha V.A. Treatment of mice with stem bark extract of *Aphanamixis polystachya* reduces radiation-induced chromosome damage, Int J Radiat Biol. 82 (2006) 197–209.

Janssen R., Hadders R., Henkelman M., Bosli A. Exposure to operating staff during cardiac catheterization measured by thermoluminescence dosimetry, Radiat Prot.Dosim. 43 (1992) 175–177.

Jeggo P.A., Pearl L.H., Carr A.M. DNA repair, genome stability and cancer: a historical perspective, Nature Reviews. 16 (2016) 35-42.

Jha A.N., Sharma T. Enhanced frequency of chromosome aberrations in workers occupationally exposed to diagnostic X-rays, Mutat Res. 260 (1991) 343–348.

Joiner M.C., Thomas R.A., Grever W.E., Smolinski J.M., Divine G.W., Konski A.A., Auner G.W., Tucker J.D. Developing point of care and high-throughput biological assays for determining absorbed radiation dose, Radiother Oncol. 101 (2011) 233–236.

Joseph J., Patwardhan U. M., Samuel A. B. Frequency of micronuclei in peripheral blood lymphocytes from subjects occupationally exposed to low levels of ionizing radiation, Mutat. Res. 564 (2004) 83–88.

Joseph L.J., Patwardhan U.N., Samuel A.M. Frequency of micronuclei in peripheral blood lymphocytes from subjects occupationally exposed to low levels of ionizing radiation, Mutat. Res. 564 (2004) 83–88.

Kalaiselvi K., Rajaguru P., Palanivel M., Usharani M.V., Ramu G. Chromosomal aberration, micronucleus and Comet assays on peripheral blood lymphocytes of leprosy patients undergoing multidrug treatment. Mutagenesis. 17 (2002) 309–312.

Kaplan D.J., Patel J.N., Liporace F.A., Yoon R.S. Intraoperative radiation safety in orthopedics: a review of the ALARA (As low as reasonably achievable) principle, Patient Saf Surg. 10 (2016) 27.

Karppinen J., Parvianen T., Servomaa A., Komppa T. Radiation risk and exposure of radiologists and patients during coronary angiography and percutaneous transluminal coronary angioplasty (PTCA), Radiat Prot.Dosim. 57 (1995) 481–485.

Khanna K.K., Jackson S.P. DNA double-strand breaks: signaling, repair and the cancer connection, Nat Genet. 27 (2001) 247–254.

Khisroon M., Khan A., Naseem M., Ali N., Khan S., Rasheed S.B. Evaluation of DNA damage in lymphocytes of radiology personnel by comet assay, J Occup Health. 57 (2015) 268–274.

Kim J.S., Kim Y.J., Kim T.Y., Song J.Y., Cho Y.H., Park Y.C., Chung H.W. Association of ALDH2 polymorphism with sensitivity to acetaldehyde-

induced micronuclei and facial flushing after alcohol intake, Toxicology. 210 (2005) 169–174.

Kim K.P., Miller D.L., Balter S., Kleinerman R.A., Linet M.S., Kwon D., Simon S.L. Occupational radiation doses to operators performing cardiac catheterization procedures, Health Phys. 94 (2008) 211–227.

Kirsch-Volders M., Plas G., Elhajouji A., Lukamowicz M., Gonzalez L., Loock K.V., Decordier I. The *in-vitro* MN assay in 2011: Origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance, Arch. Toxicol. 85 (2011) 873–899.

Knops K., Boldt S., Wolkenhauer O., Kriehuber R. Gene expression in low- and high-dose-irradiated human peripheral blood lymphocytes: possible applications for biodosimetry, Radiat. Res. 178 (2012) 304–312.

Kobashigawa S., Suzuki K., Yamashita S. Ionizing radiation accelerates Drp1-dependent mitochondrial fission, which involves delayed mitochondrial reactive oxygen species production in normal human fibroblast-like cells, Biochem. Biophys. Res. Commun. 414 (2011) 795–800.

Kohanoff J., Artacho E. Water radiolysis by low-energy carbon projectiles from first-principles molecular dynamics, PLoS One. 12 (2017) 0171820.

Kono Y., Fridovich I. Superoxide radical inhibits catalase, J. Biol. Chem. 257 (1982) 5751–5754.

Kopec R., Budzanowski M., Budzyniska A., Czepczynski R., Dziuk M., Sowinski J., Wyszomirska A. On the relationship between whole body, extremity and eye lens doses for medical staff in the preparation and application of radiopharmaceuticals in nuclear medicine, Radiat. Meas. 46 (2011) 1295–1298.

Kopjar N., Garaj-Vrhovac V. Application of the alkaline comet assay in biomonitoring of medical personnel occupationally exposed to ionizing radiation, European IRPA congress 2002: Proceedings Firenca, (2002) 1-10.

Koyama S., Narita E., Shinohara N., Miyakoshi J. Effect of low-dose X-ray irradiation on micronucleus formation in human embryo, newborn and child cells, Int J Radiat Biol. 92 (2016) 790–795.

Kruszewski M., Wojewodzka M., Iwanenko T., Collins A.R., Szumiel I. Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation. II. Base damage, Mutat. Res. 416 (1998) 37–57.

