GENOTOXIC RISK ASSESSMENT OF PETROL STATION ATTENDANTS AT OLUKU AXIS, BENIN CITY: A MICRONUCLEUS ASSAY STUDY OF THE BUCCAL EPITHELIUM CELLS

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BENIN CITY

NOVEMBER, 2025.

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AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research titled **"GENOTOXIC RISK ASSESSMENT OF PETROL STATION ATTENDANTS AT OLUKU AXIS, BENIN CITY: A MICRONUCLEUS ASSAY STUDY OF THE BUCCAL EPITHELIUM CELLS "** was carried out by **Anne Ayomide EDO (MISS)** with Matriculation Number: **LSC2009918** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

_____ _____

PROF. D.I. OLORUNFEMI **DATE**

(Project Supervisor)

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PROF. (MRS.) E.T. AISIEN **DATE**

(Head of Department)

DECLARATION

I **'ANNE AYOMIDE EDO' (MISS)** declare that **'GENOTOXIC RISK ASSESSMENT OF PETROL STATION ATTENDANTS AT OLUKU AXIS, BENIN CITY: A MICRONUCLEUS ASSAY STUDY OF THE BUCCAL EPITHELIUM CELLS'** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

_____ _____

ANNE AYOMIDE EDO **DATE**

DEDICATION

I dedicate this project work to God Almighty for bringing it to reality and making it a success.

ACKNOWLEDGEMENTS

I am grateful to God Almighty for his grace, mercy and protection over my life throughout the project work. I would like to express my sincere gratitude to my Project Supervisor, Prof. D.I. Olorunfemi for his support and guidance throughout the project work. Special thanks to the Head of Department Prof. (Mrs.) E.T. Aisien, my amazing Course Adviser and Project Coordinator Dr. A.F. Eghomwanre and the entire lecturers in the department for their investment in my academic development. I am forever grateful to my amazing parents, Mr. and Mrs. Edo for their unwavering support and sacrifices to ensure that I have the essential resources. To my beloved siblings, thank you all so much. To my colleagues, thank you all for your help towards the success of this project. I am also grateful to my entire family, friends, roommates and coursemates for their support, encouragement and contributions towards the success of this project. May the Good Lord bless you all richly.

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ABSTRACT

Petrol station attendants are occupationally exposed to petroleum products and it's fumes which represents complex mixture of genotoxic agents that possesses an increased risk of various cancers such as that of the oral mucosa. This study assessed the level of genotoxic damage in exfoliated buccal cells obtained from petrol station attendants within Oluku Axis, Benin City, Edo State, Nigeria. Twenty - five exposed petrol station attendants were recruited from five filling stations which includes; thirteen females and twelve males whose duration of exposure is within zero to five years while nine unexposed healthy individuals were recruited from the University of Benin. Buccal cell samples were collected by scrapping of the inner cheeks which was fixed with Carnoy's reagent and stained using May - Grunwald and Giemsa stains, after which it was analyzed using a light microscope at 100x magnification. A total of 3400 cells were examined for micronuclei and other anomalies such as binucleated and anucleated cells. A significant increase in the frequencies of micronucleated cells, binucleated cells and anucleated cells (12.50, 6.17 and 6.00) with ($p < 0.05$) were found in the workers exposed as compared to the controls (0.67, 4.50 and 3.33) respectively. These findings indicate that continuous exposure to benzene have the potential to induce anomalies reflecting DNA strand breaks. This study concludes that petrol station attendants within Oluku Axis experience measurable genetic damage linked to occupational exposure to petroleum products. It recommends that protective strategies should be implemented by the concerned authorities to minimize exposure to petroleum products.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Approximately 95% of the chemicals in gasoline vapour are aliphatic and alicyclic with fewer than 2% being aromatics. Petrol is a complete mixture of hydrocarbons. Benzene, toluene and xylene (BTX) are the main aromatic chemicals found in gasoline. According to toxicological analysis, benzene is the most dangerous ingredient and has been identified by the International Agency for Research on Cancer as a human carcinogen (IARC,1989).

Petrol station attendants are exposed to petrol fumes either through fuel manipulation or through the refueling of vehicles and also through the inhalation of volatile vapors emitted by engines during refilling. These exposure makes them more prone to the development of several types of cancer such as urinary tract, skin, laryngeal, pancreatic cancer and leukemias. Also, exposure by either nasal inhalation or oral ingestion of these compounds has carcinogenic effects over the oral mucosa (Benites *et al*., 2006).

Studies have shown increased cytogenic damage in peripheral blood lymphocytes of petrol station attendants using different genetic endpoints such as DNA strand breaks, sister chromatid exchange (SCE) and micronuclei (MN) (Rajkokila *et al*., 2010). In addition, other alterations in the morphology of the nucleus including karyorrhexis (KH), pyknosis (P), karyolysis (KL) and binucleus (BN) are also proof of cytogenic damage. The buccal epithelium cells is composed of four strata such as; the basal cell

layer, prickle cell layer, intermediate cell layer and the superficial layers. The oral mucosa maintains itself through a system of continuous cell renewal in which new cells produced by the process of mitosis in the basal cell layer moves towards the surface to replace those that are shed. Thus, the mucosa contains the progenitor and maturing populations of cells (Celik *et al*., 2003).

Micronucleus are small extra nuclear bodies that arise from chromosome fragments or a whole chromatids or chromosomes lagging behind at the anaphase stage if dividing cells and are not added to the main nucleus stage but instead enwrapped by the nuclear membrane to resemble the structure of the daughter nucleus although way smaller (Fenech *et al*., 2011; Sedelnikova *et al*., 2007). Genotoxic events that occurred in the dividing basal layer are reflected by the micronucleus in the exfoliated buccal epithelial cells. Micronucleus assay is the most suitable biomonitoring approach because the epithelia is the origin of more than 90% of all human cancers (Ozkul *et al*., 1997). Micronucleus assay have been proven scientifically as a promising tool for studying cytogenetic and genotoxic changes in petrol station attendants (Celik *et al*., 2003).

1.2 Aim of Study

This study is aimed to investigate the genotoxic damage in exfoliated buccal cells obtained from petrol station attendants and control subjects using the micronucleus assay.

1.3 Objectives of Study

- To assess the genotoxic risk among petrol station attendants within Oluku Axis, Benin City using the micronucleus assay in buccal epithelial cells as a biomarker of DNA damage.
- To evaluate other nuclear abnormalities such as (pyknosis, karyolysis, binucleated cells, karyorrhexis, anucleated cells) in the buccal cells of the exposed and non - exposed groups.
- To compare the duration of exposure (years of work) and the frequency of micronuclei in petrol station attendants within Oluku Axis, Benin City.
- To investigate the impact of safety measures or personal protective equipment on the frequency of micronuclei in exposed workers.
- To determine the frequency of micronuclei (MN) in buccal cells of petrol station attendants within Oluku Axis, Benin City compared to a control group (non exposed individuals).
- To recommend preventive measures (improved workplace safety, PPE, regular health monitoring) based on study findings.

1.4 Statement of Research Problem

Despite the widespread use of petrol and it's association with hazardous chemicals such as benzene, toluene and xylene (BTX), there is limited information on the genotoxic risks faced by petrol station attendants especially within Oluku Axis, Benin City, Nigeria. These workers are constantly exposed to fuel vapors and petroleum products, which may lead to chromosomal aberrations, DNA damage and increased

cancer risk. Micronucleus assay in buccal epithelial cells is a well known biomarker for genotoxic damage, yet it's application in the assessment of occupational exposure among petrol station attendants within Oluku Axis in Benin City remains under explored. The long term health effects for these workers remain a mystery thereby potentially leading to weak safety measures and regulatory oversight due to improper risk assessments.

By comparing the frequency of micronuclei and other nuclear abnormalities in the buccal cells of petrol station attendants to non - exposed controls. This study seems to close the gap and assess the degree of genotoxic damage linked to their occupational exposure and the results will support evidence based suggestions for enhanced health money and occupational safety in this high risk population.

1.5 Scope and Limitation of the Study

This study focuses on petrol station attendants within Oluku Axis in Benin City, Nigeria, who are occupationally exposed to petroleum products and vapours. Their buccal epithelial cells will be analyzed using the micronucleus (MN) assay to assess genotoxic damage and the frequency of micronuclei (MN) as well as other nuclear abnormalities (e.g. binucleated cells, anucleated cells) to serve as a primary biomarker for DNA damage. Results will be compared with a control group (non-exposed individuals) to determine occupational risk and work duration will be taken into consideration to evaluate the dose-response relationships. Results will contribute to occupational safety recommendations for petrol station attendants.

