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Cytology Analysis of Urine among Cigarette Smokers

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Abstract

Introduction: This is a descriptive study carried out in Owo Town, Ondo State**,** during the period of February (2015) to August (2015).
Aim: The main purpose of carrying out this research work is to evaluate the cytomorphological features of urine smears (using papanicoulaou stain) among cigarette smokers in Owo town, Ondo State, Nigeria.
Materials and Method: 250 subjects were used for this research work, 200 subjects were cigarette smokers while 50 subjects were non- cigarette smokers. The numbers of years of cigarette smoking were different and the numbers of cigarette sticks smoked per day were also variable among the test group. Individuals with urinary tract infection were not included in this research work and individuals with less than five (5) years of cigarette smoking were also not included in this research work. From each urine sample collected, smears were obtained from the sediments after centrifuging and were immediately fixed with a cytology-spray fixative for at least 30 minutes, before staining smears with Papanicolaou stain.
Results and Discussion: The stained smears were examined under a light microscope and revealed a high cellular turnover among 70% of the test group when compared with the control group which are non-smokers, showing few normal urothelia cells. Enlargement in nuclear cytoplasm ratio, irregular nuclear borders, necrosis, cluster of cells showing dysplastic changes, moderate haemorrhage, heavy infiltrates of inflammatory cells, hyperchromatism, pleomorphysms and neoplastic transformation were among the features observed in smears of the test group.
Conclusion: On the basis of this research work, cigarette smoking has been seen to be one of the leading causes of renal diseases.

Keywords: Cytomorphological, cigarettes, papanicolaou, pleomorphysm.

1. Introduction

Cytology is the study of free cells from different organs of the body [1]. These cells may have been shed by the body itself, aspirated by tube or needle, or scraped or washed from tissue surfaces. Effusions, secretions, aspirates and scrapings are all used in diagnostic cytology [1]. Exfoliative cytology is the study of cells which are shed spontaneously from epithelial surfaces (e.g. vagina, oral mucosa, etc) of the body or removed by physical means from various parts of the body or cells that are found in body fluids as effusions [2]. This spontaneous shedding is a function of normal epithelium. The epithelium surfaces undergo constant growth and so they continue to shed worn out cells which are replaced by new ones. However, malignant tumour cells exfoliate more readily than those from healthy tissues. The detection of malignant cells in clinical specimens under microscopic examination is the most important role of diagnostic cytology. Diagnostic cytology is an art and science of interpretation of cells that are obtained from different tissues. Many factors are attributed to the professional and social acceptance of cytological technique, the simplicity of the method and the reasonable accuracy. Urine cytology comprises of large proportion of non-gynecological specimens, processed in most routine cytology laboratories. Cytological examination of urine specimen is simple, safe and cost effective inexpensive method [2]. Cigarette smoking has been identified as the most important source of preventable morbidity and premature mortality worldwide. Smoking-related diseases claim an estimated 443,000 Nigerian lives each year, including those affected indirectly such as babies born prematurely due to prenatal maternal smoking and victims of second hand exposure to tobacco carcinogens [3]. Cigarette smoking appears to be the single greatest risk factor for bladder cancer [4], because cancer-causing chemicals (carcinogens) in tobacco can become concentrated in urine and eventually damage the lining of the bladder. This damage can increase the chances of a cancer-causing genetic mutation. Cigarette smokers are at least twice more likely to develop bladder cancer than the non-cigarette smokers. The risk increases with the number of cigarette sticks smoked per day and the number of years an individual has been smoking [5]. There are approximately 600 ingredients in cigarettes. When burned, they create more than 7,000 chemicals, at least 69 of these chemicals are known to cause cancer and many are poisonous. Many of these chemicals are also found in consumer products, but these products have warning labels, which warns the public about the danger of the poisons in these products [6]. Scientists have laid out the entire chemical composition of human urine, revealing that more than 3,000 compounds are found in the fluid and base on previous studies, it was discovered that at least 3,079 compounds can be detected in urine, 72 of these compounds are made by bacteria, while 1,453 come from the body itself. Another 2,282 come from diet, drugs, cosmetics or environmental exposure [7].

2. Material and Method

2.2 Study Area (Site)

This research work was conducted on human populations who are cigarette smokers in Owo town, Ondo State, Nigeria.

2.3 Duration of the Research Project

This research work took the duration of six (6) months.

2.4 Sample Size Determination

Data from World Health Organisation (WHO) shows that incidence rate of cigarette smokers in men and women in Nigeria, is 18.1% [8]. Therefore in this study, sample size determination shall employ P (reported prevalence of cigarette smokers in Nigeria) = 0.181.

Sample size for this research work was determined using:

$$n = \frac{Z^2 P (1-P)}{d^2} \quad [9]$$

n = Required sample size

Z = Confidence level at 95% (standard value of 1.96)

P = Estimated prevalence (18.1%)

d = Accepted error (5%)

$$n = \frac{1.96^2 \text{ x } 0.181\ (1 - 0.181)}{0.05^2}$$

n = 228

The minimum sample size that was used to carry out this research work was 250 subjects; 200 subjects were cigarette smokers while 50 subjects were non-cigarette smokers.

2.5 Ethical clearance

Approval for this research work was obtained from the Research Ethics Committee of the Federal Medical Centre, Owo, Ondo State, Nigeria.

2.6 Study Population

The study population was divided into cigarette smokers (n = 200) and non-cigarette/tobacco smokers (n = 50).