Le Caer S. Water radiolysis: influence of oxide surfaces on H_2 production under ionizing radiation, Water. 3 (2011) 235–253.

Lehnert S. Biomolecular action of ionizing radiation (Series in Medical Physics and Biomedical Engineering). Taylor and Francis Group, New York (2007).

Leuraud K., Richardson D.B., Cardis E., Daniels R.D., Gillies M., O'Hagan J.A., Hamra G.B., Haylock R., Laurier D., Moissonnier M. Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): An international cohort study, Lancet. Haematol. 2 (2015) 276–281.

Li M.J., Wang W.W., Chen S.W., Shen Q., Min R. Radiation dose effect of DNA repair-related gene expression in mouse white blood cells, Med Sci Monitor: Int Med J Exp Clin Res. 17 (2011) 290–297.

Linet M.S., Slovis T.L., Miller D.L. Cancer risks associated with external radiation from diagnostic imaging procedures, CA Cancer J Clin. 62(2) (2012) 75-100.

Liu G., Gong P., Bernstein L.R. Apoptotic cell death induced by low-dose radiation in male germ cells: Hormesis and adaptation, Crit. Rev. Toxicol. 37 (2007) 587-605.

Livak K.J., Schmittgen T.D., Analysis of relative gene expression data using real-time quantitative PCR and the 2(–Delta Delta C(T)) method, Methods. 25 (2001) 402-408.

Lloyd D.C., Purrott R.J., Reeder E.J. The incidence of unstable chromosome aberration in peripheral blood lymphocytes from irradiated and occupationally exposed people, Mutat Res. 72 (1980) 523–532.

Lonergan P.E., Martin D.S.D., Horrobin D.F., Lynch M.A. Neuroprotective effect of eicosapentaenoic acid in hippocampus of rats exposed to gamma-irradiation, J Biol Chem. 277 (2002) 20804–20811.

López P.O., Dauer L.T., Loose R., Martin C.J., Miller D.L., Vañó E., Doruff M., Padovani R., Massera G., Yoder C. Authors on Behalf of ICRP. ICRP Publication 139: Occupational Radiological Protection in Interventional Procedures, Ann ICRP. 47(2) (2018) 1-118.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. Protein measurement with the folin phenol reagent, J Biol Chem. 193 (1951) 265–275.

Lucas J., Dressman H.K., Suchindran S., Nakamura M., Chao N.J., Himburg H., Minor K., Phillips G., Ross J., Abedi M., Terbrueggen R., Chute J.P. A translatable predictor of human radiation exposure, PLoS One. 9 (2014) e107897.

Lusiyanti Y., Alatas Z., Syaifudin M., Purnami S. Establishment of a dose-response curve for X-ray-induced micronuclei in human lymphocytes, Genome Integr. 7 (2016) 7-15.

Lusiyanti Y., Kurnia I., Suvifan V.A., Sardini S., Purnami S., Rahajeng N. Evaluation of chromosomal aberrations and micronuclei in medical workers chronically exposed to low dose ionizing radiation, J Biol Biol Edu. 9 (2017) 585–591.

Maffei F., Angelini S., Forti G.C., Lodi V., Violante F.S., Mattioli S., Hrelia P. Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors, Mutagenesis. 17 (2002) 405–409.

Maluf S.W., Passos F.D., Bacelar A., Speit G., Erdtmann B. Assessment of DNA damage in lymphocytes of workers exposed to X-radiation using the micronucleus test and the comet assay, Environ Mol Mutagen. 38 (2001) 311-5.

Manning G., Macaeva E., Majewski M., Kriehuber R., Brzóska K. Comparable dose estimates of blinded whole blood samples are obtained independently of culture conditions and analytical approaches. Second RENEB gene expression study, Int J Radiat Biol. 93 (2017) 87–98.

Martínez A., Coleman M., Romero- Talamás C.A., Frías S. An assessment of immediate DNA damage to occupationally exposed workers to low dose ionizing radiation by using the comet assay, Rev Invest Clin. 62 (1) (2010) 23-30

Mavragani I.V., Nikitaki Z., Souli M.P., Aziz A., Nowsheen S., Aziz K., Rogakou E., Georgakilas A.G. Complex DNA Damage: a route to radiation-induced genomic instability and carcinogenesis, Cancers. 9 (2017) 91.

McCurdy D., Tai L.Q., Frias S., Wang Z. Delayed Repair of DNA damage by ionizing radiation in cells from patients with juvenile systemic lupus erythematosus and rheumatoid arthritis, Radiat Res. 147 (1997) 48-54.

Meadows S.K., Dressman H.K., Muramoto G.G. Gene expression signatures of radiation response are specific, durable and accurate in mice and humans, PLoS One. 3(4) (2008) e1912.

Medvedeva N.G., Panyutin I.V., Panyutin I.G., Neumann R.D. Phosphorylation of histone H2AX in radiation induced micronuclei, Radiat Res. 168 (2007) 493–498.

Meng A., Yu T., Chen G., Brown S.A., Wang Y., Thompson J.S., Zhou D. Cellular origin of ionizing radiation induced NF-kappa B activation *in vivo* and role of NF kappa B in ionizing radiation-induced lymphocyte apoptosis, Int J Radiat Biol. 79 (2003) 849–861.

