EVALUATION OF CYTOLOGICAL FEATURES OF SPUTUM SMEARS OBTAINED FROM FUEL STATION ATTENDANTS EXPOSED TO GASOLINE FUMES

[1*]Ajileye, A. B., [2]Soladoye, E. A. and [3]Esan E. O.

[1]Biomedical Laboratory Science Department, College of Medicine, University of Ibadan, Ibadan, Nigeria.

[2]Medical Laboratory Science Department, Lead City University, Ibadan, Ibadan, Nigeria.

[3]Department of Medical Laboratory Science, University of Medical Sciences, Ondo City, Ondo State.

Corresponding Author: *Ajileye, Ayodeji Blessing. E-mail: ayobless05@gmail.com or ab.ajileye@ui.edu.ng **ORCID NUMBER**: 0000-0002-1576-6207, **Cell Phone:** +2348030445624

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Abstract

Background: Fuel pump attendants are individuals that work in filling stations. They are constantly exposed to fumes of petrol, gas, diesel and automobile engine products from vehicle exhaust. This increases the risk of acute and chronic respiratory diseases and carcinogenesis among them. The risk of health complications increase with the duration of exposure. Aim: This study aimed to evaluate the cytological features of sputum smears obtained from fuel station attendants exposed to gasoline fumes.

Methods: Sputum samples were collected from 150 subjects, of which 100 were fuel pump attendants (test group) and 50 were control subjects who are not fuel pump attendants. Questionnaire was administered to both groups prior to sample collection. Samples collected were processed and smears were made immediately and fixed in 95% alcohol. The fixed smears were stained with Heamatoxylin and Eosin and Papanicolaou staining techniques. Stained smears were examined under the microscope and photomicrographs were taken.

Results: The smears revealed that 82(82%), 37(37%) and 23(23%) of fuel pump attendants had infiltrate of inflammatory cells, atypical cells and cytoplasmic granulation respectively. About 19(19%), 22(22%) and 48(48%) of fuel pump attendants had pyknotic cells, multinucleated squamous cells and increased nucleo-cytoplasmic ratio.

Conclusion: This study revealed respiratory health risks in fuel pump attendants, emphasizing the importance of comprehensive safety measures.

Keywords: atypical cells, cytoplasmic granulation, gasoline fumes, inflammatory cells, carcinogenesis

INTRODUCTION

Sputum is produced in the lungs and in the airways leading to the lungs (Jain and Roy, 2018). Sputum, or phlegm, is the fluid that is secreted by cells in the lower respiratory tract such as the bronchi and the trachea. It differs from saliva, in that it contains cells that line the respiratory passages (Jain and Roy, 2018). Sputum specimen can be obtained from a subject either spontaneously or by aerosol–induced method. Several studies have revealed that morning specimen resulting from overnight accumulation of secretion yields best result, and about 3 to 5 consecutive days' sputum samples should be examined to ensure maximum diagnostic accuracy (Shen and Sergi, 2024).

Sputum cytology is carried out to help detect certain non-cancerous lung conditions. It may also be done when lung cancer is suspected (Jain and Roy, 2018; Chinoca *et al.,* 2022). Sputum sample may be collected by a person coughing up mucus, by breathing in a saltwater (saline) mist and then coughing, after which endoscopic technique of visualizing the inside of the airways for diagnostic and therapeutic purposes can be carried out (Al-Abbadi, 2011).

Gasoline also known as petrol is a human-made substance that people use primarily to fuel vehicles and other machines that use an engine. Having exposure to gasoline or gasoline vapors in large amounts or over an extended period can cause serious health complications (Abubakae *et al.,* 2015; Fan *et al.,* 2021). Ingesting even a small quantity of gasoline can be fatal. Gasoline is a toxic and extremely flammable liquid. At room temperature, it is usually colorless, pale brown, or pale pink. Gasoline comprises compounds called hydrocarbons, which include alkanes, benzene, toluene, and xylene. When even small quantities of hydrocarbons enter the bloodstream, it can reduce the function ability of the central nervous system (CNS) and cause organ damage (McGregor *et al*., 2009). A person can sustain damage to the skin, eyes, and lungs when they come into contact with gasoline liquid or the fumes or vapors of gasoline (McGregor *et al.,* 2009; Abeer, 2022). Inhaling gasoline vapors can irritate the lung tissues, and some chemicals can enter the bloodstream (Abeer, 2022). Once in the bloodstream, some of these chemicals can make it difficult for the body to move oxygen around the body tissues, leading to necrosis (Bonney *et al.,* 2013).

Some of the constituents of gasoline are known to be highly toxic or carcinogenic to humans. Many of the toxicological effects associated with the exposure to gasoline can be attributed to specific components of gasoline, such as benzene, toluene, ethylene and xylene, which are also known as volatile organic compounds (VOCs) (Abdel-Maksoud *et al.,* 2019). A number of studies have demonstrated that occurrences of various health problems are closely associated with occupational exposures to VOCs. Exposure to benzene from petrol vapour caused haematotoxicity among petrol station workers (Abdel-Maksoud *et al.,* 2019). Occupational exposure to petroleum and its derivatives is a significant concern for the health and safety of workers in the petroleum industry. Fuel station workers in particular, are exposed to various hazardous substances present in gasoline fumes and other petroleum products. These substances have been linked to respiratory tract disorders among other health complications (Schneider *et al.,* 2011).

The cytological evaluation of sputum smears can provide valuable information about the cellular changes associated with respiratory disorders. Abnormalities such as cellular atypia, inflammation, and the presence of malignant cells can be detected through this analysis. By comparing the cytological findings of fuel station workers with those of individuals not exposed to petroleum products, it is possible to establish a correlation between occupational exposure and cellular abnormalities (Schifter *et al.,* 2002). The aim of carrying out this study was to evaluate the cytological features of sputum smears obtained from fuel station attendants exposed to premium motor spirit (PMS) in Ibadan, Oyo State.