2.61 Exposed Subjects

Urine samples were gotten from cigarette smokers in beer parlours, motor parks and in other places where people do smoke cigarettes. The subjects (Cigarette smokers) were first given questionnaires to fill before giving them a sterile universal (urinalysis) bottles and for those who were unable to fill the questionnaires, assistance was made in helping them to fill the questionnaire.

2.62 Control (Non-exposed) Subjects

The control (non- smokers) subjects for this study were both male and female subjects that are not smoking any type of Tobacco.

2.7 Inclusion Criteria

Subjects for this study include people who have been smoking cigarettes for at least 5 years.

Control subjects were apparently males and females who are not Tobacco smokers. Such subjects have no previous demographic and medical history showing incidence of kidney disease.

2.8 Exclusion Criteria

Subjects who have not been smoking cigarettes for a period up to five (5) years at the time of sample collection were not considered suitable for this study and subjects for control were individuals who have never smoked cigarette. Subjects with history of any form of kidney disease and subjects who take other type of Tobacco were not considered as part of the subjects (control group) for this research work.

2.9 Collection of samples

Urine samples were collected from smokers in beer parlors, joints and in other places where people do smoke. Questionnaires were first given to the smokers to fill before giving them a sterile universal (urinalysis) bottles and for those who were unable to fill the questionnaire, assistance was made in helping them to fill the questionnaire. Urine samples that were used for this research work were mainly collected from 200 cigarette smokers (test samples) and 50 non-cigarette/tobacco smokers (control samples) in Owo town, Ondo State, Nigeria.

2.10 Methods

Urine samples were collected each inside a clean sterile universal bottle from 200 cigarette smokers (test group) and 50 non-cigarette smokers (control group). Each of the urine samples was treated as follows:

5-10mls of each of the urine samples was transferred into a sterile clean test tubes, the test tubes containing the urine samples were arranged inside the centrifuge and were spun for 10minutes at 1,500 rpm [revolution per minute], Following centrifugation, the supernatant in the test tubes containing the spun urine samples were poured off, and the sediments left in the test tubes were used to make smears on a clean frosted end slides. The smears were immediately fixed with a cytological spray fixative and were kept fixed for at least 30 minutes before staining the smears with Papanicolaou stain (Method Adopted from [1]. The slides were analyzed with the aid of a light microscope for cytomorphological changes. The slides were viewed and captured on a Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular).

2.11 Staining Procedures for Papanicolaou Stain

Smears were fixed with a cytological spray fixative for at least 30 minutes.

Fixed smears were rinsed in descending grades of alcohol (80%, 70%, 50%) and water for 10 seconds each.

Smears were stained in Harris Haematoxylin for 4 minutes.

Smears were rinsed in tap water.

Smears were briefly differentiated in 1% acid alcohol.

Smears were washed and blued in tap water for 10 minutes.

Smears were transferred to 70% alcohol, and then transferred to 95% alcohol for a few seconds. Smears were stained in OG6 for 2 minutes.

Smears were rinsed in 2 changes of 95% alcohol for few seconds each.

Smears were stained in EA 50 for 2 minutes.

Smears were rinsed in 2 changes of 95% alcohol for a few seconds each.

Smears were dehydrated in absolute alcohol for a few seconds.

Cleared in xylene and mounted with DPX [10].

2.12 Statistical Analysis

All the information, results and data gotten from this research work was analysed using SPSS program; frequency distribution, pie-chart, bar-chart and cross-tabulation values were calculated.

3. Results

Table 1: Sex of test group and control group.

Variables	Test		Control	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Male	177	87	40	80
Female	23	13	10	20
Total	**200**	**100**	**50**	**100**

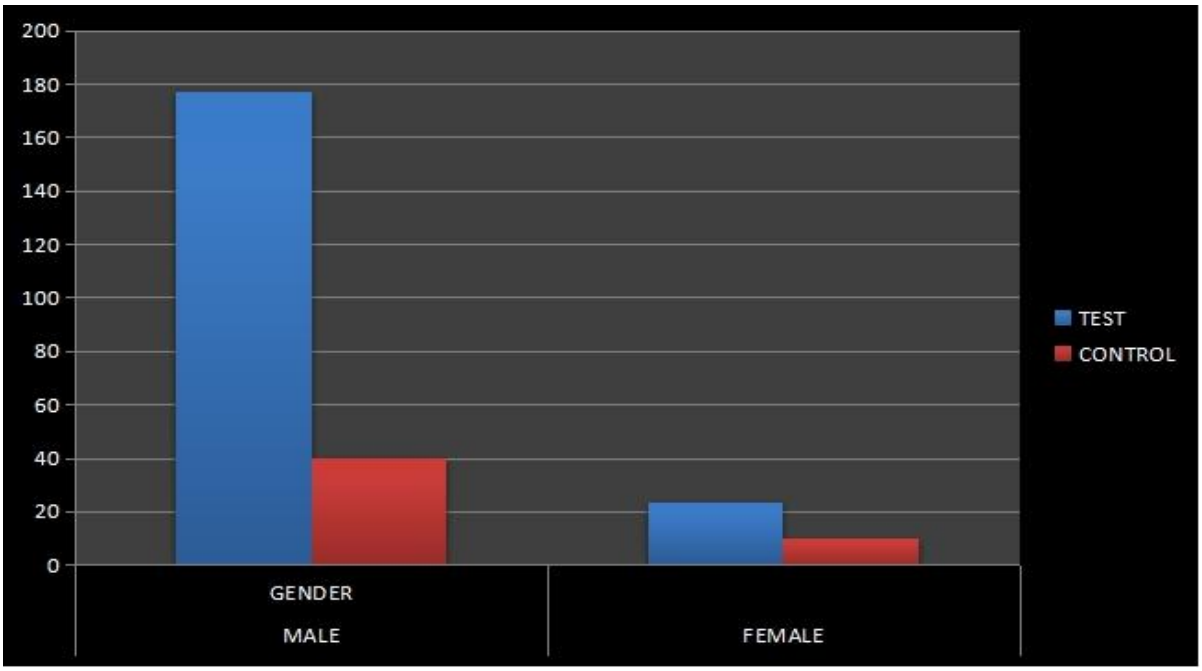

Figure 1: Distribution of sex among the test group and the control group.