Mettler Jr. F.A., Huda F.A., Yoshizumi T.T., Mahesh M. Effective doses in radiology and diagnostic nuclear medicine: a catalog, Radiology 248 (2008) 254–263.

Migliore L., Parrini M., Sbrana I., Biagini C., Battaglia A., Loprieno N. Micronucleated lymphocytes in people occupationally exposed to potential environmental contaminants: the age effect, Mutat Res. 256 (1991) 13–19.

Mihalache D.A., Preoteasa V., Petrescu A. Incidence of radiation-induced micronuclei in occupationally exposed subjects, Romanian J Biophys. 17 (2007) 119–128.

Miszczyk J., Rawojć K., Panek A., Swakoń J., Prasanna P.G., Rydygier M. Response of human lymphocytes to proton radiation of 60 MeV compared to 250 kV X-rays by the cytokinesis-block micronucleus assay, Radiother. Oncol. 115 (2015) 128-134.

Moller P., L. Knudsen E., S. Loft, H. Wallin. The comet assay as a rapid test in biomonitoring occupational exposure to DNA damaging agents and effect of confounding factors, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 1005–1015.

Momota H., Ichimiya S., Kondo N., Kikuchi T., Torigoe T., Yamaki T., Houkin K., Sato N. Histone H2AX sensitizes glioma cells to genotoxic stimuli by recruiting DNA double-strand break repair proteins, Int J Oncol. 23 (2003) 311–315.

Morgan W.F., Day J.P., Kaplan M.I., McGhee E.M., Limoli C.L. Genomic instability induced by ionizing radiation, Radiat. Res. 146 (1996) 247-258.

Moron M.S., Depierre J.W., Mannervik B. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver, Biochim. Biophys. Acta., 582 (1979) 67–78.

Muirhead C.R., Goodill A.A., Haylock R.G.E., Vokes J., Little M.P., Jackson D.A., O'Hagan J.A., Thomas J.M., Kendall G.M., Silk T.J. Occupational radiation exposure and mortality: second analysis of the national registry for radiation workers, J Radiol Prot. 19 (1999) 3–26.

Natarajan A.T. Mechanisms for induction of mutations and chromosome alterations, Environ. Health Perspect. 101 (suppl. 3) (1993) 225–229.

Niklason L., Marx M., Chan H. The estimation of occupational effective dose in diagnostic radiology with two dosimeters, Health Phys. 67 (6) (1994) 611–615.

O'Brien G., Cruz-Garcia L., Majewski M., Grepl J., Abend M. FDXR is a biomarker of radiation exposure *in vivo*, Scientific rep. 8 (2018) 684.

Olive P.L. DNA damage and repair in individual cells: applications of the comet assay in radiobiology, Int J Radiat Biol. 75 (1999) 395-405.

Olivieri G., Bodycote J., Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine, Science. 223 (1984) 594-597.

Organization for Economic Co-Operation and Development (OECD). 2016. Radiological protection science and application. NEA-7265. http://www.oecd-nea.org/rp/pubs/2016/7265-rp-science-application.pdf

Ostling O., Johanson K.J. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells, Bioche Biophys Res Commun. 123 (1984) 291-8.

Padmaja T., Maddileti U., Prasad H. Reproductive epidemiology in radiographers exposed to diagnostic X-rays, Hum Ecol. 20 (2006) 233-5.

Paul S., Amundson S.A. Development of gene expression signatures for practical radiation biodosimetry, Int J Radiat Oncol Biol Phys. 71(4) (2008) 1236–44.

Paul S., Barker C.A., Turner H.C., McLane A., Wolden S.L. Prediction of in vivo radiation dose status in radiotherapy patients using *ex vivo* and *in vivo* gene expression signatures, Radiat res. 175 (2011) 257–265.

Paul S., Amundson S.A. Gene expression signatures of radiation exposure in peripheral white blood cells of smokers and non-smokers, Int. J. Radiat. Biol. 87 (2011) 791–801.

Pearce M.S., Salotti J.A., Little M.P., McHugh K., Lee C., Kim K.P., Howe N.L., Ronckers C.M., Rajaraman P., Sir Craft, Parker A.W, L., Berrington de González A. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study, Lancet. 380 (2012) 499-505.

Petrini J.H.J., Stracker T.H. The cellular response to DNA double-strand breaks; defining the sensors and mediators, Trends Cell Biol. 13 (2003) 458- 462.

Picano E. Risk of Cancer from diagnostic X-rays: impressive but underestimated, Lancet. 363 (2004) 1909-10.

Piperakis S.M., Visvardis E.E., Sagnou M., Tassiou A.M. Effects of smoking and aging on oxidative DNA damage of human lymphocytes. Carcinogenesis. 19 (1998) 695-698.

Plappert U.G., Stocker B., Fender H., Fliedner T.M. Changes in the repair capacity of blood cells as biomarker for chronic low-dose exposure to ionizing radiation, Environ. Mol. Mutagen. 30 (1997) 153–160.

Qian Q.Z., Cao X.K., Shen F.H., Wang Q. Effects of ionizing radiation on micronucleus formation and chromosomal aberrations in Chinese radiation workers, Radiat Prot Dosim. 168 (2016) 197–203.