MATERIALS AND METHODS

Study Area

Oyo State is an inland state in southwestern Nigeria. Its capital is Ibadan, the third most populous city in the country and formerly the second most populous city in Africa. Oyo State is bordered to the north by Kwara State, to the east by Osun State, and to the southwest by Ogun State and the Republic of Benin. With a projected population of 7,840,864 in 2016. Oyo State is the fifth most populous in Nigeria. Cassava, cocoa, and tobacco are among the most important crops to Oyo State's economy (Akinjogbin, 2006).

Study Site

This study was carried out in four local government area in Ibadan which are; Ibadan North East LGA, Ibadan Southeast LGA, Ibadan South-West and Oluyole LGA

Study Design

This study was an Analytical cross sectional study.

Ethical Considerations

Ethical approval to carry out this study was sought and obtained from the ministry of health ethical review committee, secretariat Ibadan, Oyo state, with reference number NHREC/OYOSHRIEC/10/11/22.

Confidentiality and privacy of respondents were duly respected during and after the period of collecting and collating data. Serial numbers rather than the names of the participants were used to ensure confidentiality. Participants were provided with informed consent and those unable to read and write were duly assisted and guided in a language they understand, and verbal consent was obtained.

Sample Size Determination

The sample size nf= n/1+ (n) /N

=384/1 + (384/150)

~ 108

About 150 subjects were totally recruited for this study, consisting of 100 test subjects and 50 control subjects.

Inclusion Criteria

i. Participants who have been working at the fuel station for at least 1 year
ii. Participants who could produce sputum for cytological evaluation.
iii. Individuals who provide informed consent to participate in the study.
iv. Individuals not employed as petrol station attendants or those who have never worked at a petrol station were included as the control group.

Exclusion Criteria

i. Individuals not employed as fuel station attendants or those who have never worked at a fuel station were not recruited for the test group
ii. Participants who did not provide informed consent to participate in the study.
iii. Individuals with pre-existing medical conditions that may affect sputum production or the interpretation of cytological smears (e.g. individuals with chronic respiratory diseases like asthma or chronic obstructive pulmonary disease).
iv. Individuals who have taken antibiotics within a specific time frame before sputum collection (as antibiotics can affect sputum microbiology).
v. Individuals who were unable to produce an adequate sputum sample for evaluation.

Sample Collection and Processing

Questionnaire and informed consent were given to participants before collecting the sample in the morning, participants were given water to rinse their mouth before collection of sample.

department of Biomedical Laboratory Science, College of Medicine, University of Ibadan, for processing and analysis. From each of the samples collected from the subjects, a small amount of sputum was spread on clean grease free glass slides using a sterile wooden applicator, multiple smears were prepared to ensure adequate cell representation. The smears were fixed in 95% alcohol. A set of smears made from each sample collected was stained using Hematoxylin and Eosin techniques while another set was stained using papanicolaou staining technique.

Papanicolaou Staining Procedure for Sputum.

The sputum smears were fixed while still wet in 95% alcohol for at least 30 minutes, after which were rinsed in water, then stained in Harris Heamatoxylin for 5min then rinsed in water.

The smears were differentiated in 0.5% acid alcohol and were rinsed in water, then blued in running tap water for 10minute, rinsed in 70% alcohol and 2 changes of 95% alcohol (6dips), stained in Orange G6 for 2minutes, then rinsed in 2 changes of 95% alcohol (6dips), transferred to Eosin Azure 50 for 2 minutes then rinsed in 2 changes of 95% alcohol (6dips), after which were transferred to absolute alcohol, cleared in xylene and mounted with DPX.

H & E Staining Procedure for Sputum

The smears were fixed in 95% alcohol for at least 30 minutes, hydrated in descending grades of alcohol (Absolute, 90%, 70%, 50%), rinsed in water. The smeared slides were stained with Harris heamatoxylin for 10minutes, rinsed in water. The smears were differentiated with 1% acid alcohol briefly. Smears were blued in running tap water for 10minutes. Smears were counterstained with Eosin for 2minutes, then rinsed in water. Smears were dehydrated in ascending grades of alcohol (50%, 70%, 95% and Absolute alcohol), after they were cleared in xylene and mounted with DPX.

Data Analysis

Analysis was done using the Statistical Package for the Social Sciences (SPSS) version 25. Descriptive statistics was used to provide information on the age and years of employment at the fuel station of the respondents, while regression analysis was used to assess the correlation between cytological abnormalities and the duration of occupational exposure, age, smoking history, and other relevant factors.

RESULTS

Table 1 shows the socio-demographic profiles of both the fuel attendants and control subjects. Gender distribution of respondents showed that majority of them involving 55(55%) fuel pump attendants and 36(72%) control of individual are females while minority of the respondents involving 45 fuel attendants and 14 control group of individuals are males. Age distribution of respondents ranged between less than 20 and greater than 60 years. Majority of them involving 72(72%) fuel pump attendants and 38(76%) control group of individuals are 20-30years of age, while the minority of the respondents involving 5 fuel attendants and 4 control group of individuals representing 5% and 8% are 41-50years of age and less than 20 years of age respectively. The educational level of respondents showed that the majority of them involving 65(65%) fuel attendants and 24(48%) control groups of individuals have attained secondary education and tertiary education while the minority of the respondent with 1(1%) fuel pump attendants and 1(2%) control group of individual have attended primary education. Based on the years of employment of the fuel pump attendants, the majority of them involving 75(75%) fuel pump attendants were within the range of 0-2years of employment in fuel stations, while the minority involving the fuel pump attendants which represent 1% was within 10-12years of employment in fuel stations. No significant association was found on the gender between the fuel pump attendants and the control group ($p>0.05$). A significant association was observed however, on the age groups and educational backgrounds between the fuel pump attendants and the control group ($p<0.05$).