Table 2: Age of test group.

Age Range (years)	Frequency	Percentage (%)
11-20	14	7
21-30	105	52
31-40	49	24
41-50	18	9
51-60	11	6
61-70	03	2
Total	**200**	**100**

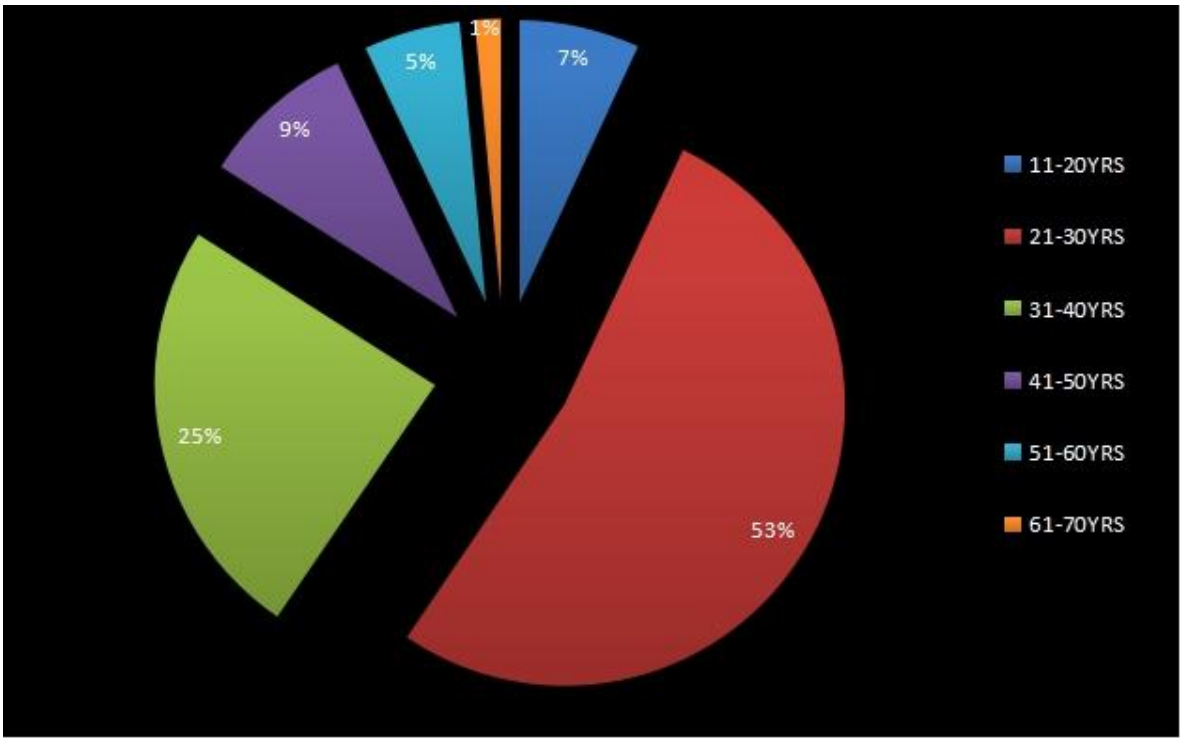

Figure 2: Percentage of the test group by age.

Table 3: Age of test group and control group.

Age Range (Years)	Test Group		Control Group	
	Frequency	Percentage (%)	Frequency	Percentage (%)
11-20	14	7	8	16
21-30	105	52	24	48
31-40	49	24	10	20
41-50	18	9	6	12
51-60	11	6	2	4
61-70	3	2	0	0
Total	**200**	**100**	**50**	**100**