Ramalho A., Sunjevaric I., Natarajan A.T. Use of the frequencies of micronuclei as quantitative indicators of X-ray-induced chromosomal aberrations in human peripheral blood lymphocytes: comparison of two methods, Mutat. Res. 207 (1988) 141–146.

Ramalho A.T., Costa M.L.P., Oliveira M.S. Conventional radiation biological dosimetry using frequencies of unstable chromosome aberrations, Mutat Res. 404 (1998) 97–100.

Ray M., Yunis R., Chen X., Rocke D.M. Comparison of low and high dose ionising radiation using topological analysis of gene coexpression networks, Bmc Genom. 13 (2012) 190.

Reisz J.A., Bansal N., Qian J., Zhao W., Furdui C.M. Effects of ionizing radiation on biological molecules--mechanisms of damage and emerging methods of detection, Antioxid Redox Signal. 21(2) (2014) 260-292.

Reus A.A., Reisinger K., Downs T.R., Carr G.J., Zeller A., Corvi R., Krul C.A., Pfuhler S. Comet assay in reconstructed 3D human epidermal skin models--investigation of intra- and inter-laboratory reproducibility with coded chemicals. Mutagenesis. 28 (2013) 709-20.

Richardson D.B., Cardis E., Daniels R.D., Gillies M., Haylock R., Leuraud K. Site-specific solid cancer mortality after exposure to ionizing radiation: a cohort study of workers (INWORKS), Epidemiology. 29(1) (2018) 31–40.

Richardson D.B., Cardis E., Daniels R.D., Gillies M., O'Hagan J.A., Hamra G.B., Haylock R., Laurier D., Leuraud K., Moissonnier M. Risk of cancer from occupational exposure to ionizing radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS), BMJ. 351 (2015) h5359.

Rieger K.E., Chu G. Portrait of transcriptional responses to ultraviolet and ionizing radiation in human cells, Nucleic Acids Res. 32 (2004) 4786–4803.

Riley P.A. Free radicals in biology: oxidative stress and the effects of ionizing radiation, Int J Radiat Biol. 65 (1994) 27–33.

Rodrigues-Moreira S., Moreno S.G., Ghinatti G., Lewandowski D., Hoffschir F., Ferri F., Gallouet A.S., Gay D., Motohashi H., Yamamoto M.s Low-dose irradiation promotes persistent oxidative stress and decreases selfrenewal in hematopoietic stem cells, Cell Rep. 20 (2017) 3199–3211.

Rong C., Meinert E.F., Hess J. Estrogen receptor signaling in radiotherapy: from molecular mechanisms to clinical studies, Int J Mol Sci. 19 (2018) 713.

Roobottom C.A., Mitchell G., Morgan-Hughes G. Radiation-reduction strategies in cardiac computed tomographic angiography, Cli Radiol. 65 (2010) 859-67.

Ropolo M., Balia C., Roggieri P., Lodi V., Nucci M.C., Violante F.S., Silingardi P., Colacci A., Bolognesi C. The micronucleus assay as a biological dosimeter in hospital workers exposed to low doses of ionizing radiation, Mutat Res. 747 (2012) 7–13.

Rosenberger A., Ro¨ssler U., Hornhardt S., Sauter W., Bickebo¨ller H., Wichmann H.E. Validation of a fully automated COMET assay: 1.75 million single cells measured over a 5 year period, DNA Repair (Amst.) 10 (2011) 322–337.

Rozgaj R., Kasuba V., Sentija K., Prlic I. Radiation induced chromosomal aberrations and haematological alterations in hospital workers, Occup Med (Lond). 49 (1999) 353–360.

Russo G.L., Tedesco I., Russo M., Cioppa A., Andreassi M.G., Picano E. Cellular adaptive response to chronic radiation exposure in interventional cardiologists, Eur Heart J. 33 (2012) 408–414.

Sahin A., Tatar A., Oztas S., Seven B., Varoglu E., Yesilyurt A., Ayan A.K. Evaluation of the genotoxic effects of chronic low-dose ionizing radiation exposure on nuclear medicine workers, Nucl Med Biol. 36 (2009) 575–578.

Sakamoto-Hojo E.T., Mello S.S., Pereira E., Fachin A.L., Cardoso R.S., Junta C.M., Sandrin-Garcia P., Donadi E.A., Passos G.A. Gene expression profiles in human cells submitted to genotoxic stress, Mutat Res. 544 (2003) 403–413.

Sakly A., Gaspar J.F., Kerkeni E., Silva S., Teixeira J.P., Chaari N., Cheikh H.B. Genotoxic damage in hospital workers exposed to ionizing radiation and metabolic gene polymorphisms, J Toxicol Environ Hlth. 75 (2012) 934–946.

Santivasi W.L., Xia F. Ionizing radiation-induced DNA damage, response, and repair, Antioxid Redox Signal. 21 (2014) 251–259.

Seo S., Lee D., Seong K.M., Park S., Kim S.G., Won J.U., Jin Y.W. Radiation-related occupational cancer and its recognition criteria in South Korea. Ann Occup Environ Med. 30 (2018) 018-219.