Table 1: Socio-demographic Profiles of the Participants

Variables	Test Group (n=100) Frequency (%)	Control Group (n=50) Frequency (%)	*p*-value
Gender			
Male	45(45)	14(28)	0.107
Female	55(55)	36(72)	
Age (years)			
<20	12(12)	4(8)	0.0001*
20-30	72(72)	38(76)	
31-40	11(11)	6(12)	
41-50	5(5)	0(0)	
51 and above	0(0)	2(4)	
Educational Background			
Primary School	1(1)	1(2)	0.0001*
SSCE	65(65)	12(24)	
Technical	2(2)	2(4)	
OND	22(22)	5(10)	
HND	4(4)	4(8)	
NCE	2(2)	2(4)	
Bsc	4(4)	24(48)	
Years of employment (yrs)			
1-5	89(89)	-	
6-10	10(10)	-	
11-15	1(1)	-	

*significant at p<0.05

Table 2: Distribution of work schedule and exposure to gasoline fumes among the respondents

Work schedule	**Frequency (%) n=100**
0-40hrs/week	11(11)
41-70hrs/week	42(42)
71-100hrs/week	38(38)
101-130hrs/week	9(9)
Exposure to gasoline fumes	
Daily	70(70)
Weekly	29(29)
Monthly	1(1)
Involvement in refueling vehicles	
Yes	82(82)
No	18(18)

The frequency distribution of work schedule of respondents showed that majority of them involving fuel pump attendants representing 42% worked for more than 40hours per week while the minority of the fuel pump attendants which represent 9% worked more than 100hours per week. Exposure to fuel fumes by respondent showed that 70(70%) of test subjects are exposed to gasoline fumes daily; 29(29%) are exposed to gasoline fuel fumes weekly while 1(1%) is exposed to fuel fumes monthly.

The frequency distribution of the respondent involved in refueling vehicles showed that majority of them involving petrol attendants representing 82% are directly involved in refueling vehicles while the minority involving 18 fuel attendants are not involved in refueling vehicles.

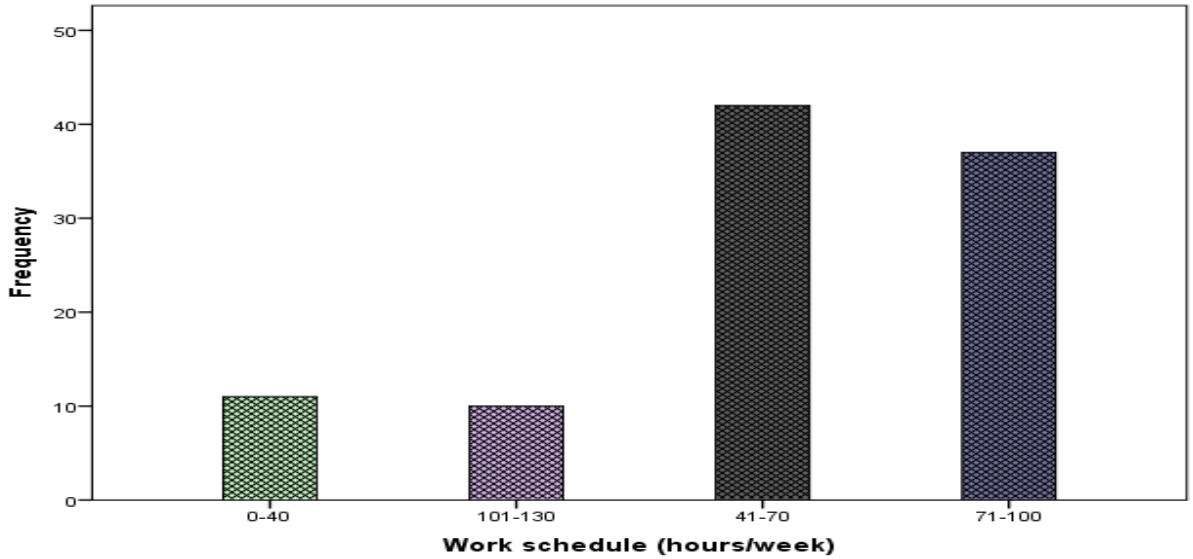

Figure 1: Distribution of the work schedule of Respondents

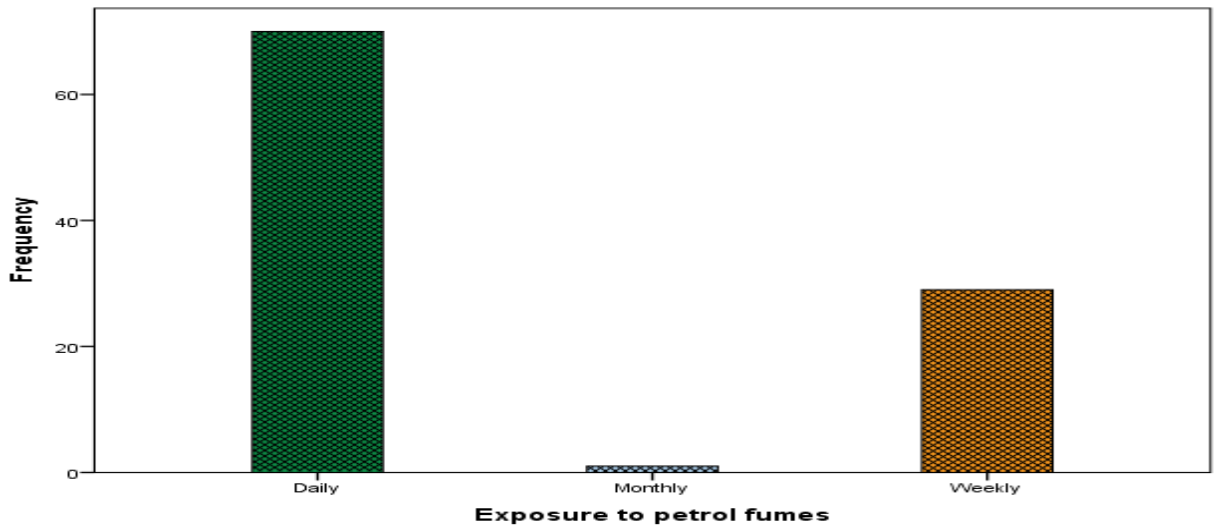

Figure 2: Distribution of respondents exposed to gasoline fumes

Table 3: Distribution of previous training and the use of Personal Protective Equipment (PPE) among the respondents