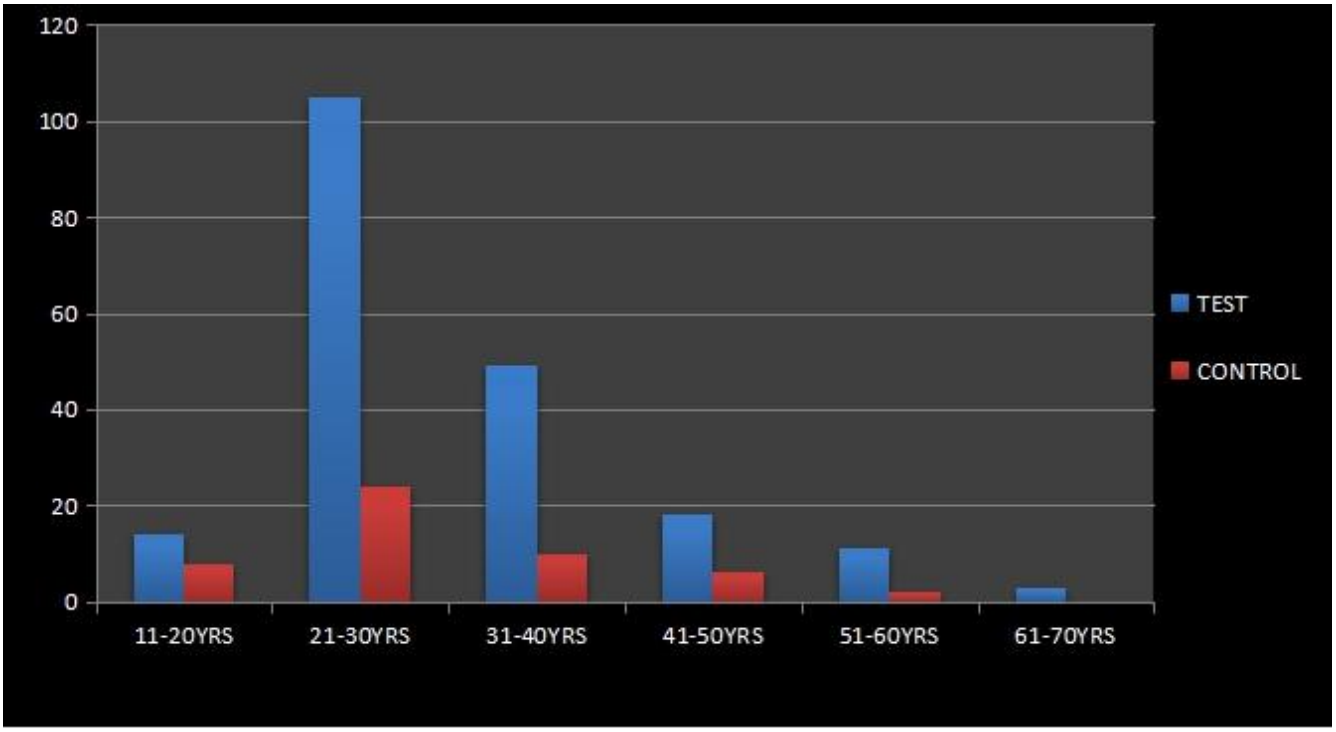

Figure 3: Distribution of age group among the test group and the control group.

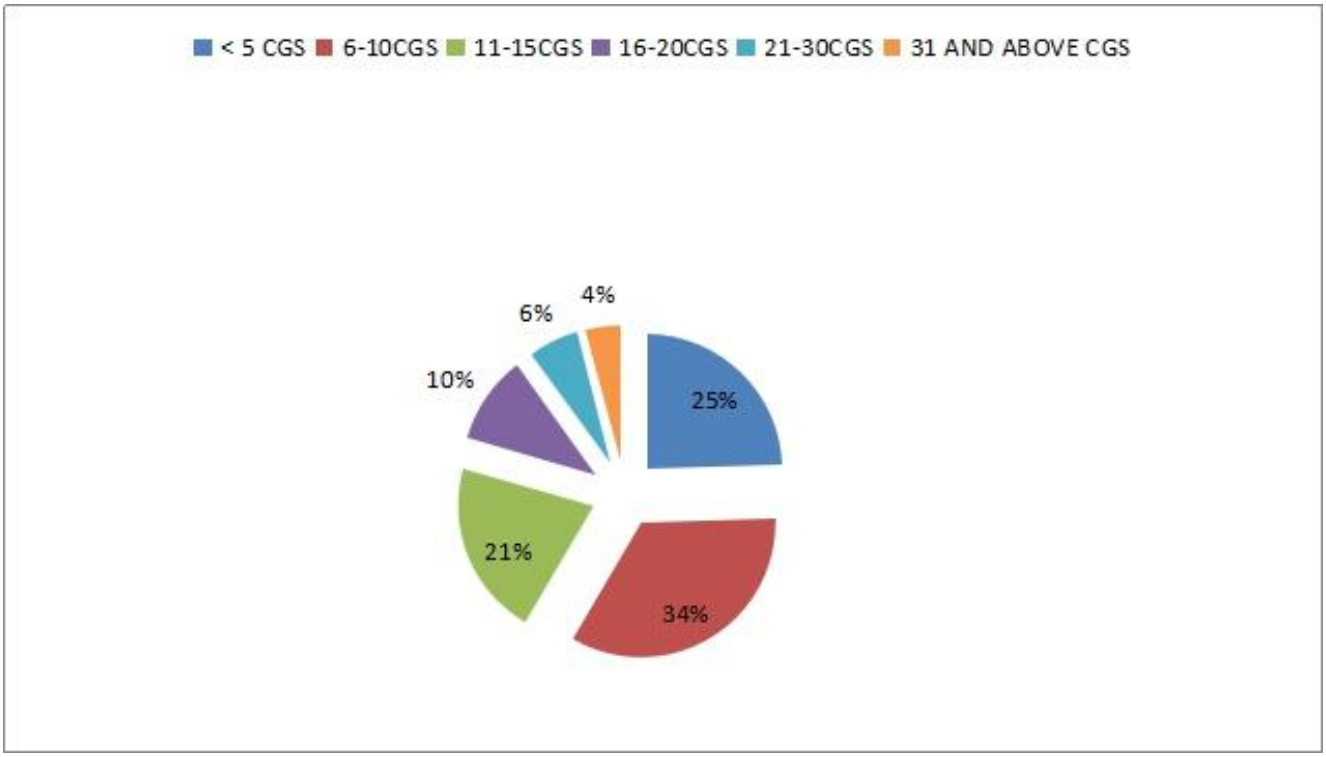

Figure 4: Distribution by Pie chart of number of cigarette sticks smoked per day among the test group.