It is limited to petrol station attendants within Oluku Axis in Benin City and the results may not apply to other axis. Micronucleus assay covers only one aspect of genotoxicity while other biomarkers such as (comet assay, chromosomal aberrations) are not included. Factors like (smoking, alcohol, diet) may influence genotoxic damage and they cannot be fully controlled. This study evaluates current genotoxic damage but cannot determine the long - term health effects (e.g., cancer development) and the exposure levels may differ between petrol station attendants due to differences in ventilation, fuel types, and safety measures observed.

1.6 Justification and Significance of Study

This study is justified by the occupational health risks faced by the petrol station attendants within Oluku Axis in Benin City, Nigeria, who are exposed to genotoxic substances (e.g., benzene, PAHs, and VOCs) found in petroleum products. Based on the lack of strict safety regulations and policies in many Nigerian petrol stations, attendants are susceptible to DNA damage and long-term health effects, including cancer. The buccal micronucleus (MN) assay help provide a safe, sensitive, and cost - effective method to assess genotoxic damage, by offering deep insights into early biological effects. Furthermore, there are limited data on occupational health hazard in Benin City, thereby making this study essential for evidence - based policymaking, workplace safety improvements, and preventive healthcare strategies for this vulnerable group.

1.7 Hypotheses

Null Hypothesis (H_0):

"There are no significant increase in micronucleus frequency in the buccal cells of petrol station attendants within Oluku Axis in Benin City compared to non-exposed individuals, thereby suggesting no genotoxic risk from occupational exposure."

Alternative Hypothesis (H_1):

"Petrol station attendants within Oluku Axis in Benin City showed an increase in the frequency of micronuclei in their buccal cells compared to non-exposed individuals, thereby indicating genotoxic damage due to long term exposure to petroleum products."

CHAPTER TWO

LITERATURE REVIEW

2.1 Genotoxicity and Occupational Hazards

Genotoxicity is a word used in genetics which describes substances that has destructive effect on the genetic material of the cell (DNA/ RNA) thereby affecting the integrity of the cell. Genotoxins can be chemical substances as well as radiation. Genetic toxicology is the branch of science that deals with the study of substances or agents that can damage the cell's DNA and chromosomes. Most of the times, genotoxicity is confused with mutagenicity. All mutagens are genotoxic but not all genotoxins are mutagenic (De and Izzotti, 2007). The damage is distinct as it specifically damages the molecules responsible for storing and transmitting genetic information and the damage includes; gene mutations which are the alterations in the nucleotide sequence of the DNA such as insertions, base substitutions (point mutation), or deletions (frameshift mutation) and chromosomal aberrations such as the structural aberrations or the numerical aberrations (Claxton *et al*., 2010). Genotoxins can be any of the following depending on it's effects; Carcinogens (cancer causing agents), Mutagens (mutation causing agents), Teratogens (birth defects causing agents) and the common sources of genotoxic exposure in occupational settings includes; chemical manufacturing, pesticide application, coal mining and petrol stations. Workers in these settings may face risks from inhalation skin contact or ingestion of these agents. The term Genotoxicity studies refers to a variety of *in-vitro* and *in-vivo* tests intended to detect any drug or substances that may directly or

indirectly harm genetic material through a variety of methods. These tests ought to make it possible to determine the risk of DNA damage and fixation (Cimino, 2006). Genetic change only contributes to the intricate process of malignancy and heritable consequences, which includes the repair of DNA damage caused by gene mutation, extensive chromosomal damage, recombination, or numerical chromosomal changes. By detecting positive results, these assays are crucial in determining if a substance has the capacity to produce genotoxicity and carcinogenicity (Shah, 2012).

2.2 Petroleum Products Genotoxic Risks

Petrol is a complete mixture of hydrocarbons. Benzene, toluene and xylene (BTX) are the main aromatic chemicals found in gasoline. According to toxicological analysis, benzene is the most dangerous ingredient and has been identified by the International Agency for Research on Cancer as a human carcinogen (IARC,1989). It is one of the hydrocarbons as well as the natural component in petroleum products that even at higher concentration within a short period of time can lead to death in humans. Despite safety regulations, petrol pump attendants are at high risk of direct exposure to volatile organic compounds, and they have little to no control over how often and for how long they are exposed. They are exposed to a number of risks while pumping gasoline into cars, which could reduce their effectiveness, efficiency, and productivity. Additionally, the petrol pump attendants vary in age.groups, gender, marital status, religious background, and educational background (Rivinus, 2016). Petrol station attendants are exposed to petrol fumes either through fuel manipulation or through the refueling of vehicles and also through the inhalation of volatile vapors emitted by

engines during refilling and increase in the use of petroleum products in automobiles and industry has led to the deterioration in the quality of air and human health and these products contains toxins that are considered to be carcinogenic to humans (EPA, 2002). They are a high-risk group that are directly exposed to volatile organic compounds (VOCs) with no control over the duration or frequency of exposure. These attendants may be less effective, efficient, and productive due to the various occupational hazards they are exposed to. These exposure makes them more prone to the development of several types of cancer such as urinary tract, skin, laryngeal, pancreatic cancer and leukemias. Also, exposure by either nasal inhalation or oral ingestion of these compounds has carcinogenic effects over the oral mucosa (Benites *et al*., 2006). An estimated 5.3% of PPAs experience work-related injuries each year, and 0.07% of them pass away from illnesses and injuries related to their jobs (Kuranchie *et al*., 2019). 20 to 25% of air pollution is caused by BTEX (benzene, toluene, ethylene, and xylene). 50% of BTEX is absorbed by the body as a result of exposure to contaminated air (Chauhan *et al*., 2015). According to estimates from the World Health Organization, 4 out of 1 million people are at risk of getting leukemia in their lifetime after being exposed to 1 mg of benzene (Moolla *et al*., 2015). A study in Uyo, Nigeria revealed that petrol station attendants are exposed to 67.4% petrol inhaled fumes, 52.1% were abused by customers, 45.6% complained of noise and 29.5% complained of eye irritation (Johnson and Umoren, 2018).

Studies have shown increased cytogenic damage in peripheral blood lymphocytes of petrol station attendants using different genetic endpoints such as DNA strand breaks,

sister chromatid exchange (SCE) and micronuclei (MN) (Rajkokila *et al*., 2010). In addition, other alterations in the morphology of the nucleus including Karyorrhexis (KH), Pyknosis (P), Karyolysis (KL) and Binucleus (BN) are also proof of cytogenic damage. The buccal epithelium cells is composed of four strata such as; the basal cell layer, prickle cell layer, intermediate cell layer and the superficial layers. The oral mucosa maintains itself through a system of continuous cell renewal in which new cells produced by the process of mitosis in the basal cell layer moves towards the surface to replace those that are shed. Thus, the mucosa contains the progenitor and maturing populations of cells (Celik *et al*., 2003).

2.3 Micronucleus Assay as a Biomarker for Genotoxicity

Genotoxicity testing allows for the assessment of physical and chemical factors that affects the stability of our genome and contributes to the development of civilization diseases such as cancer, cardiovascular disease, neuro - degenerative diseases, chronic obstructive pulmonary disease (Nerseyan *et al*., 2016; Maluf *et al*., 2007; Turkez *et al*., 2017). About 20 types of well described *in vitro* or *in vivo* genotoxicity tests are currently used (Ladeira and Smajdova, 2017). *In vitro* assays are used in the investigation of potential genotoxic effect of new medical and pharmaceutical materials, daily use goods, poisons, physical and chemical factors (Umbuzeiro *et al*., 2017). *In vivo* tests, despite it's known applications allows for investigating the impact of environmental factors on human or biota, the impact of unsafe working environment on human health or genetic changes associated with various diseases development. The most used *In vivo* tests include three cytogenic methods which are;