Previous training	**Frequency (%) n=100**
Yes	15(15)
No	75(75)
Use of PPE	
Yes	08(8)
No	92(92)

Table 3 shows the distribution of respondents that received training on PPE. Fuel station attendants that did not receive training on PPE were 75(75%), while those that received training on the use of PPE were 15(15%). The frequency distribution of the use of personal protective equipment (PPE) by the respondents revealed that 92(92%) of fuel pump attendants do not always use PPE, while 8(8%) of fuel pump attendants always use PPE when on duty.

Table 4: Cytomorphological findings

Cell types	Test group n = 100		Control group n=50		P-value
	Yes (%)	No (%)	Yes (%)	No (%)	
Infiltrate of Inflammatory cells	82(82)	18(18)	08(16)	42(84)	0.041
Atypical cells	37(37)	63(63)	0(0)	50(100)	0.190
Cytoplasmic granulation	23(23)	77(77)	03(6)	47(94)	0.0001
Pyknotic cells	19(19)	81(81)	02(4)	48(96)	0.0001
multinucleated squamous cells	22(22)	78(78)	0(0)	50(100)	0.058
Increased nucleo-cytoplasmic ratio	48(48)	52(52)	02(4)	48(96)	0.034

Power of significance at $p \leq 0.05$

Table 5 presents the cytological findings of both the test group and the control group. The table revealed different cell types observed. Significant differences ($p<0.05$) were observed in the infiltrate of inflammatory cells, cytoplasmic granulation, pyknotic cells, and increased nucleocytoplasmic ratio between the test and control groups. Atypical cells and multinucleated squamous cells did not show significant differences between the groups ($p>0.05$).

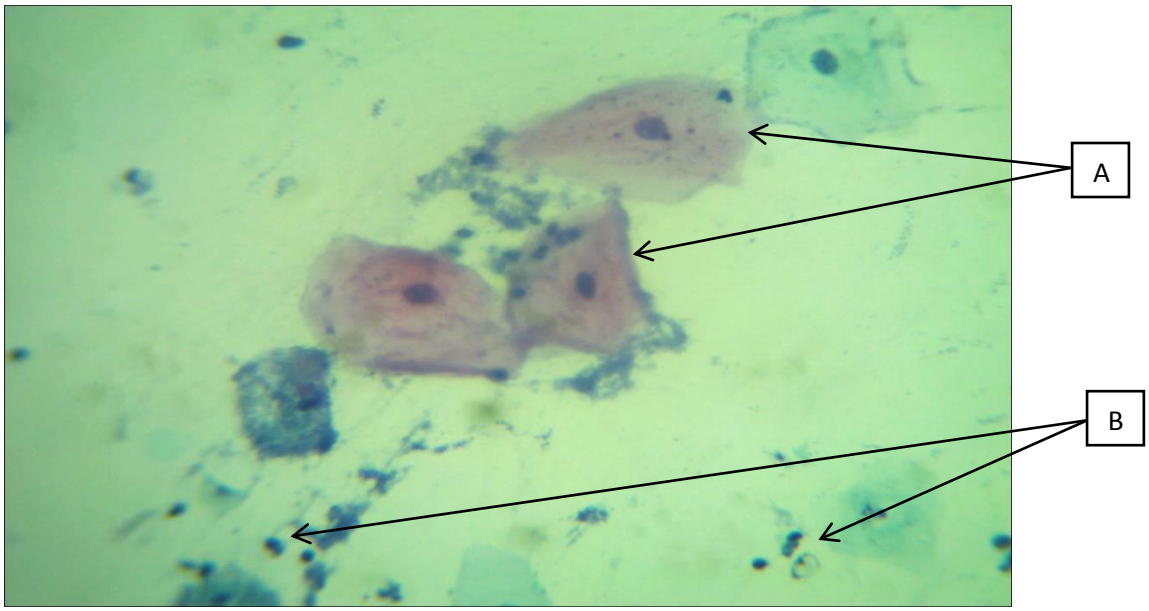

Plate 1: Sputum smear from a control subject showing [A] superficial squamous cells [B] mild infiltrate of inflammatory cells (Pap x 400).