Shah D.J., Sachs R.K., Wilson D.J. Radiation-induced cancer: a modern view, Br J Radiol. 85 (2012) 1166–1173.

Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. Nat. Rev. Cancer. 3 (2003) 155–168.

Sigurdson A.J., Bhatti P., Preston D.L., Doody M.M., Kampa D., Alexander B.H., Petibone D., Yong L.C., Edwards A.A., Ron E. Routine diagnostic X-ray examinations and increased frequency of chromosome translocations among US radiologic technologists, Cancer Res. 68 (2008) 8825–8831.

Singh N.P., Danner D.B., Tice R.R., Pearson J.D., Brant L.J., Morrel C.H., Schneider E.L. Basal DNA damage in individual human lymphocytes with age, Mutat Res. 248 (1991) 285–289.

Singh N.P., McCoy M.T., Tice R.R., Schneider E.L. A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988) 184–191.

Smith P.G., Doll R. Mortality from cancer and other causes among British radiologists, Bjr. 4 (1981) 187–194.

Snyder A.R., Morgan W.F. Gene expression profiling after irradiation: clues to understanding acute and persistent responses? Cancer Metastasis Rev. 23 (2004) 259–268.

Somorovská M., Szabová E., Vodicka P. Biomonitoring of genotoxic risk in workers in a rubber factory: comparison of the Comet assay with cytogenetic methods and immunology, Mutat. Res. 445 (1999) 181–192.

Sram R.J., Podrazilova K., Dejmek J., Mrackova G., Pilcik T. Single cell gel electrophoresis assay: sensitivity of peripheral white blood cells in human population studies. Mutagenesis. 13 (1998) 99-103.

Stoyanova T., Roy N., Kopanja D. DDB2 (damaged-DNA binding protein 2) in nucleotide excision repair and DNA damage response, Cell Cycle. 8(24) (2009) 4067–71.

Streffer C., Müller Wu., Kryscio A., Böcker W. Micronuclei-biological indicator for retrospective dosimetry after exposure to ionizing radiation, Mutat. Res. 404 (1998) 101-105.

Surniyantoro H.N.E., Darlina., Rahardjo T. Alkaline Comet Assay as a Predictor of DNA Damage in Medical Radiation Workers, J Physi: Conf. Series. 1436 (2020) 012023.

Sutherland B.M., Bennett P.V., Sidorkina O., Laval J. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation, Proc. Natl. acad. Sci. USA. 97 (2000) 103-108.

Tessner T.G., Muhale F., Schloemann S., Cohn S.M., Morrison A.R., Stenson W.F. Ionizing radiation up-regulates cyclooxygenase-2 in I407 cells through p38 mitogen-activated protein kinase, Carcinogenesis. 25 (2004) 37–45.

Thierens H., Vral A., Barbe M., Meijlaers M., Baeyens A., De Ridder L. Chromosomal radiosensensitivity study of temporary nuclear workers and the support of the adaptive response induced by occupational exposure, Int J Radiat Biol. 78 (2002) 1117-1126.

Thierens H., Vral A., De Ridder L. A cytogenetic study of radiological workers: effect of age, smoking and radiation burden on the micronucleus frequency, Mutat Res. 360 (1996) 75–82.

Thierens H., Vral A., Morthier R., Aousalah B., De Ridder L. Cytogenetic monitoring of hospital workers occupationally exposed to ionizing radiation using the micronucleus centromere assay. Mutagenesis. 15 (2000) 254–249.

Tice R.R., Andrew P.W., Hirai O., Singh N.P. The single cell (SCG) assay: an electrophoretic technique for the detection of DNA damage in individual cells. Biological Reactive Intermediates. Vol IV. Plenum Press (1990) 157-164.

Tice R.R., Andrews P.W., Hirai O., Singh N.P. The single cell gel (SCG) assay: an electrophoretic technique for the detection of DNA damage in individual cells, Adv Exp Med Biol. 283 (1991) 157-64.

Tucker J.D., Grever W.E., Joiner M.C., Konski A.A., Thomas R.A. Gene expression-based detection of radiation exposure in mice after treatment with granulocyte colony-stimulating factor and lipopolysaccharide, Radiat res. 177 (2012) 209–219.

Tusher V.G., Tibshirani R., Chu G. Significance analysis of microarrays applied to the ionizing radiation response, Proc Natl Acad Sci U S A. 98 (2001) 5116–5121.

Umegaki K., Sugisawa A., Shin S.J., Yamada K., Sano M. Different onsets of oxidative damage to DNA and lipids in bone marrow and liver in rats given total body irradiation, Free Radic Biol Med. 31 (2001) 1066–1074.

United Nations Scientific Committee on the effects of Atomic Radiation (UNSCEAR). Sources and effects of ionizing radiations. (2014). https://www.unscear.org/docs/publications/1994/ UNSCEAR1994Report.pdf

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation). Adaptive responses to radiation in cells and organisms. Unscear report sources & effects of ionizing radiation. New York, NY: United Nations (1994).

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation). Effects of Ionizing Radiation, Volume I, United Nations, New York., (2006). www.unscear.org/reports/2006.html

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation). Biological Mechanisms of Radiation Actions at Low Doses; United Nations: New York, NY, USA, 2012.