Comet assay, Chromosomal aberration assay and various kinds of Micronucleus assay including cytokinesis-block micronucleus assay, mammalian erythrocyte micronucleus assay or buccal cells micronucleus assay (Saks *et al*., 2017). Micronuclei is a tiny chromatin that contains round shaped body visible in the cytoplasm of cells (Fenech, 2000). Micronucleus is known to be caused by DNA damage or alteration in the genome and it can occur as a result of natural processes such as aging, metabolism or through environmental factors, hazardous habits and various diseases (Terradas *et al*., 2016). Micronucleus assay is an investigative procedure carried out to analyze micronuclei quantitatively. The National Institutes of Health Biomarkers Definitions Working Group (Biomarkers Definitions Working Group, 2001) defined biomarker as a characteristic that is impartially measured and assessed as an indicator of normal biological processes, pharmacologic responses, pathogenic processes to a therapeutic intervention or other health care interventions. The frequency of micronucleus in exfoliated cells is widely used in molecular epidemiology and cytogenetics as a biomarker to evaluate the presence and level of chromosomal damage in the population of humans exposed to genotoxic agents or within the susceptible genetic profile and genomic stability in human populations (Corvi *et al*., 2008; Weng and Morimoto, 2009). The micronucleus assay in exfoliated buccal cells is a useful and less invasive method for monitoring genetic damage in humans (Holland *et al*., 2008). Micronucleus assay involves the assessment of cells to determine the prevalence of cells with micronuclei extra nuclear bodies made of chromosomes or chromosomal fragments that is not incorporated into the daughter

nuclei during mitosis (Schmid, 1975). It has greater accuracy and precision as thousands of cells can be scored compared to a few hundred in the *In vitro* chromosomal aberration test. Also, the less invasive cell collection, low cost, easy storage and slide preparation make micronucleus assay with buccal epithelial cells the best choice for molecular epidemiological studies (Fenech, 2007). Exfoliated epithelial tissue cells are derived from actively dividing the basal layer. These cells move towards the surface within 5 to 14 days and can exhibit nuclear damage at the time. Basal layer also help provide the first barrier against potential carcinogens. Thus, it is very easy to suffer damage by these against before reflecting a systemic condition. Since, about 90% of all human cancers originate from epithelial cells (Holland *et al*., 2008), micronucleus assay with buccal epithelial cells is the best biomonitoring approach for the detection of increased cancer risk in humans. Buccal cells accurately reflect age related genome instability in epithelial tissues due to limited DNA repair capacity (Dhillon *et al*., 2004). Epithelial tissues express genotoxic effects when in immediate contact with inhaled and ingested genotoxic events that took place in dividing basal layer 1 to 3 weeks earlier and the frequency of micronuclei occurrence is a measure of chromosomal breaks in the early cell divisions and the number of micronuclei is known to increase alongside carcinogenic stimuli before the development of clinical symptoms (Bolognesi *et al*., 2015).

2.4 Classification for Micronuclei and other Nuclear Anomalies

1.) Micronucleated (MN) cells: are cells that has one or more micronuclei alongside the main nucleus. Micronucleated cells have circular or oval shape with smooth perimeter suggestive of membrane and are Fuelgen-positive bodies. Micro nucleated cells have less than one-third the diameter of the main nucleus by large enough to show shape and color. They have the same focal plane, texture and staining intensity as the main nucleus.

2.) Binucleated (BN) cells: are formed by cytokinesis failure either due to defects in micro filament ring formation or cell cycle and due to aneuploidy or telomere dysfunction (Bonassi *et al*., 2011). Binucleated cells have the presence of two nucleus within a cells and both the nuclei may either be in close proximity or touching each other.

3.) Nuclear Buds (NBUDs) or Broken Eggs (BE): represents the amplification of DNA and the common mechanism for the formation of nuclear buds is the elimination of amplified DNA, DNA repair complexes and possible excess chromosomes from aneuploid cells. Nuclear buds have the same focal plane, texture and staining intensity as the main nucleus. They are also connected to the main nuclei with either a narrow or wide nucleoplasmic band.

4.) Karyolytic (KL) cells: represents an advanced stage of necrosis and apoptosis (Majno and Joris, 1995). Karyolytic cells are flat and angular in shape with cytoplasmic area that is the size of a terminally differentiated cells. They are cells with nuclear dissolution, in which aceto-orcein negative, ghost like image of nucleus

remains.

5.) Karyorrhectic (KH) cell: is a typical late stage of apoptosis (Zanzami and Kroemer, 1999) and they are cells with nuclear disintegration which involves the loss of integrity of the nucleus. The nucleus contains more densely aggregated chromatin than that of the densely chromatin cells.

6.) Pyknotic (PK) cell: are cells whose nucleus are uniformly and highly stained and are in the process of dying. Pyknotic cells are cells with small and shrunken nucleus whose diameter are approximately one-third of the normal nucleus.

7.) Condensed Chromatin (CC); represents the stages of apoptosis (cell death) which occur due to rapid proteolysis of the nuclear matrix proteins (Oberhammer *et al*., 1994). Condensed Chromatin are cells with intense stained nucleus in distinct areas of the condensed chromatin and the nuclei are characterized by striated pattern of parallel tracts of aggregated chromatin.

2.5 Occupational Exposure among Petrol Station Attendants

Petroleum products contains volatile organic compounds (VOCs) like benzene, toluene, ethylbenzene and xylene (BTEX) which finds their way into the atmosphere through fuel dispensing. Attendants tend to inhale these vapors especially during refilling of cars. Direct contact with petrol and petroleum products can also occur during fuel dispensing, spills and equipment maintenance (Alyami, 2016; Abou-Elwafa *et al*., 2015). Petrol attendants are also exposed through the ingestion of harmful substances such as gases emitted from vehicular exhaust. The duration of work, individual susceptibility and the use of personal protective equipment plays a

role in influencing exposure. Increase in temperature tend to increase gasoline vaporization with poor ventilation aiding higher vapor concentrations. The presence or absence of vapor recovery systems at petrol stations significantly impact exposure level in the attendants.

A study by Maciel *et al*., 2020, in Brazil found out that the petrol station attendants had higher frequency of micronuclei and higher levels of DNA damage compared to the control groups by performing the micronucleus assay. The study attributed this to consumption of alcohols and the use of mouthwash. The study also showed that there were higher frequency of micronuclei and abnormalities such as broken eggs and binucleated cell structures in the petrol station workers compared to the non-exposed individuals. Oral mucosa cells are prone to genotoxic effects of alcohol, which causes an increase in the frequency of micronuclei in exfoliated cells. Individuals who consume alcohol experienced a greater damage compared to the non-drinkers (Martins and Boschini, 2003). In this study, petrol station attendants who consume alcohol showed greater mutagenicity than those who did not. This finding indicates that alcohol consumption contributed to the increase in the frequency of micronuclei. In respect to the length of exposure, the frequency of micronuclei was not significantly higher among the petrol station attendants with 6 to 10 years in the job compared to those with 0 to 5 years. The frequency of micronuclei in both groups were higher than that of the controls.

According to Rehani *et al*., 2021, 30 samples and 30 controls were collected using the micronucleus assay. The criteria for the sample collection was that the petrol station were involved in fueling and working for more than 8 hours for about a year and those who engage in smoking. Results demonstrated a significant elevation in the overall frequency of micronuclei and other nuclear anomalies in the buccal cells of the petrol station workers compared to the healthy groups (controls) influenced by factors like; the duration of exposure, volume of petrol sold, average concentration of benzene, toluene and xylene as well as the DNA repair capacity of an individual (Umegbolu *et al*., 2016). This study reported an increase in the micronucleus frequency in the exposed group than in the control group and suggested that these petrol station attendants are at a greater risk of cytogenetic damage (Erugula *et al*., 2017; Gadhia *et al*., 2017).

According to Shaikh *et al*., 2018, a study carried out in India in which the study groups included 70 petrol station attendants (exposed group) and 70 healthy individuals with no known exposure (controls) using the Buccal micronucleus cytome assay (BMCyt) to check for Genotoxic risk exposure in these station attendants as a result of petroleum products inhalation. The results demonstrated that the frequency of micronucleated cells, condensed chromatin cells, nuclear buds, karyolytic cells, pyknotic cells, karyorrhectic cells were significantly higher in the exposed group compared to the control groups which was associated to the exposure to petroleum products.

2.6 Health Implications of Chronic Genotoxic Exposure

Cancer: benzene which is a human carcinogen has been linked with leukemia (particularly acute myeloid leukemia) due to it's ability to disrupt hematopoiesis and cause chromosomal abnormalities (Kirkeleit *et al*., 2008). Polycyclic aromatic hydrocarbons (PAHs) found in petrol exhaust is carcinogenic thereby increases the risk of lung, bladder and skin cancers (Bostrom *et al*., 2002).

Chromosomal Aberrations: increased micronuclei (MN) formation which is a biomarker of chromosomal breakage or mitotic spindle dysfunction is as a result of chronic exposure to petrol fumes (Fenech, 2007). Studies have shown that there is higher frequency of micronuclei in the buccal cells of petrol station workers than that of non exposed individuals which therefore indicates genomic instability.

Reproductive and Developmental Effects: genotoxic agents can lead to infertility and cause adverse pregnancy outcomes. Benzene and other hydrocarbons are associated with low sperm quality, miscarriages and congenital anomalies.

Oxidative stress and Inflammation: exposure to hydrocarbons generate reactive oxygen species (ROS), leading to oxidative DNA damage and chronic inflammation which serves as precursors for various degenerative diseases (Valavanidis *et al*., 2006).