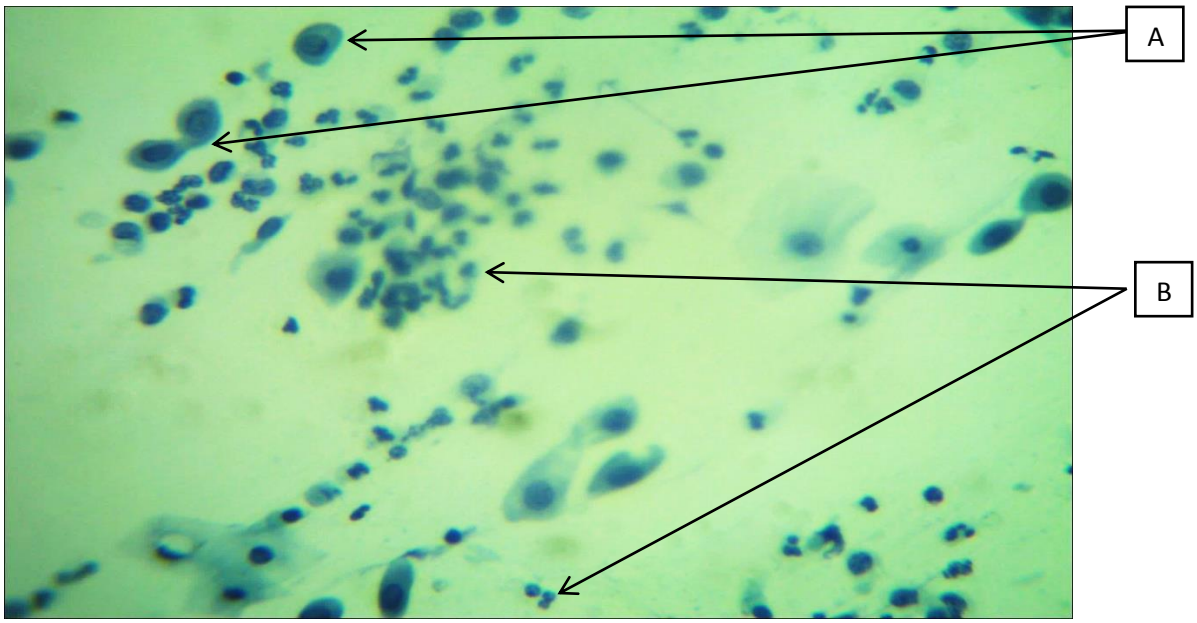

Plate 2: Sputum smear from a test subject showing [A] atypical cells with increased nucleo-cytoplasmic ratio [B] heavy infiltrate of inflammatory cells (Pap x400).

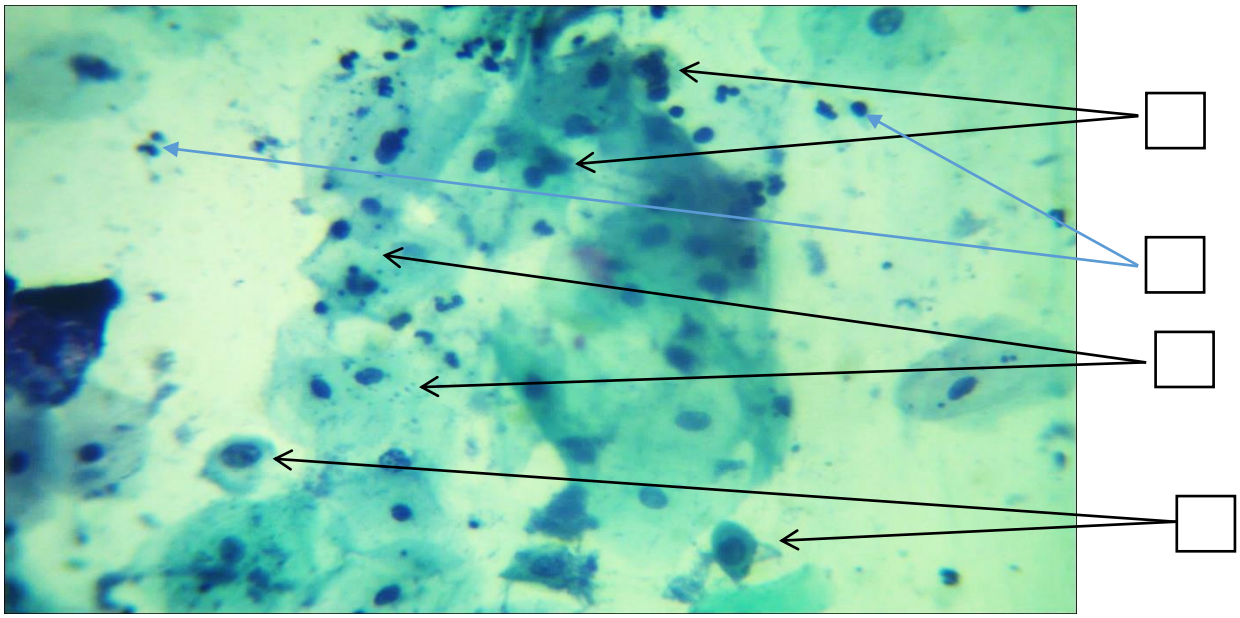

Plate 3: Sputum smear from a test subject showing [A] Binucleated squamous cells [B] infiltrate of inflammatory cells, [C] squamous cells with cytoplasmic granulation [D] atypical squamous cells (Pap x400).