UNSCEAR 2008 Report Vol. II. Sources and effects of ionizing radiation, https://www.unscear.org/docs/publications/2008/UNSCEAR_2008_Annex-E.pdf

UNSCEAR. Sources and Effects of Ionizing Radiation, Report to the General Assembly with Scientific Annexes; UNSCEAR: New York, NY, USA, 2008.

Upton A., Luntz C., Silverman M., Burton J., Tobias M., Cornelius M., Asokendu M. Radiation Encyclopedia Britannica. (2021) https://www.britannica.com/science/radiation

USNRC (United States Nuclear Regulatory Commission) 2014, Reactor Concept Manual: Biological Effects of Radiation. Retrieved from: https://www.nuclear-power.com/nuclear-engineering/radiation protection/radiobiology/deterministic effects/

V. Garaj-Vrhovac, N. Nevenka Kopjar, The alkaline Comet assay as biomarker in assessment of DNA damage in medical personnel occupationally exposed to ionizing radiation, Mutagenesis. 18 (3) (2003) 265–271.

Vaiserman A., Koliada A., Zabuga O., Socol Y. Health Impacts of Low-Dose Ionizing Radiation: Current Scientific Debates and Regulatory Issues, Dose-Response: An International Journal. (2018) 1-27.

Valavanidis A., Vlachogianni T., Fiotakis K. Tobacco smoke: Involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles, Int. J. Environ. Res. Public Health. 6 (2009) 445-462.

Valko M., Izakovic M., Mazur M., Rhodes C.J., Telser J. Role of oxygen radicals in DNA damage and cancer incidence, Mol. Cell Biochem. 266 (2004) 37–56.

Valverde M., Rojas E. Environmental and occupational biomonitoring using the Comet assay, Mutat Res. 681 (1) (2009) 93-109.

Vasquez M.Z. Combining the in vivo comet and micronucleus assays: a practical approach to genotoxicity testing and data interpretation. Mutagenesis. 25(2010) 187–199.

Vellingiri B., Shanmugam S., Subramanian M.D., Balasubramanian B., Meyyazhagan A., Alagamuthu K., Prakash V., Shafiahammedkhan M., Kathannan S., Pappuswamy M. Cytogenic endpoints and xenobiotic gene polymorphism in lymphocytes of hospital workers chronically exposed to ionizing radiation in cardiology, radiology and orthopedic laboratories, Ecotoxicol. Environ. Saf. 100 (2014) 266–274.

Vera G.V., Aleksandra F., Dragan K., Andrija H. Assessment of genome damage in occupational exposure to ionizing radiation and ultrasound, Mutat Res 395 (1997) 101–105.

Visweswaran S., Joseph S., Vinay Hedge S, Annalakshmi O., Jose M.T., Perumal V. DNA damage and gene expression changes in patients exposed to lowdose X-radiation during neuro-interventional radiology procedures, Mutat Res. 844 (2019) 54-61.

Wang F., Sun Q., Wang J., Yu N. Risk of developing cancers due to low-dose radiation exposure among medical X-ray workers in China—results of a prospective study, Int J Clin Exp Pathol. 9 (2016) 11897–11903.

Wang J.S., Wang H.J., Qian H.L. Biological effects of radiation on cancer cells, Mil Med Res. 5(1) (2018) 20.

Wang W., Mani A.M., Wu Z.H. DNA damage-induced nuclear factor-kappa B activation and its roles in cancer progression, J Cancer Metastasis Treat. 3 (2017) 45–49.

Ward J. F. DNA Damage Produced by Ionising Radiation in Mammalian Cells: Identities, Mechanisms of Formation, and Repairability, Prog. Nucl. Acid. Res. Mol. Biol. 35 (1988) 95–125.

Ward J.F. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol. 35 (1998) 95–125.

Watters D. Molecular mechanisms of ionizing radiation-induced apoptosis. Immunol Cell Biol. 77 (1999) 263–271.

Wojewodzka M., Kruszewski M., Iwanenko T., Collins A.R., Szumiel I. Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation I. Strand breakage, Mutat Res. 416 (1998) 21–35.

Wojewodzka M., Kruszewski M., Iwanenko T., Collins A.R., Szumiel I. Lack of adverse effect of smoking habit on DNA strand breakage and base damage, as revealed by the alkaline comet assay. Mutat. Res. 440 (1999) 19-25.

Yim J.H., Yun J.M., Kim J.Y., Nam S.Y., Kim C.S. Estimation of low-dose radiation-responsive proteins in the absence of genomic instability in normal human fibroblast cells, Int J Radiat Biol. 93 (2017) 1197–1206.

Yin E., Nelson D.O., Coleman M.A., Peterson L.E., Wyrobek A.J. Gene expression changes in mouse brain after exposure to low-dose ionizing radiation, Int J Radiat Biol. 79 (2003) 759–775.

Yin H., Xu L., Porter N.A. Free radical lipid peroxidation: mechanisms and analysis. Chem Rev. 111 (2011) 5944–5972.

Yoo J.C., Pae H.O., Choi B.M., Kim W.I., Kim J.D., Kim Y.M., Chung H.T. Ionizing radiation potentiates the induction of nitric oxide synthase by interferon-gamma (Ifn-gamma) or Ifn-gamma and lipopolysaccharide in bnl cl.2 murine embryonic liver cells: Role of hydrogen peroxide, Free. Radic. Biol. Med. 28 (2000) 390–396.