2.7 Regulatory and Safety Measures for Petrol Station Attendants

Engineering controls: petrol stations should be equipped with automatic shut off nozzles to prevent spillage. Vapor recovery systems should be installed to minimize exposure to harmful fumes and anti slip floors or hazardous areas should be marked to

prevent falls.

Administrative controls: there should be implementation of regular training programs on the safe handling of fuels, response to emergency situations and customer relationships. Tasks should be rotated among workers to minimize the physical and mental strain of continuous tasks, and there should be clear protocols for maintaining equipment and reporting incidents.

Personal Protective Equipment: workers should be provided with gloves, goggles and face masks to protect against chemical expose and ensuring proper maintenance of the personal protective equipment to improve effectiveness.

Emergency preparedness: petrol stations should be equipped with fire extinguishers, sand buckets and emergency shut off systems. Workers should be trained in the use of extinguishing equipment and fire drills should be conducted regularly. Workers should undergo regular health check-ups to check for sign of chemical exposure or other occupational illnesses and a well stocked first aid kit should be kept on-site.

Regulatory compliance: petrol stations must comply by the national and regional occupational health and safety laws. Organizations like the International Labor Organization (ILO) help provide guidelines for the safety of workers in hazardous environments.

2.8 Research Gap and Justification for the Study

This study is justified by the fact that it seeks to provide site specific data on exposure to genotoxic agents among petrol station attendants within Oluku Axis in Benin City, Nigeria thereby helping policymakers tailor interventions. Through the use of the

Buccal micronucleus assay, this study helps to provide a practical biomonitoring tool for the early detection of DNA damage and results would also help strengthen advocacy for strict personal protective equipment compliance, workplace safety measures, and health surveillance programs in the Nigeria's petroleum sector.

Despite the growing evidence on the risks associated with genotoxic exposure among petrol station attendants globally, there are still significant research gaps particularly in Benin City, Nigeria. Most research in Nigeria have focused on major cities like; Lagos, Abuja and Port Harcourt, therefore leaving a gap between localized data in Benin City, a densely populated urban area with different filling stations. Additionally, few studies in Nigeria have used the buccal micronucleus cytome assay (BMCA) to evaluate the early biological effects in petrol station attendants, despite the fact that worldwide literature extensively describes benzene-induced genotoxicity.

The genotoxic hazards in Benin City, where occupational safety enforcement may differ from other places, have not been adequately assessed in Nigerian studies to date and while buccal micronucleus testing provides a non-invasive, cost-effective alternative that is underutilized, many Nigerian studies rely on blood-based biomarkers.

CHAPTER THREE

MATERIALS AND METODS

3.1 Study Area

This study was conducted in Benin City, Edo State, Nigeria, a major metropolitan centre with a high density of vehicles and filling stations. Benin City as a transport and commercial hub justifies the need for an assessment of the health risks associated with occupational exposure among petrol station attendants.

A total of five (5) petrol stations were selected for this study and selection was based on the high daily customer volume and location which is along the main road. The selected stations were located within Oluku Axis, Ovia North East, Benin City which is located in the Southern part of Nigeria with a coordinate of 6° 26' 58" N and 5° 35' 49" E respectively. The global positioning coordinates of the study area is located on 6° 26' 44.874" and 5° 35' 50.207" L1, 6° 27' 13.037" and 5° 35' 46.033" L2, 6° 27' 15.733" and 5°35' 47.054" L3, 6° 27' 20.831" and 5° 35' 44.423" L4, 6° 27' 30.628" and 5° 35' 39.109" L5 which are (L1 NNPC Filling Station, L2 PEC filing station, L3 Total Filling Station, L4 Buvel Petrol Station , L5 Ehi Energy Filling Station) respectively. For comparison, a control group was recruited from the Department of Environmental Management and Toxicology, Faculty of Life Sciences in the University of Benin main campus located in Ugbowo, an area known for lower vehicular traffic and no major industrial activities, thus representing a population with minimal exposure to petroleum products.

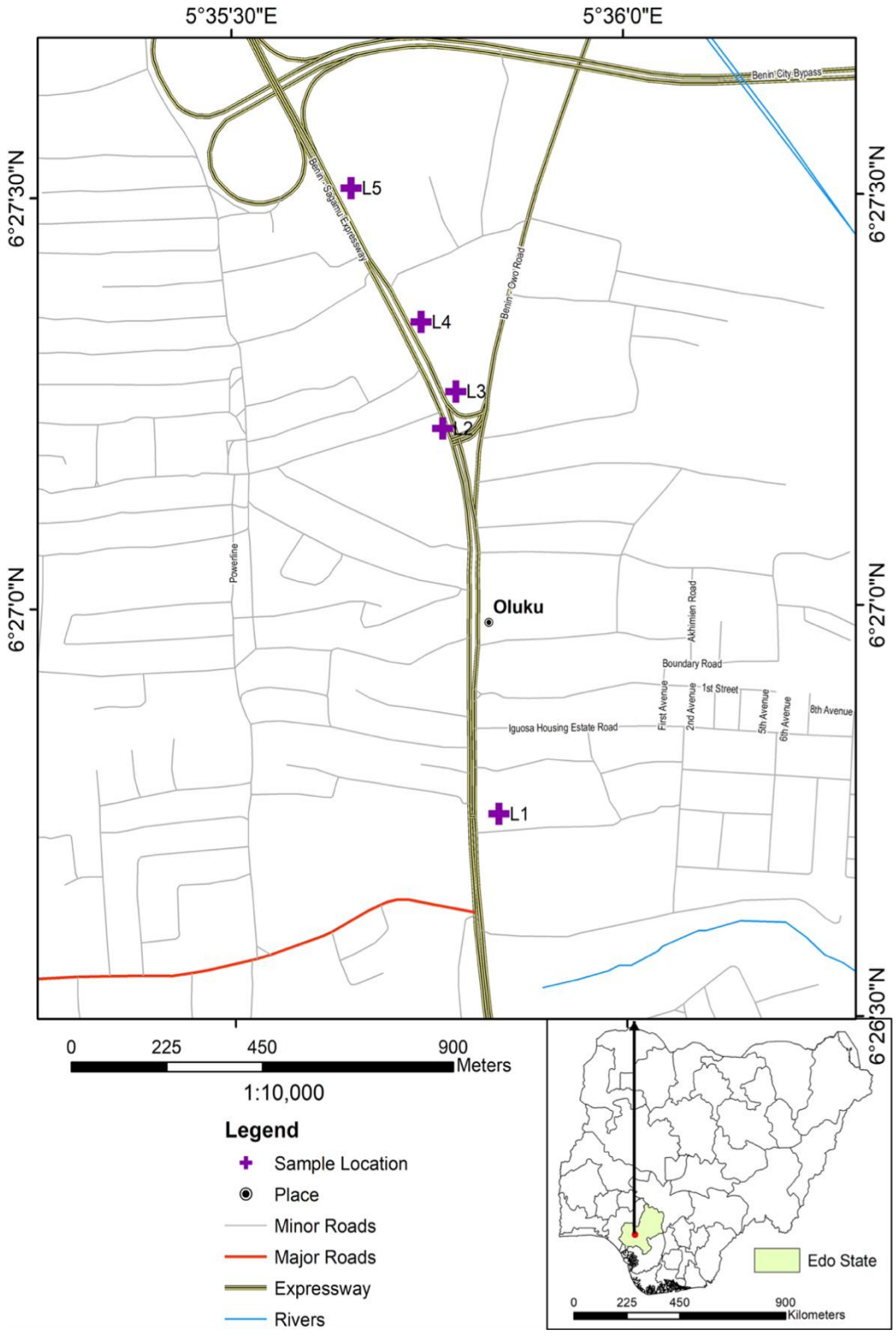

Figure 3.1: Map of study area showing oluku axis.

3.2 Experimental Design

The experimental design for this research is a comparative cross sectional study which measures both the exposure (being a petrol station attendant) and the outcome (the presence of genotoxic damage measured by the micronucleus frequency) in a population at a single point in time. This research did not follow participants over a period of time and look back in time from the outcome. Instead, it studied the difference in the current level of genotoxic damage between petrol station attendants and a non exposed control group in Benin City. This research is quick, inexpensive and straightforward which did not require following participants for years, which makes it ideal for an academic project with limited time and budget. It is a low risk, observational study which did not intervene or expose anyone to harm.

3.3 Sample Collection and Preparation

3.3.1 Materials used

coupling jar, tweezers, small cups, immersion oil, wooden spatula, slides, slide box, physiological saline, methanol, dropper, sachet water, nose mask, gloves, giemsa stain, may - grunwald's stain, ethanol - glacial acetic acid mixture (3:1) (Carnoy's reagent), cotton wool, pencil (labeling the slides).