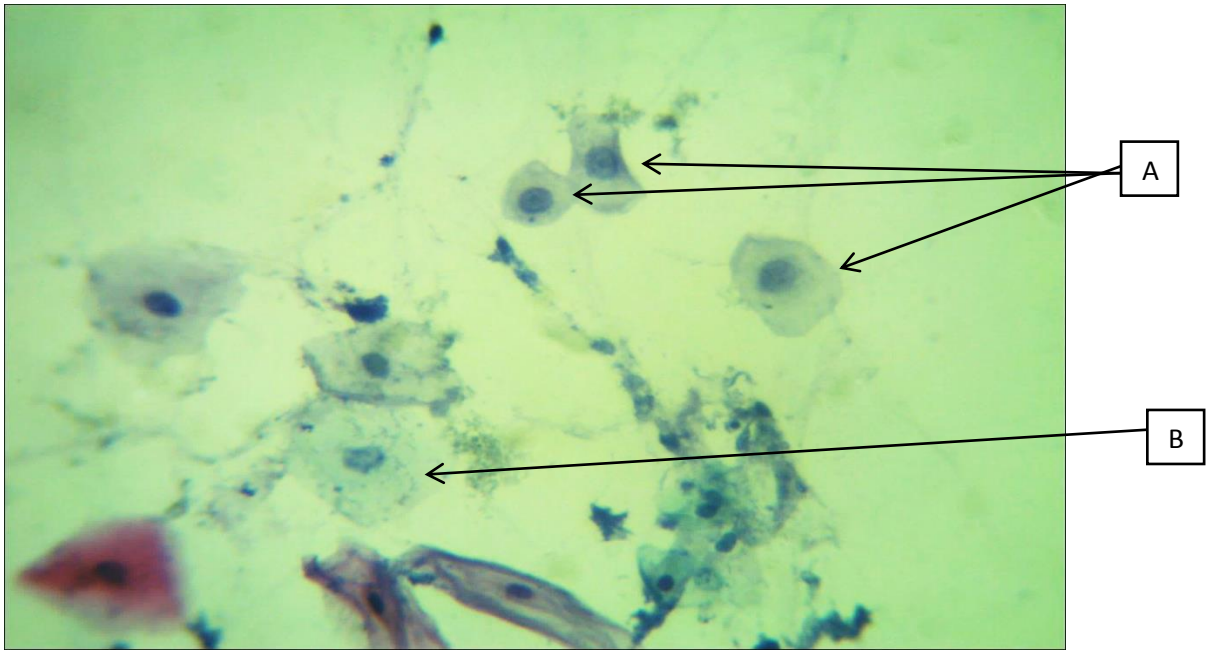

Plate 4: Sputum smear from a test subject showing [A] increased nucleo-cytoplasmic ratio [B] Squamous cells with cytoplasmic granulations (Pap x400)

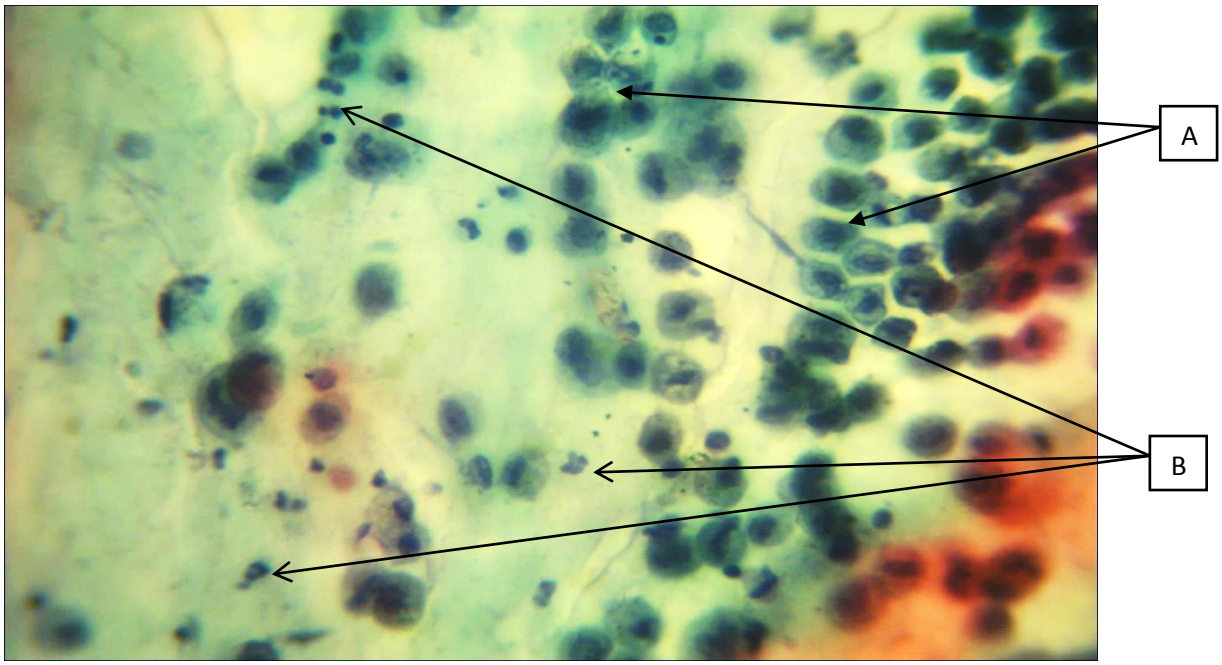

Plate 5: Sputum smear from a test subject showing [A] Atypical cells [B] infiltrate of inflammatory cells (Pap x400).

Plate 6: Sputum smear from a control subject showing [A] normal superficial squamous cells (H & E x400).