Table 4: Number of Cigarettes smoked per day.

Cigarette Range (Cigarettes)	Frequency	Percentage (%)
<5	49	24
6-10	68	34
11-15	42	21
16-20	21	11
21-30	12	6
31 and above	08	4

Table 5: Different cell types among the Test and Control groups.

Cell Types		Test		Control		Total	P-value
		Present	Absent	Present	Absent		
Dysplastic cells		123	77	nil	50	250	0.000
Inflammatory Cells	Heavy infiltrates of inflammatory cells	140	60	nil	50	250	0.000
	Mild infiltrates of Inflammatory cells	61	139	20	30	250	0.180
Red blood cells		40	160	nil	50	250	0.000
Cast		111	89	15	35	250	0.002

Power of significance = 0.05.

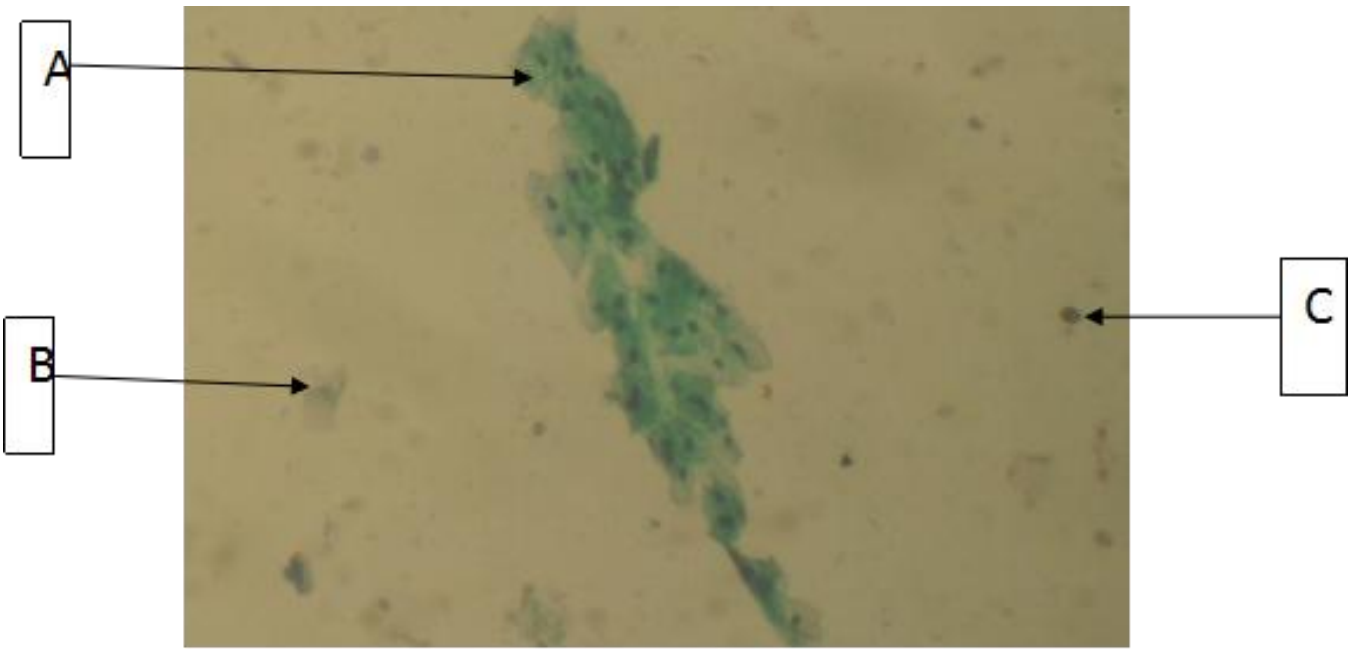

Figure 5: (Control) Urine sample of a non-cigarette smoker showing [A] Intermediate Squamous cells. [B] Superficial cells and [C] Mild Inflammatory cells (PAP X100).