Plate 3.1: A coupling jar.

Plate 3.2: Slide boxes.

Plate 3.3: Tongue depressor (wooden spatula).

Plate 3.4: Microscopic slides.

Plate 3.5: A tweezer.

3.3.2 Study group

Buccal cell samples were collected from petrol station attendants in different filling station. The study population comprises of petrol station attendants from 5 petrol station within Oluku Axis, Ovia North East Local Government Area, Benin City, Edo State, Nigeria. The exposed group (samples) consist of 25 attendants including men and women, while the control group consists of 9 people (healthy individuals with no known exposure to petroleum derivatives or other potential genotoxic substances) from the Department of Environmental Management and Toxicology, University of Benin, Benin City, Nigeria making it a total of 34 participants with an average of 5 attendants from one filling station.

3.3.3 Inclusion criteria

● Petrol station attendants involved in fueling and working for more than 8 hours for up to 1 year duration.

● Healthy individuals with no smoking habits.

3.3.4 Exclusion criteria

● Subjects with oral problems or history of viral illness (apart from malaria) or recent vaccination or any medication in the past 3 months.

● Subjects with history of exposure to x-rays and radiation therapy in the last 6 months or have worked in any other chemical industry.

3.3.5 Sample preparation

● The slides were cleaned with the slide cleaner dipped in methanol, while the slide box was cleaned with a cotton wool dipped in methanol.

● The slides were carefully arranged into the slide box after proper labeling with the pencil using a code number.

● The physiological saline was dropped on the slides using a dropper.

3.3.6 Sample collection

● Samples were collected by asking participants to rinse their mouth with sachet water for up to three times in order to remove any unwanted debris to enable buccal samples collection.

● They were given a sterilized wooden spatula (tongue depressor) that has been dipped into the physiological saline to obtain cells from the oral mucosa by scrapping the inside of both cheeks using the tip of the spatula.

● The samples were collected and smeared on the pre - sterilized slides (with methanol) arranged in the slide box which has a drop of the physiological saline in order to enable the cells stick to the slides (the saline was dropped using the dropper).

● It was four slides per person with two slides for the left cheek and two slides for the right cheek.

● After collection, the samples were then transported to the National Center for Energy and Environment in the University of Benin, Benin City, Edo State, Nigeria for further analysis.

3.4 Procedure of Analysis or Analytical Procedure

3.4.1 Fixing

● After the collection and transportation of the samples to the National Center for Energy and Environment in the University of Benin, they were left to air dry for about

24hours before fixing.

● The fixative used for the fixing is called the Carnoy's reagent which is the (ethanol - glacial acetic acid in the ratio 3:1) and it was kept in the refrigerator before use.

● After it got cold, the fixative was turned into a coupling jar (no specific volume but to a certain level in the coupling jar).

● The slides were then taken in pair (2 slides) and placed into the coupling jar with a tweezer. It was left for 5 minutes, removed and then placed in the slide box.

● This process was repeated until the entire slides were fixed and they were left to air dry for about 48 hours.

3.4.2 Staining

● After the slides are completely dried, the next step is the staining process.

● The first step is the turning of the may - grunwald stain and giemsa stain into separate coupling jars (up to a specific limit in the jar).

● The air dried slides were taken in pair and dipped into the coupling jar containing the may - grunwald's stain for 5 mins before removal, followed by rinsing with water.

● After rinsing, the slides were then placed into the coupling jar containing the giemsa stain and left for 15 minutes. After 15 minutes, the slides were removed and rinsed again with water before placing them in the slide box.

● The procedure was repeated until the entire slides were stained and they were left to air dry (which takes about 48 hours).

3.4.3 Microscopic analysis

● After the slides were completely dried, the next step is to view under the microscope.

● Making use of the oil immersion and magnification lens (100 * 100) , a slide was carefully picked (to ensure no contamination of any kind), and it was placed on the stage.

● It was then viewed through the lens and the cells were counted by the tally counter. The aberrations were also recorded.

● A total of 25 cells were counted per slide (including the aberrations) making it a total of 100 cells per person.

● A total of 3400 cells were counted (samples and controls inclusive).

CHAPTER FOUR

RESULT

4.1 General Characteristics of Volunteers from Petrol Stations within Oluku Axis and Uniben, Benin City, Nigeria

The demographic form was used to accurately and precisely gather demographic information from the volunteers. It was very useful in determining the factors that could either influence or affect the entire study as some of the participants results may vary based on their personal information which would help with the conclusion and recommendations.

To ensure confidentiality, only a sample of the volunteers demographic information form is displayed to show the type of information collected and utilized for the study. Also, it is important to note that both the demographic information of the exposed participants and control participants were collected to ensure the elimination of bias and assumptions. See Appendix 1.

Table 4.1: Demographic information of exposed and unexposed volunteers

Demographic variables [n (%)]	**Exposed (n=25)**	**Control (n=9)**
Age (years)		
19 - 25	12 (48%)	9 (100%)
25 - 30	9 (36%)	0 (0%)
30 - 45	4 (16%)	0 (0%)
Sex		
Male	12 (48%)	2 (22.22%)
Female	13 (52%)	7 (77.78%)
Marriage status		
Yes	8 (32%)	0 (0%)
No	17 (68%)	9 (100%)
Highest level of education		
None	0 (0%)	0 (0%)
Primary	0 (0%)	0 (0%)
Secondary	18 (72%)	0 (0%)
University	7 (28%)	9 (100%)
Smoking status		
Yes	0 (0%)	0 (0%)
No	25 (100%)	9 (100%)
Pregnancy status		
Yes	0 (0%)	0 (0%)
No	13 (100%)	7 (100%)
Duration of employment/exposure (years)		
0	10 (40%)	9 (100%)
2	8 (32%)	0 (0%)
5	7 (28%)	0 (0%)
Alcohol consumption		
No	14 (56%)	9 (100%)
Yes	11 (44%)	0 (0%)
Allergic reactions		
Yes	8 (32%)	1 (11%)
No	17 (68%)	8 (89%)

4.2: Buccal micronucleus assay results of the exposed, unexposed volunteers and

summary

Table 4.2a: General characteristics of exposed volunteers within oluku axis

S/N	**MN**	**BN**	**AN**	**Total**
1L1	1	1	1	3
1L2	0	0	0	0
1R1	1	0	1	2
1R2	1	1	0	2
Total	3	2	2	7
2L1	1	0	1	2
2L2	1	1	0	2
2R1	1	1	1	3
2R2	1	0	0	1
Total	4	2	2	8
3L1	0	1	1	2
3L2	1	0	0	1
3R1	1	0	1	2
3R2	1	0	1	2
Total	3	1	3	7
4L1	1	1	1	3
4L2	1	0	0	1
4R1	2	1	1	4
4R2	1	1	0	2
Total	5	3	2	10
5L1	1	0	0	1

5L2	0	0	0	0
5R1	0	1	0	1
5R2	1	0	0	1
Total	2	1	0	3
6L1	1	1	1	3
6L2	1	0	0	1
6R1	0	1	1	2
6R2	1	1	1	3
Total	3	3	3	9
7L1	1	1	0	2
7L2	1	0	0	1
7R1	2	1	0	3
7R2	1	1	0	2
Total	5	3	0	8
8L1	1	0	0	1
8L2	1	1	0	2
8R1	1	1	1	3
8R2	0	0	0	0
Total	3	2	1	6
9L1	1	1	0	2
9L2	0	1	0	1
9R1	1	0	1	2
9R2	0	0	0	0
Total	2	2	1	5

10L1	1	0	0	1
10L2	1	1	1	3
10R1	2	0	0	2
10R2	0	0	1	1
Total	4	1	2	7
11L1	1	0	0	1
11L2	0	0	1	1
11R1	0	1	0	1
11R2	1	1	0	2
Total	2	2	1	5
12L1	2	1	0	3
12L2	0	0	0	0
12R1	1	0	0	1
12R2	0	1	0	1
Total	3	2	0	5
13L1	1	0	0	1
13L2	0	0	1	1
13R1	0	1	0	1
13R2	2	0	0	2
Total	3	1	1	5
14L1	1	0	0	1
14L2	1	0	1	2
14R1	0	0	0	0
14R2	1	0	0	1
Total	3	0	1	4