Yoshikawa T., Naito Y. What is oxidative stress? JMAJ. 45 (2002) 271–276.

Yu Y., Cui Y., Niedernhofer L.J., Wang Y. Occurrence, biological consequences, and human health relevance of oxidative stress-induced DNA damage, Chem Res Toxicol. 29 (2016) 2008–2039.

Zakeri F., Hirobe T. A cytogenetic approach to the effects of low levels of ionizing radiations on occupationally exposed individuals, Eur J Radiol. 73 (2010) 191–195.

Zhang X.P., Liu F., Wang W. Two-phase dynamics of p53 in the DNA damage response, Proc Natl Acad Sci U S A. 108 (2011) 8990–8995.

Zhang Y., Qian Y., Zhang J., Yan W., Jung Y.S. Ferredoxin reductase is critical for p53-dependent tumor suppression via iron regulatory protein 2, Genes Dev. (2017)

Zhou X., Li N., Wang Y. Effects of X-irradiation on mitochondrial DNA damage and its supercoiling formation change, Mitochondrion. 11 (2011) 886–92.

Zothansiama, Zosangzuali M., Lalramdinpuii M., Jagetia G.C. Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood

lymphocytes of humans residing in the vicinity of mobile phone base stations, Electromagn. Biol. Med. 36 (2017) 295-305.

Zothansiama, Zosangzuali M., Vanlalruati A., Jagetia G.C., Pau K.S., Kumar N.S. Chronic low dose exposure of hospital workers to ionizing radiation leads to increased micronuclei frequency and reduced antioxidants in their peripheral blood lymphocytes, Int. J. Radiat. Biol. 95 (2019) 697-709.

ABBREVIATIONS

µl	Microlitre
µmol	Micromolar
µg	Microgram
γ-ray	Gamma radiation
cDNA	Complementary DNA
cm	Centimetre
CO_2	Carbon dioxide
Ct	Threshold cycle
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
FBS	Fetal Bovine Serum
Gy	Gray
H_2O_2	Hydrogen peroxide
hr	Hour
kVp	Kilovoltage peak
M	Molar
mA	Milliampere
mAs	Milli-ampere-second
mg	Milligram
mGy	Milligray
min	Minute
ml	Millilitre
mM	Millimolar
MN	Micronucleus
mRNA	Messenger RNA
nm	Nanometre
p53	Tumor Protein p53
PBS	Phosphate Buffer Saline.
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Real Time-PCR

RNA	Ribonucleic acid
rpm	Revolutions per minute
sec	Second
SEM	Standard error of mean
V	Voltage
X-ray	X-radiation

BRIEF BIO-DATA OF THE CANDIDATE

Name: Mary Zosangzuali

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EDUCATIONAL DETAILS:

Degree	Year	Board/ University	Subject	Division	Percentage
HSLC	2009	Mizoram Board of School Education	General	First	70.6%
HSSLC	2011	Mizoram Board of School Education	Science	First	65.4%
B.Sc	2014	North-Eastern Hill University	Zoology	First	64.6%
M.Sc	2016	Mizoram University	Zoology	First	72.1%

PUBLICATIONS

Mary Zosangzuali, L Pachuau, K.S Pau and Zothansiama. Effects of low-dose irradiation on chromosomal damage and oxidative stress in cultured human peripheral blood. *Journal of Environmental Biology*, 41: 908-914, 2020.

Mary Zosangzuali, Marina Lalremruati, C. Lalmuansangi, F. Nghakliana, Lalrinthara Pachuau, Priyanka Bandara & Zothan Siama. Effects of radiofrequency electromagnetic radiation emitted from a mobile phone base station on the redox homeostasis in different organs of Swiss albino mice. *Electromagnetic biology and medicine*, 1-15, 2021.

Zothansiama, **Mary Zosangzuali**, Annie Vanlalruati, K.S. Pau, N.S. Kumar, G. C. Jagetia GC. (2019). Chronic low dose exposure of hospital workers to ionizing radiation leads to increased micronuclei frequency and reduced antioxidants in their peripheral blood lymphocytes. *International Journal of Radiation Biology*, 2019. https://doi.org/10.1080/09553002.2019.1571255.

Zothansiama**, Mary Zosangzuali**, Miriam Lalramdinpuii, G.C Jagetia. Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood lymphocytes of humans residing in the vicinity of mobile phone base stations. *Electromagnetic Biology and Medicine*, 2017. DOI: 10.1080/15368378.2017.1350584.

C. Lalmuansangi, **Mary Zosangzuali**, Marina Lalremruati, L. Tochhawng and Zothansiama: Evaluation of the protective effects of Ganoderma applanatum against doxorubicin-induced toxicity in Dalton's Lymphoma Ascites (DLA) bearing mice. *Drug and Chemical Toxicology*, 1-11, 2020.

Zothansiama, C. Lalmuansangi, **Mary Zosangzuali**, L. Tochhawng and G. C. Jagetia. Assessment of free radical scavenging activity and antioxidant mediated hepato-protective effects of *Mallotus roxburghianus* Muell. in doxorubicin induced oxidative stress in Swiss albino mice. *International Journal of Pharmaceutical Sciences and Research*, 9 (10): 4138-4150, 2018.