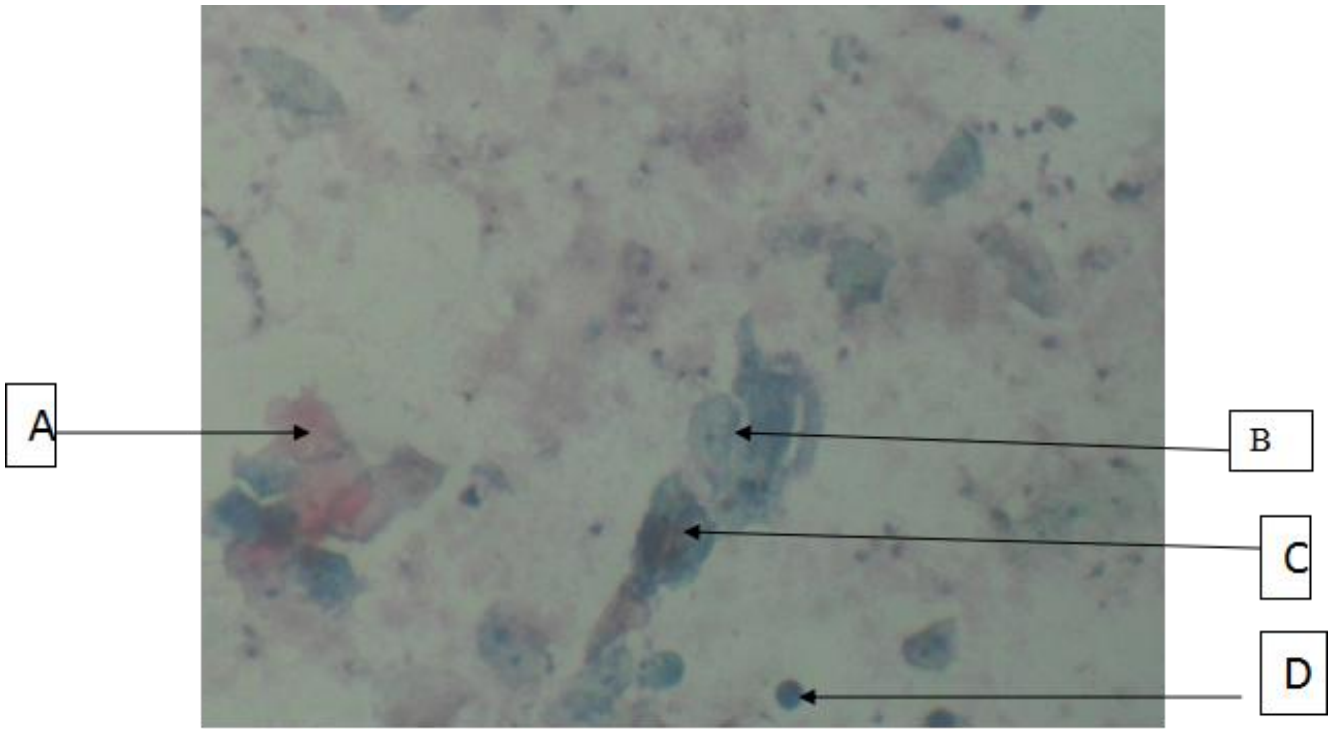

Figure 6: (Control) Urine smear of a non-cigarette smoker showing [A] Superficial Squamous cells. [B] Transitional cells [C] Cast and [D] Mild Inflammatory Cells (PAP X100).

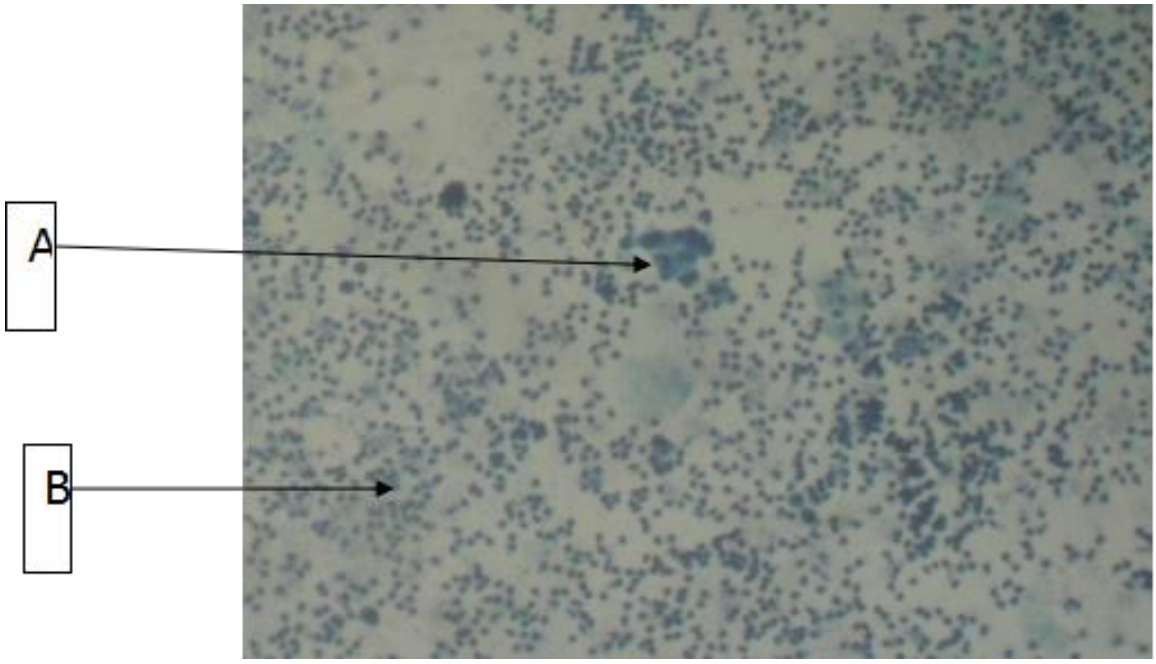

Figure 7: (Test) Urine smear of a cigarette smoker showing [A] A cluster of dysplastic epithelial cells and [B] Heavy infiltrates of inflammatory cells (PAP X 100) .