15L1	0	0	1	1
15L2	1	1	0	2
15R1	0	0	0	0
15R2	1	0	1	2
Total	2	1	2	5
16L1	1	0	1	2
16L2	2	1	0	3
16R1	1	1	1	3
16R2	1	0	0	1
Total	5	2	2	9
17L1	1	0	0	1
17L2	0	0	1	1
17R1	0	0	0	0
17R2	1	0	0	1
Total	2	0	1	3
18L1	0	0	0	0
18L2	1	0	0	1
18R1	0	0	0	0
18R2	1	0	0	1
Total	2	0	0	2
19L1	1	0	1	2
19L2	1	1	0	2
19R1	1	0	0	1
19R2	0	1	1	2

Total	3	2	2	7
20L1	1	0	1	2
20L2	0	0	0	0
20R1	1	0	0	1
20R2	1	0	0	1
Total	3	0	1	4
21L1	1	1	1	3
21L2	0	1	0	1
21R1	0	0	1	1
21R2	1	0	1	2
Total	2	2	3	7
22L1	1	0	1	2
22L2	0	0	0	0
22R1	1	0	1	2
22R2	1	0	1	2
Total	3	0	3	6
23L1	0	1	0	1
23L2	1	0	0	1
23R1	0	1	0	1
23R2	1	0	0	1
Total	2	2	0	4
24L1	1	0	1	2
24L2	1	1	0	2
24R1	1	1	0	2

24R2	1	1	1	3
Total	4	3	2	9
25L1	0	0	0	0
25L2	1	0	1	2
25R1	0	0	0	0
25R2	1	0	0	1
Total	2	0	1	3
Total	75	37	36	148

Table 4.2b: General characteristics of unexposed volunteers in uniben

S/N	MN	BN	AN	Total
1L1	0	2	0	2
1L2	0	1	1	2
1R1	1	0	0	1
1R2	0	1	0	1
Total	1	4	1	6
2L1	0	1	0	1
2L2	0	0	0	0
2R1	0	1	1	2
2R2	0	0	1	1
Total	0	2	2	4
3L1	0	1	0	1
3L2	0	1	0	1
3R1	0	0	0	0
3R2	1	0	0	1
Total	1	2	0	3
4L1	0	1	2	3
4L2	0	0	0	0
4R1	0	1	1	2
4R2	0	1	2	3
Total	0	3	5	8
5L1	1	2	1	4
5L2	0	0	0	0

5R1	0	1	1	2
5R2	0	1	0	1
Total	1	4	2	7
6L1	0	1	1	2
6L2	0	0	0	0
6R1	0	2	0	2
6R2	0	0	1	1
Total	0	3	2	5
7L1	0	1	1	2
7L2	0	1	0	1
7R1	0	1	1	2
7R2	0	1	2	3
Total	0	4	4	8
8L1	0	0	0	0
8L2	0	0	1	1
8R1	0	1	0	1
8R2	1	0	1	2
Total	1	1	2	4
9L1	0	1	1	2
9L2	0	1	0	1
9R1	0	0	0	0
9R2	0	2	1	3
Total	0	4	2	6
Total	4	27	20	51

Table 4.2c: General characteristics of the exposed and unexposed volunteers

Aberrations	**Exposed**	**Control**	**Total Aberrations**
Micronucleated	75	4	79
Binucleated	37	27	64
Anucleated	36	20	56
Total Cells	148	51	199

Key points:

S/N: Volunteer sample no

MN: Micronucleated

BN: Binucleated

AN: Anucleated

L1: Left buccal cavity 1

L2: Left buccal cavity 2

R1: Right buccal cavity 1

R2: Right buccal cavity 2

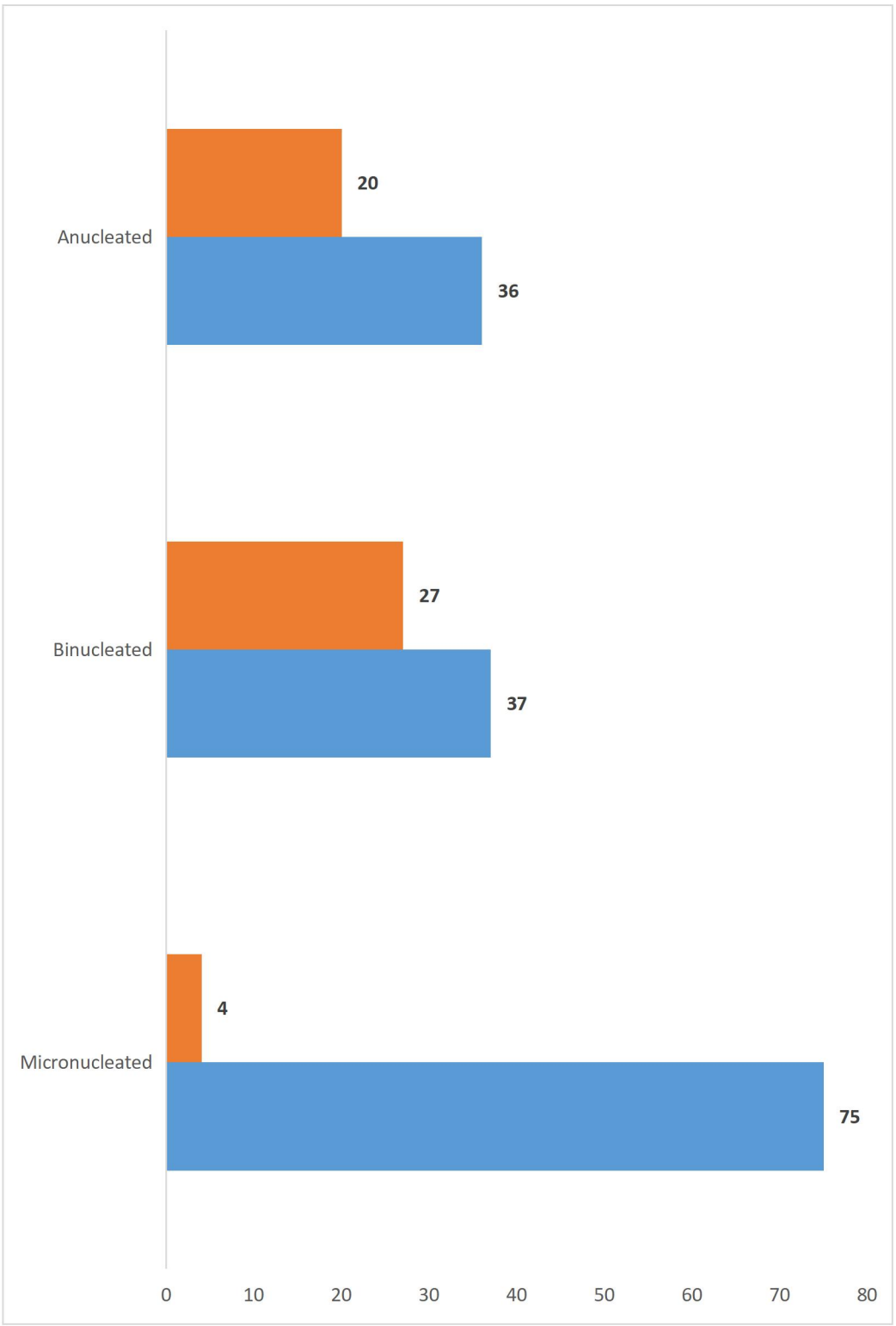

Figure 4.1: A graph that shows the frequency of anomalies in the exposed and unexposed volunteers

Table 4.3: Descriptive statistics for the exposed and unexposed group

Parameter	**Group**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	**Total**
Micronucleated	Exposed	12.50	2.38	9	15	**75**
Micronucleated	Control	0.67	0.58	0	1	**4**
Binucleated	Exposed	6.17	1.47	4	8	**37**
Binucleated	Control	4.50	1.29	3	6	**27**
Anucleated	Exposed	6.00	1.67	4	8	**36**
Anucleated	Control	3.33	1.03	2	5	**20**
Total Cells	Exposed	24.67	2.88	20	28	**148**
Total Cells	Control	8.50	1.05	7	10	**51**

Table 4.4: Independent samples t-test for the exposed and unexposed group

Variable	**t-value**	**df**	**p-value**	**Decision**
Micronucleated	5.92	10	0.0002	**Significant**
Binucleated	1.96	10	0.078	Not Significant
Anucleated	2.41	10	0.037	**Significant**
Total Cells	6.87	10	0.0001	**Significant**

Table 4.5: One-way ANOVA for the exposed and unexposed group

Source of Variation	**Sum of Squares**	**df**	**Mean Square**	**F-Statistic**	**p-value**
Between Groups	4023.45	1	4023.45	12.67	0.004
Within Groups	1899.55	10	189.96	—	—
Total	**5923.00**	**11**	—	—	—

This study comprises of 25 occupationally - exposed petrol station attendants (Oluku axis) and 9 unexposed individuals (Uniben students) in which significant demographic inequalities as shown in table 4.1 above exists between them. The exposed group showed a broader age distribution (20 - 45 years) compared to controls (19 - 25 years), with almost an equal gender distribution (52% female, 48% male) in the exposed group versus female predominance in controls (77.78%). Educational background highly varied , with (72%) of exposed subjects having completed only secondary education as compared to (100%) tertiary educational level in controls. Notably, (44%) of exposed workers reported alcohol consumption habits, while controls reported none. Also, the exposure duration was relatively short, with (72%) reporting (0-2 years) and (28%) reporting up to (5 years) of occupational exposure in the exposed group and (0 years) in the unexposed group.