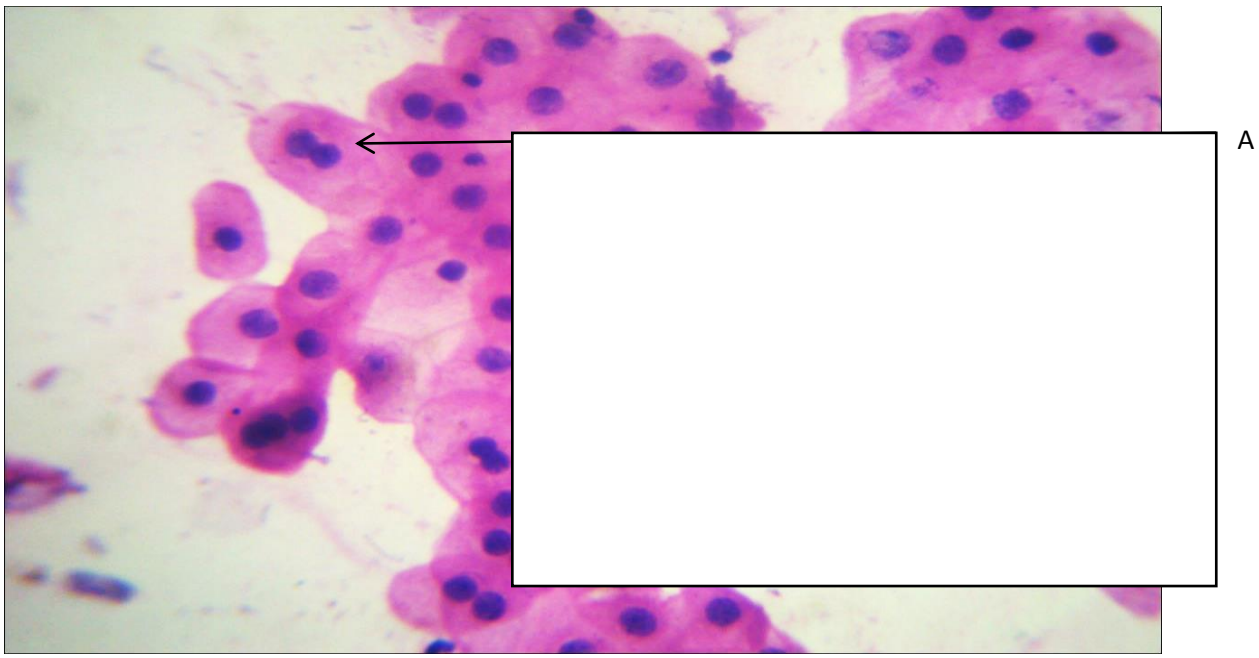

Plate 7: Sputum smear from a test subject showing [A] binucleated and hyperchromatic squamous cells with slight increased nucleo-cytoplasmic ratio (H & E x400).

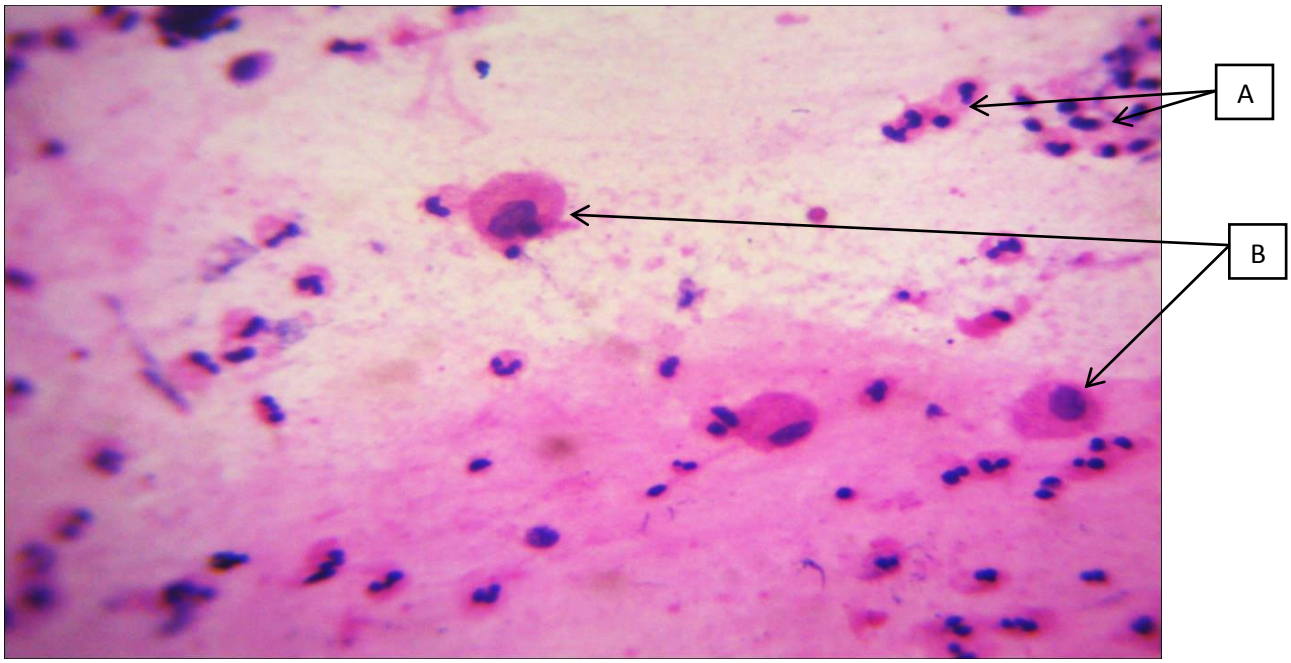

Plate 8: Sputum smear from a test subject showing [A] infiltrate of inflammatory cells [B] squamous cells with increased nucleo-cytoplasmic ratio (H & E x400).