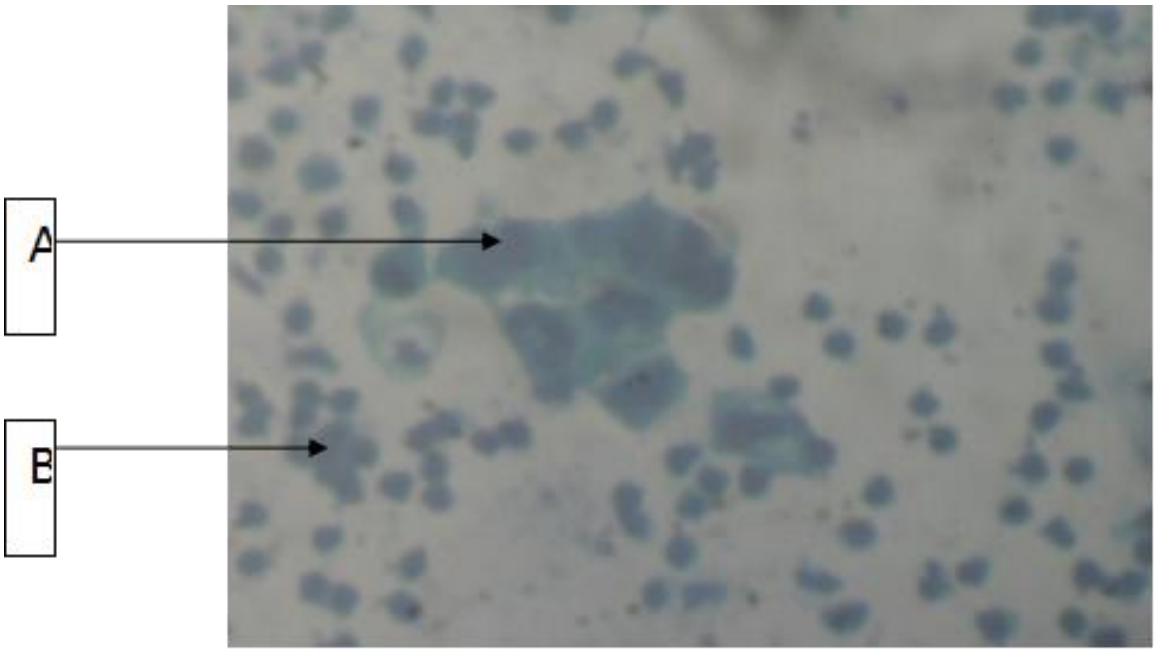

Figure 8: (Test) Urine smear of a cigarette smoker showing [A] A cluster of dysplastic epithelial cells and [B] Heavy infiltrates of inflammatory cells (PAP X 400).

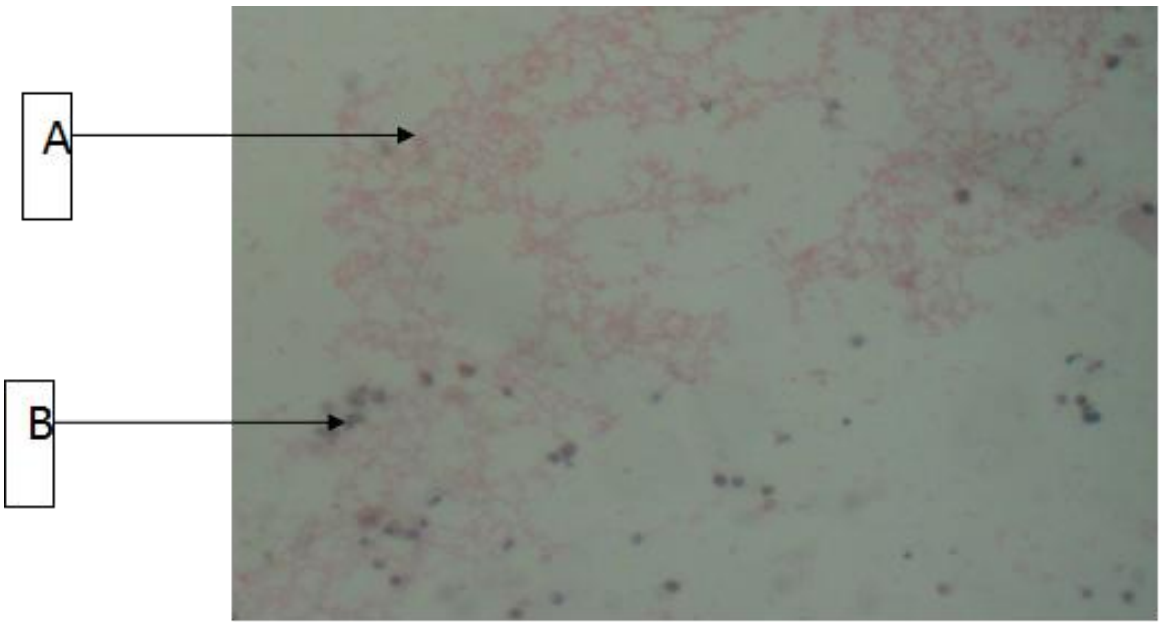

Figure 9: (Test) Urine smear of a cigarette smoker showing [A] Moderate heamorrhage and [B] Mild infiltrates of Inflammatory cells (PAP X 100).