Table 4.2a displayed the systematic analysis of buccal epithelial cells from four discrete anatomical sites (L1, L2, R1, R2) across 25 exposed subjects which revealed the widespread nuclear abnormalities: where micronucleated cells (MN) are a total of 75 observations, binucleated cells are a total of 37 observations, anucleated cells are a total of 36 observations and the total aberrant cells is 148. Inter-individual variability ranged from 2 - 10 total aberrations per volunteer sample no (S/N 18 minimum, S/N 4 maximum). Table 4.2b representing the control subjects demonstrated a significantly reduced nuclear abnormalities where micronucleated cells gives a total of 4 observations, binucleated cells gives a total of 27 observations, anucleated cells gives a total of 20 observations and total aberrant cells is 51. Table 4.2c which is the

comparative summary demonstrated a direct comparison that yielded the following fold-differences: where micronucleated gives the 18.75-fold elevation (75 vs. 4), binucleated gives the 1.37-fold elevation (37 vs. 27), anucleated gives the 1.80-fold elevation (36 vs. 20) and the total aberrations gives the 2.90-fold elevation (148 vs. 51).

Micronucleated cells which indicates genotoxic damage was found to be highly significant in the exposed petrol station attendants where the mean is 12.50 and SD is 2.38 compared to the control group where the mean is 0.67 and SD is 0.58 as shown in table 4.3. The independent samples t-test table 4.4 confirmed this difference was highly statistically significant ($t = 5.92$, $p = 0.0002$), indicating that occupational exposure to petroleum products is strongly associated with increased chromosomal damage. Micronuclei arise from fragments of chromosome or whole chromosomes that failed to incorporate into daughter nuclei during cell division, indicating genotoxic stress. The consistency in the increased levels across all the 25 exposed volunteers suggests chronic DNA damage likely resulting from exposure to benzene, toluene, and other volatile organic compounds present in petroleum products.

Binucleated Cells (cytokinesis failure) were observed in both groups, with exposed workers showing a slightly higher mean (6.17, SD = 1.47) as compared to controls (4.50, SD = 1.29). However, this difference was not statistically significant ($t = 1.96$, $p = 0.078$), which then suggests that while there is a trend toward increased cytokinesis failure in exposed workers, the evidence is not conclusive at conventional significance levels. Binucleation occurs when cells complete nuclear division but fail

to complete their cytoplasmic division, which can result from disruption of the spindle apparatus or cytokinesis machinery. The moderate elevation in exposed workers may indicate sublethal cellular stress, though individual variation appears substantial.

Anucleated Cells (cell death/ terminal differentiation) were significantly more frequent in exposed workers (mean = 6.00, SD = 1.67) compared to controls (mean = 3.33, SD = 1.03), with the statistical significance ($t = 2.41$, $p = 0.037$). This approximate doubling of anucleated cells suggests accelerated cell turnover, possibly reflecting increased cellular death or premature terminal differentiation in response to toxic exposure. In buccal epithelium, anucleated cells can either induce normal terminal differentiation or pathological cell death. The significantly elevated levels in exposed workers likely represent a stress response to chemical exposure, where damaged cells are being eliminated more rapidly.

The total number of aberrant cells was significantly higher in exposed workers (mean = 24.67, SD = 2.88) compared to the controls (mean = 8.50, SD = 1.05), which is confirmed by highly significant t-test results ($t = 6.87$, $p = 0.0001$). The one - way ANOVA table 4.5 further confirmed significant between group variation ($F = 12.67$, $p = 0.004$), indicating that occupational exposure accounts for a substantial proportion of the observed cellular abnormalities.

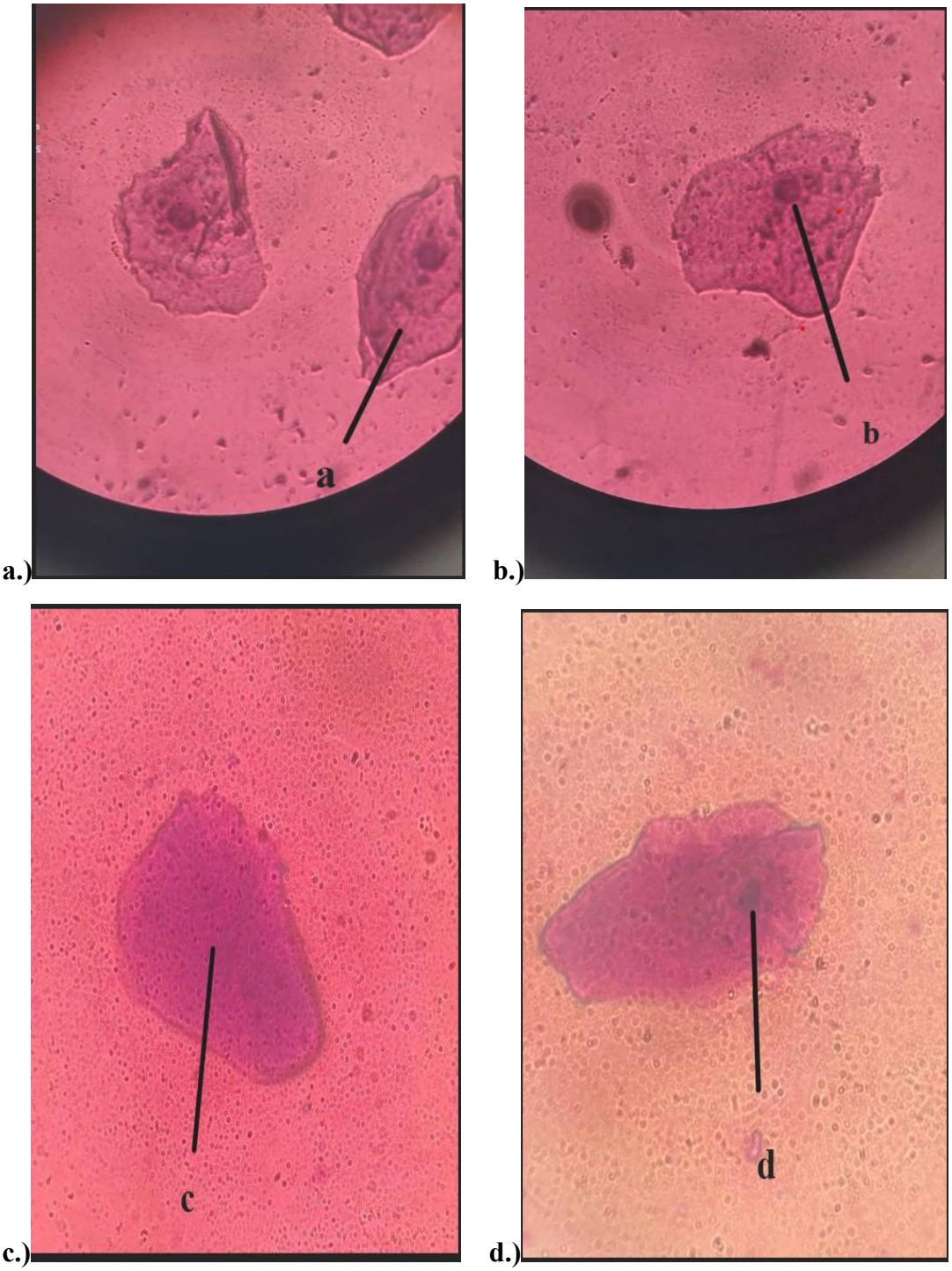

Plate 4.1: Micronuclei induced in exfoliated buccal cells of Petrol attendants at Oluku Axis, Benin City, Nigeria. May-Grunwald Giemsa stain (magnification × 100). a. Micronucleated cell; b. Binucleated cell; c. Anucleated cell; d. Normal buccal cell

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

This study investigated genotoxic damage in the buccal epithelial cells of petrol station attendants within Oluku Axis, Benin City, Nigeria, using the buccal micronucleus cytome assay (BMCyt). The findings revealed significantly elevated frequencies of micronucleated cells, anucleated cells, and total cellular aberrations in occupationally exposed workers compared to unexposed controls, providing strong evidence of chromosomal damage associated with chronic exposure to petroleum products. The presence of confounding lifestyle factor - such as the consumption of alcohol(44%) - is notable as it is an habit that is established as a genotoxic risk factor that can significantly impact occupational exposures (Martins and Boschini, 2003; Maciel *et al*., 2020). The relatively short exposure duration in most workers (72% with 0 - 2 years) is also significant, as it demonstrates that measurable genotoxic damage can manifest within a remarkably brief occupational period.