F. Nghakliana, C. Lalmuansangi**, Mary Zosangzuali**, Marina Lalremruati and Zothansiama. Anti-oxidative potential and anti-cancer activity of *Elaeagnus caudata* (Schltdl) against Type-II human lung adenocarcinoma, A549 cells. *Journal of Biochemistry and Biophysics* 58, 543-556, 2021.

Marina Lalremruati**,** C. Lalmuansangi, **Mary Zosangzuali**, Lalchhandami Tochhawng, Amit Kumar Trivedi, Nachimuthu Senthil Kumar and Zothan Siama: *Mussaenda macrophylla* Wall. exhibit anticancer activity against Dalton's lymphoma ascites (DLA) bearing mice via alterations of redox-homeostasis and apoptotic genes expression. *The Journal of Basic and Applied Zoology*, 83(6), 2022.

Book chapters

Mary Zosangzuali, Marina Lalremruati, C. Lalmuansangi, F. Nghakliana and Zothansiama. Pharmacological importance and chemical composition of *Mallotus roxburghianus* Muell.Arg. Bioactives and Pharmacology of Medicinal Plants. Apple academic Press, USA (distributed by CRC Press of Taylor & Francis)

Marina Lalremruati, C. Lalmuansangi, **Mary Zosangzuali**, F. Nghakliana and Zothansiama. *Mussaenda macrophylla* Wall.: Chemical Composition and Pharmacological Applications. Bioactives and Pharmacology of Medicinal Plants. Apple academic Press, USA (distributed by CRC Press of Taylor & Francis).

C. Lalmuansangi, Marina Lalremruati, **Mary Zosangzuali**, F. Nghakliana and Zothansiama. Medicinal properties and bioactive compounds of *Stemona tuberosa* Lour. (Family: Stemonaceae). Bioactives and Pharmacology of Medicinal Plants. Apple academic Press, USA (distributed by CRC Press of Taylor & Francis).

F. Nghakliana, **Mary Zosangzuali**, Marina Lalremruati, C. Lalmuansangi and Zothansiama. Ethnopharmacological Applications and Phytochemical compounds of the Genus *Callicarpa L*. Bioactives and Pharmacology of Medicinal Plants. Apple academic Press, USA (distributed by CRC Press of Taylor & Francis).

CONFERENCES/ SEMINAR/WORKSHOP ATTENDED

International

1. The 12th Annual Convention of Association of Biotechnology and Pharmacy (ABAP) & International conference on Biodiversity, Environment and Human Health: Innovations and Emerging trends (BEHIET) organized by School of Life Sciences, Mizoram University during 12th to 14th November, 2018.
2. International Conferences of Recent Advances in Animal Sciences (ICRAAS) organised by Department of Zoology, Pachhunga University College, November 6-8, 2019.

National

1. National workshop on 'Statistical and Computing Methods for life-science Data Analysis' organized by Biological Antropology Unit, Indian Statistical Institute, Kolkata and Department of Botany, Mizoram University, Aizawl, 5th -10th March, 2018.
2. National Seminar on 'Animal handling, Maintenance and Care' organised by Advanced level state Biotech-hub Facility, Department of Biotechnology, Mizoram University sponsored by Department of Biotechnology (DBT) New Delhi on 26th March, 2018.

State:

1. Seminar on 'Science for nation building' organised by Mizo Academy of Sciences in collaboration with Department of Science and Technology, Mizoram, catalysed and supported by the National Council for Science and Technology Communication, Department of Science and Technology, New Delhi, held at Pi Zaii Hall, Synod Conference Center, Aizawl Mizoram, 1st October, 2015.
2. State level training on 'Introduction to Bioinformatics- Tools and Methods' organized by Bioinformatics Infrastructure Facility, Department of Biotechnology, Mizoram University sponsored by Department of Biotechnology (DBT), Ministry of Science and Technology New Delhi, 20th to 24th March, 2016.
3. Mizoram Science Congress 2018 (MAS, MISTIC, STAM & BIOCONE)

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE	**:** MARY ZOSANGZUALI
DEGREE	**:** DOCTOR OF PHILOSOPHY
DEPARTMENT	**:** ZOOLOGY
TITLE OF THESIS	**:**STUDIES ON GENOTOXICITY AND ANTIOXIDANT STATUS IN THE PERIPHERAL BLOOD LYMPHOCYTES OF INDIVIDUALS OCCUPATIONALLY EXPOSED TO IONIZING RADIATIONS
DATE OF ADMISSION:	**:** 19.09.2017

APPROVAL OF RESEARCH PROPOSAL

1. DRC	**:** 21.03.2018
2. BOS	**:** 27.04.2018
3. SCHOOL BOARD	**:** 03.05.2018
MZU REGISTRATION NO.	**:** 118 of 2015
Ph.D. REGISTRATION NO. & DATE	**:** MZU/Ph.D./1109 of 03.05.2018
EXTENSION	**:** NIL

(Prof. H.T. LALREMSANGA)

HEAD

DEPARTMENT OF ZOOLOGY

MIZORAM UNIVERSITY