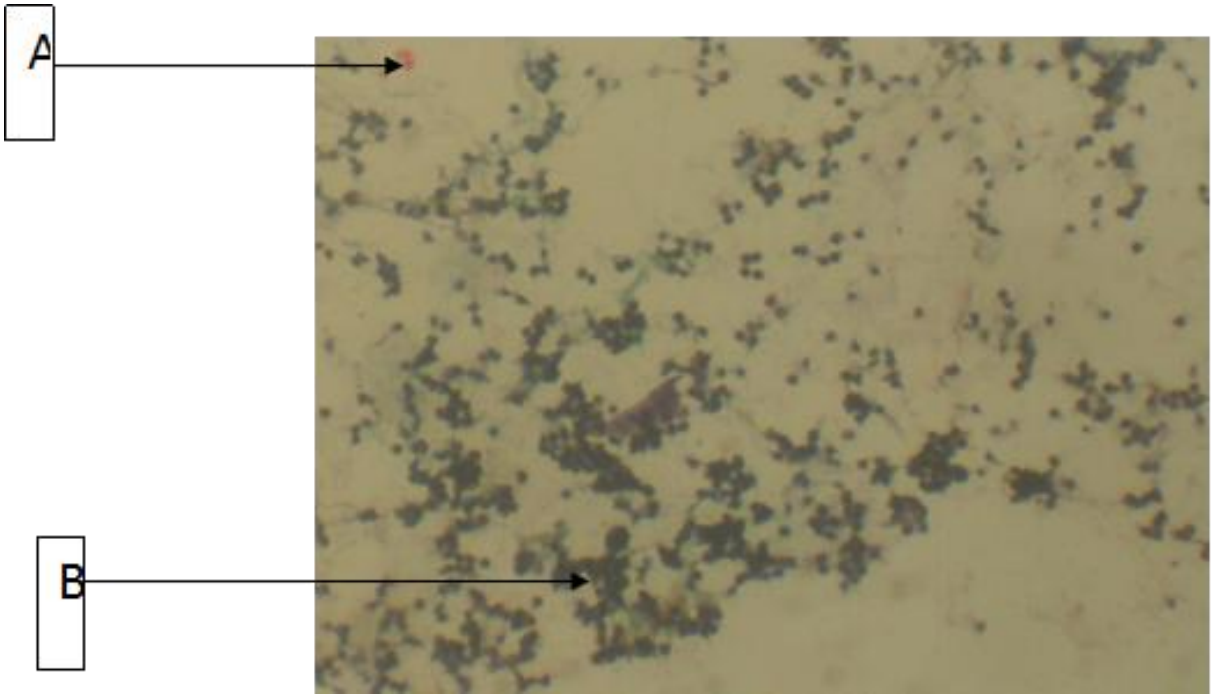

Figure 10: (Test) Urine smear of a cigarette smoker showing [A] Few red blood cells and [B] Heavy infiltrates of Inflammatory cells (PAP X 100).

4. Discussion

Based on the studied test population, 87% were males while 13% were females and this is in agreement with the reported prevalence value given by WHO [8], indicating a higher percentage of male cigarette smokers, compared to that of female cigarette smokers

in the south/west geo-political region in Nigeria. Their ages ranges from 11years to 70 years with a mean age of 41years. 59% of the test group population ranges in ages between 11years to 30years which showed a high percentage of youths involved in cigarette smoking in Owo town, Ondo State, Nigeria. The number of cigarette sticks smoked per day among the test group varies widely with 34% smoking 6 to 10 cigarettes per day. According to [11], the effects of cigarette smoking on the body depends on the number of years an individual smokes and the number of cigarette sticks an individual smoked per day, starting smoking earlier in life and smoking cigarettes higher in tar increases the risk of renal diseases. Few urothelial cells like crystals, cast, necrotic debris, mild inflammatory cells, transitional epithelium and squamous epithelium were observed consistently in most of the urine smears in the control group who are non-cigarette smokers which is in agreement with [12]. According to [13], cigarette smoking has been one of the leading causes of preventable death and a major public health concern, cigarette smoking causes most of the commonly diseases affecting the heart, lungs and the renal system [14]. In this research study, High cellular turnover was detected among 140 (70%) of the test group, this is due to the fact that when smokers inhale some of the carcinogens (cancer causing chemicals) in cigarettes, smokes are absorbed from the lungs and get into the blood, and from the blood, they are filtered by the kidneys and they are concentrated in urine in the bladder, thereby damaging the cells that line the inside of the bladder, leading to high cellular turnover and eventually causing bladder cancer, depending on how often and how long an individual continues to engage in cigarettes smoking. Red blood cells were also detected among 20% of the test group and this is due to tumour diathesis and necrosis which provokes bleeding in the urinary bladder thereby causing injury to the renal system and then leading to end stage renal disease, in agreement with [15]. The distribution of heavy infiltrates of inflammatory cells found among 140 (70%) of the test group include; neutrophils, eosinophils, lymphocytes, macrophages and plasma cells, which is in agreement with [16]. Cigarette smoke contains a high percentage of tar which increases the risk of having renal damage and it also contains several carcinogenic pyrolytic products that binds to DNA and can cause many genetic mutations [11]. Other features detected among the urine smears of cigarette smokers include: cluster of cell showing dysplastic changes, pleomorphisms, hyperchromatism, increase in nuclear cytoplasm ratio, irregular nuclear border and many of the cells becoming transformed. These cytological changes observed in the urine smears of cigarette smokers (test group) are the features of tumour diathesis which is in agreement with [17]. This research work detected significant differences between the urine cytology of cigarette smokers and that of non-cigarette smokers, indicating the adverse health effects of cigarette smoke to the renal system.

5. Conclusion

On the basis of this study and review of relevant literatures, cigarette smoking increases the risk of developing bladder cancer, kidney cancer and tumour diathesis. The consistent detection of high cellular turnover, necrotic cells, cluster of cell showing dysplastic changes, enlargement in nuclear cytoplasm ratio, irregular nuclear border, moderate haemorrhage, heavy infiltrates of inflammatory cells, hyperchromatism, pleomorphysms and neoplastic transformation among the urine smears of the test group, are caused as a result of the harmful chemicals carcinogenic substances present in cigarette smoke.

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