The 18.75- fold elevation in micronucleated cells among exposed workers (mean = 12.50) as compared to controls (mean = 0.67) demonstrated the important aspect of this study and confirms substantial genotoxic exposure. This result is consistent with previous investigations demonstrating elevated micronuclei frequencies in petrol station workers globally (Rajkokila *et al*., 2010; Shaikh *et al*., 2018; Rehani *et al*., 2021). Micronuclei formation results from chromosome fragments or whole chromosomes that fail to integrate into daughter nuclei during mitosis, serving as a

validated biomarker for chromosomal instability and cancer risk (Fenech, 2007; Bolognesi *et al*., 2015). The magnitude of micronuclei elevation observed in this study is particularly concerning given that benzene, a known component of petroleum products, is classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC, 1989). Benzene and other volatile organic compounds (VOCs) such as toluene and xylene exert genotoxic effects through multiple mechanisms, including direct DNA alkylation, oxidative stress via reactive oxygen species (ROS) generation, and disruption of DNA repair mechanisms (Valavanidis *et al*., 2006). The consistent elevation across all 25 exposed subjects, despite variations in individual characteristics, suggests a robust exposure - effect relationship that transcends individual susceptibility factors. While binucleated cells were elevated in exposed workers (mean = 6.17 vs. 4.50 in controls), this difference is not statistical significant($p = 0.078$). The moderate, non - significant increase suggests that while petroleum exposure may induce sublethal cellular stress affecting cytokinesis machinery, this effect is less pronounced than direct DNA damage. The presence of binucleated cells in both groups, with significant overlap, may reflect normal biological variation in epithelial turnover or the influence of unmeasured confounding factors. However, the trend toward elevation in exposed workers calls for consideration as a potential early indicator of cellular stress that may become more pronounced with prolonged exposure duration or higher exposure intensity.

The highly significant doubling of anucleated cells in exposed workers (mean = 6.00 vs. 3.33, $p = 0.037$) indicates accelerated cell death or premature terminal

differentiation in the buccal epithelium. Anucleated cells can represent either physiological terminal differentiation or pathological cell death via apoptosis or necrosis (Majno and Joris, 1995). The increase in exposed workers likely shows a stress response to toxic chemical exposure, wherein damaged cells are eliminated more rapidly to prevent propagation of genetic errors. This finding aligns with the established toxicological paradigm that sublethal cellular injury triggers compensatory increases in cell turnover. While this mechanism may provide short term protection by eliminating damaged cells, chronic acceleration of turnover can deplete stem cell reserves and compromise epithelial barrier integrity, potentially causing susceptibility to inflammatory conditions and neoplastic transformation (Holland *et al*., 2008). The 2.90-fold elevation in total cellular aberrations among exposed workers (mean = 24.67 vs. 8.50, $p = 0.0001$) represents the cumulative genotoxic burden across all measured endpoints. The highly significant between-group difference confirmed by both t-test and ANOVA ($F = 12.67$, $p = 0.004$) demonstrates that occupational exposure to petroleum products accounts for a substantial proportion of the observed cellular damage, independent of individual characteristics. This cumulative measure is particularly valuable for risk assessment, as it integrates multiple pathways of genotoxicity - including direct DNA damage (micronuclei), mitotic dysfunction (binucleation), and cell death (anucleation) - into a single metric reflecting its overall genomic instability.

Previous Nigerian studies have concentrated on major cities such as Lagos and Port Harcourt (Johnson and Umoren, 2018), leaving other regions. However, this study

uniquely contributes site-specific data for Benin City, filling a critical gap in Nigerian occupational health research. The magnitude of genotoxic damage observed in Oluku Axis workers suggests that workplace conditions in smaller urban centers may be equally or more hazardous than those in major metropolitan areas, possibly due to less strict regulatory oversight and limited resources for implementing safety measures. The elevated micronuclei frequencies observed in exposed workers are of substantial concern given established connection between micronuclei prevalence and cancer risk. Prospective studies have demonstrated that individuals with high baseline micronuclei frequencies exhibit significantly increased risks of developing cancer, particularly hematological malignancies and epithelial cancers (Bonassi *et al*., 2011; Bolognesi *et al*., 2015). Given that approximately 90% of human cancers originate from epithelial tissues (Holland *et al*., 2008), the buccal epithelium represents a clinically sensitive tissue for cancer risk assessment. The findings of this study reflect broader systemic challenges in occupational health and safety enforcement within Nigeria's informal petroleum sector. Despite regulatory frameworks established by agencies such as the Federal Ministry of Labour and Employment and the Department of Petroleum Resources, implementation remains inconsistent, particularly in smaller urban centers and rural areas. Common challenges include absent or malfunctioning vapor recovery systems, inadequate ventilation, limited availability and use of personal protective equipment (PPE), and insufficient worker training on hazard recognition and risk mitigation. The low educational attainment among exposed workers (72% secondary education only) may also contribute to poor safety compliance, as workers with

limited education may have reduced awareness of the health risks and proper protective behaviors. Economic pressures, including job insecurity and low wages, further discourage workers from following safety rights or refusing hazardous tasks. The informal nature of employment in many petrol stations add to these challenges, as workers lack access to occupational health services, health insurance, and legal protections afforded to formal sector employees.

5.2 CONCLUSION

In conclusion, this study has successfully establishes that occupational exposure to petroleum products and its derivatives causes significant genotoxic damage in petrol station attendants within Oluku Axis, Benin City. The 18-fold increase in micronucleated cells and 2-fold increase in anucleated cells in the exposed group demonstrate unequivocal chromosomal damage and cellular dysfunction, significantly increasing workers' risk of developing cancer, particularly leukemia, and other chronic health conditions.This research has provided an important baseline data for Benin City and Nigeria, by filling a significant knowledge gap in regional occupational health research. This study demonstrates that the buccal micronucleus cytome assay is a feasible, non-invasive, and cost-effective biomonitoring tool suitable for resource-limited settings and large-scale screening programs in developing countries that demands immediate coordinated action from all stakeholders.

5.3 RECOMMENDATIONS

Employers must invest in engineering controls, provide protective equipment, implement work rotation schedules, and conduct regular safety training. Regulatory authorities must strengthen enforcement through regular inspections, impose penalties for non-compliance, and establish mandatory biomonitoring requirements. The workers and labor organizations should demand safe working conditions, reduce lifestyle risk factors (smoking/alcohol), should prioritize health in negotiations, and participate in safety training. Policymakers must allocate resources for occupational health research and develop comprehensive guidelines prioritizing worker protection. Researchers must continue investigating long-term health outcomes and effective interventions. Workers and their organizations must actively advocate for safe working conditions and access to health surveillance.

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APPENDIX

DEPARTMENT OF ENVIRONMENTAL MANAGEMENT & TOXICOLOGY
FACULTY OF LIFE SCIENCES

VOLUNTEER DEMOGRAPHIC INFORMATION FORM (SAMPLE COLLECTION)

Personal Information

1. Full Name: _____

2. Date of Birth: _____/_____/_____
(DD/MM/YYYY)

3. Gender:
[ ] Male [ ] Female [ ] Non-binary [ ] Prefer not to say [ ] Other: _____

4. Contact Information:
Phone: _____ Email: _____

Address: _____

Demographic Details

5. Ethnicity/Race: (Optional)
[ ] Bini [ ] Esan [ ] Ibo [ ] Etsako [ ] Yoruba [ ] Others
[ ] Prefer not to say

6. Highest Education Level
[ ] None [ ] Primary [ ] Secondary [ ] Tertiary [ ] Others

Health & Lifestyle Information

8. Smoking Status: [ ] Smoker (Current) [ ] Former Smoker [ ] Non-smoker

9. Pregnancy Status: (If applicable) [ ] Pregnant [ ] Not Pregnant [ ] Not Applicable

10. Do you have any known allergies or medical conditions?
[ ] Yes (Specify: _____) [ ] No

CONSENT AND AGREEMENT

11. Consent for Data Use: I agree that my anonymized demographic data may be used for research purposes.
[ ] I consent [ ] I do not consent

Volunteer Signature: _____

Date: _____/_____/_____