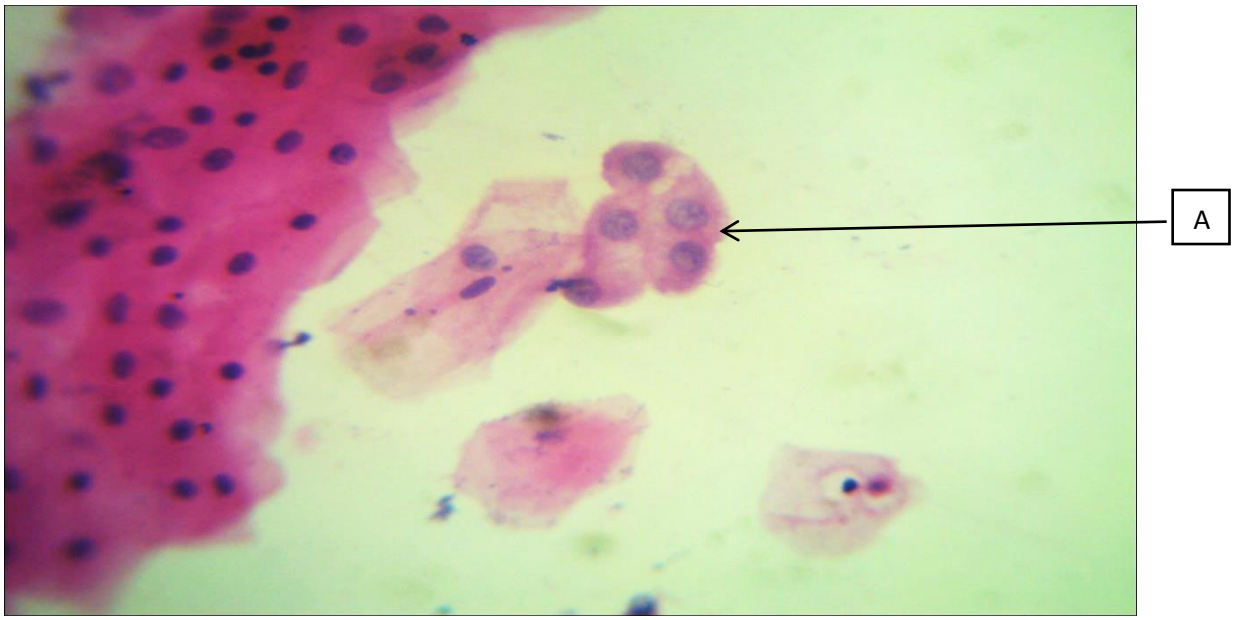

Plate 9: Sputum smear from a test subject showing [A] atypical cells (H & E x400).

DISCUSSION

Exposure to gasoline fumes is associated with serious health problems of which the respiratory system is one of the major organs affected (Salih et al., 2017), not only in those working in gasoline stations like the gasoline pump attendants but also in other people who live near or commute to work through such routes (kumar *et al*., 2018). Occupational health has been gaining importance for the fact that long-term exposure to pollution can lead to morbidity (Levsan, 2008).

The acute health risks involved are minimal provided that the products are used under appropriate health and safety practices. Gasoline pump attendants are liable to inhale not only the product in the gasoline fumes but also the product emitted from the car engine.

Fuel pump attendants are exposed to a variety of occupational hazards, including airborne pollutants such as hydrocarbons and particulate matters (Adekunle *et al.*, 2023). The respiratory system is particularly vulnerable to these exposures, and monitoring the cytological changes in sputum smears can provide valuable insights into the potential health effects on the workers (Ebensperger, 2020). The primary objective of the study was to assess the cytological changes in the sputum of fuel station workers, with a focus on identifying the abnormalities of respiratory distress.

Female gasoline pump attendants were more than male pump attendants in this study. This was noticed to be in contrast to what was observed in the study conducted by Adeniyi *et al.* (2014) in South-West Nigeria, where males were more than females among the gasoline pump attendants. This may be due to the fact that more females were working as gasoline pump attendants at the time of conducting this study compared to the study by Adeniyi et al. (2014). The general differences across participant was not found to be statistically significant ($p>0.05$) (table 1.0).

Majority of the participants for this study fell in the 21-30 years age range. This demographic pattern is expected as individuals within this age bracket are more likely to engage in active employment. Regarding educational attainment, the higher prevalence of secondary education among fuel attendants is consistent with the qualifications typically sought for service-oriented roles. This finding is corroborated by a study conducted by Ndubuisi and Emerho, (2023) who found that over half of petroleum attendants had at least a secondary education. A significant association was observed on educational backgrounds between the fuel pump attendants and the control group ($p<0.05$) (table 1.0). The age distribution is also consistent with the general workforce trends, as young energetic population are more likely to engage in active jobs (Becker *et al*., 2022). Majority of the participants reported no respiratory symptoms. This finding aligns with the general expectation that a significant portion of the population may be asymptomatic regarding respiratory issues (Mozaffari *et al*., 2023). The age group of participants were found to be statistically significant ($p<0.05$) (table 1.0). Use of Personal Protective Equipment (PPE) among respondents revealed that majority of them do not use PPE, only few fuel pump attendants use nose mask. This shows that fuel pump attendants are more at risk of respiratory disease and its symptoms (table 3.0).

Although several studies had precisely investigated sputum cytology among gasoline workers. Studies by Dutta *et al*. (2013), Al-Fawzan *et al*. (2018) and Rehani *et al.* (2021) had demonstrated an association between exposure to gasoline and its cellular alterations in the respiratory system of fuel pump attendants.

According to this study, cytological findings from sputum smears of both control and test subjects revealed insights into potential respiratory health impacts associated with occupational exposures among the test group. The observed cytological changes include alterations in nucleo-cytoplasmic ratio, presence of atypical cells, infiltrates of inflammatory cells, and variations in squamous cell characteristics. The findings in this study resonates that of Al-Fawzan *et al*. (2018) who identified significant cytological atypical among fuel station workers. This finding is also supported by Dutta *et al*. (2013) who reported infiltrates of inflammatory cells among subjects who are occupationally exposed to gasoline fumes.

The presence of an increased nucleo-cytoplasmic ratio, particularly in squamous cells and generally the cellular changes observed in this study, is an indicative of potential stress or response to environmental exposures. This may signify early signs of cellular injury or adaptation (Margaret and James, 2017).

The identification of atypical cells suggests cellular abnormalities, and their presence in sputum smears could be a manifestation of abnormal cellular morphology, including changes in cell size, shape, and organization including potential carcinogenic effects. This finding aligns with a study by Connellan (2017) who reported associating occupational exposures to hydrocarbon with increased inflammation in the respiratory tract.

The validity of the results observed from this findings is supported by the consistency of cytological changes across multiple smears in the test subjects. The inclusion of control subjects, along with the application of different staining techniques strengthened the validity of the findings. Statistical analyses conducted also further enhanced the robustness of the observed cytological findings in this study. The identified cytological changes underscored the need for targeted occupational health interventions. Regular health monitoring and screenings for fuel pump attendants can facilitate early detection and intervention, potentially mitigating the impact of occupational exposures. Practical implications include the implementation and reinforcement of stringent occupational safety measures, including the promotion of personal protective equipment (PPE) usage, improved ventilation, and reduction of exposure to harmful substances in the workplace.

CONCLUSION

This study observed respiratory health risks in fuel pump attendants exposed to gasoline fumes, emphasizing the importance of comprehensive safety measures. Abnormal cells revealed in the smears of fuel pump attendants is as a result of the potential toxic substances present in gasoline fumes.

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