

# ORAL CYTOPATHOLOGICAL CHANGES IN HABITUAL SNUFF (NASWAR) USERS

Mohyman Sarfraz, A.H. Nagi

Department of Oral Pathology  
University of Health Sciences,  
Lahore, Pakistan



# INTRODUCTION

- Dipping tobacco, traditionally known as moist/wet snuff, is a type of finely ground or shredded, moistened smokeless tobacco product (1).
- It is commonly known by various terms such as dip and rubs or chews, also known as naswar in native language of Urdu. The act of using it is called dipping, packing or more specifically packing a lip (2).

1. Axell, T., Mornstad, H. and Sundstorm, B., 1976. The relation of the clinical picture to the histopathology and histochemical study on snuff dipper's lesion in a Swedish population. *J. Oral Pathol.*, 5: 229-236.

2. Asma, S., Backinger, C., Blatt, B., Rosaria, M., Grana, R., Ricardo, G., Prakash, G., Ali, I., Brian, J., Michelle, R. and Susan G.R., 2002. Smokeless Tobacco Fact Sheets. 3rd International Conference on Smokeless Tobacco. Advancing Science & Protecting Public Health. Stockholm, Sweden.

- Wet snuff, also known as dipping tobacco, is made from fresh tobacco leaves, calcium oxide, wood ash, colouring and flavouring agents. Wet snuff is held in the mouth for 10 to 15 minutes and is not chewed, thus people take it on hourly basis.

- The smokeless tobacco products which carry specific mutagenicity are toombak used in Sudan, shamma in Saudi Arabia while powdered tobacco and alkali mixtures such as naswar are used in Central Asia and Pakistan.

- A number of studies from United States, South Africa and Scandinavia have confirmed that the lesions in the oral mucosa appear in the anatomical location where the snuff is placed.

- Greer, R.O., Poulson, T.C. and Boone, M.E., 1986. Smokeless tobacco-associated oral changes in juvenile, adult and geriatric patients. Clinical and histomorphological features. *Gerodontic*, 2: 87-98.

- In a retrospective Swedish study, 41 cases of cancer occurred on the anterior vestibular part of the oral cavity in male snuff dippers and in 29 cases (71 %) the tumor had arisen in the exact location where the snuff had been placed.

- Sundstorm, B., Mornstad, H. and Axell, T., 1982. Oral carcinomas associated with snuff dipping, some clinical and histological characteristics of 23 tumors in Swedish male. *J. Oral Pathol.*, **11**: 245-251.

- The easy availability, low cost and lack of awareness of its negative impact on health increases its consumption.



# OBJECTIVE

- To observe the cytopathological changes in oral mucosa of habitual wet snuff users\*.

\*The operational definition of habitual wet snuff users refers to people who have been using naswar (wet snuff) regularly for more than 1 year.

# MATERIAL AND METHODS

## **STUDY DESIGN:**

This was an observational, descriptive study.

## **SETTING:**

The study was conducted in the Dept. of Morbid Anatomy and Histopathology at University of Health Sciences, Lahore, Pakistan

## **DURATION OF STUDY:**

The study was completed in eight months (FEB – SEPT 2013) after approval of synopsis by Advance Studies and Research Board of UHS, Lahore, Pakistan.

### **SAMPLE SIZE:**

The calculated sample size was  
100  
(Calculated by P.A.S.S 2008)<sup>1</sup>

### **SAMPLE TECHNIQUE:**

Non-probability,  
Convenient sampling

### **SAMPLE SELECTION:**

#### ***Inclusion Criteria***

- Habitual snuff users of minimum age 15 years
- Minimal duration of usage 1 year
- Males only

#### ***Exclusion Criteria***

- Inadequate smear
- Cigarette smokers
- Cases who are using other forms of smokeless tobacco like paan, betel nut, quid, chalia etc
- Patients having associated debilitating co-morbid conditions
- Patients with immune disorders.
- Patients who are undergoing treatment/therapy for already diagnosed lesions

## **Clinical Details:**

- Socio-demographic information (name, age, occupation, full address, family/medical history) was obtained along with relevant clinical information.
- All the information was collected on specially designed proformas and recorded.

- After informed consent oral cytological smears were acquired from 100 patients randomly from different areas within the city of Lahore, Pakistan.
- Smears were prepared from oral mucosa scrapings and examined microscopically using H/E and special stains including Papanicolou, Gomori Methenamine Silver and Giemsa stain.

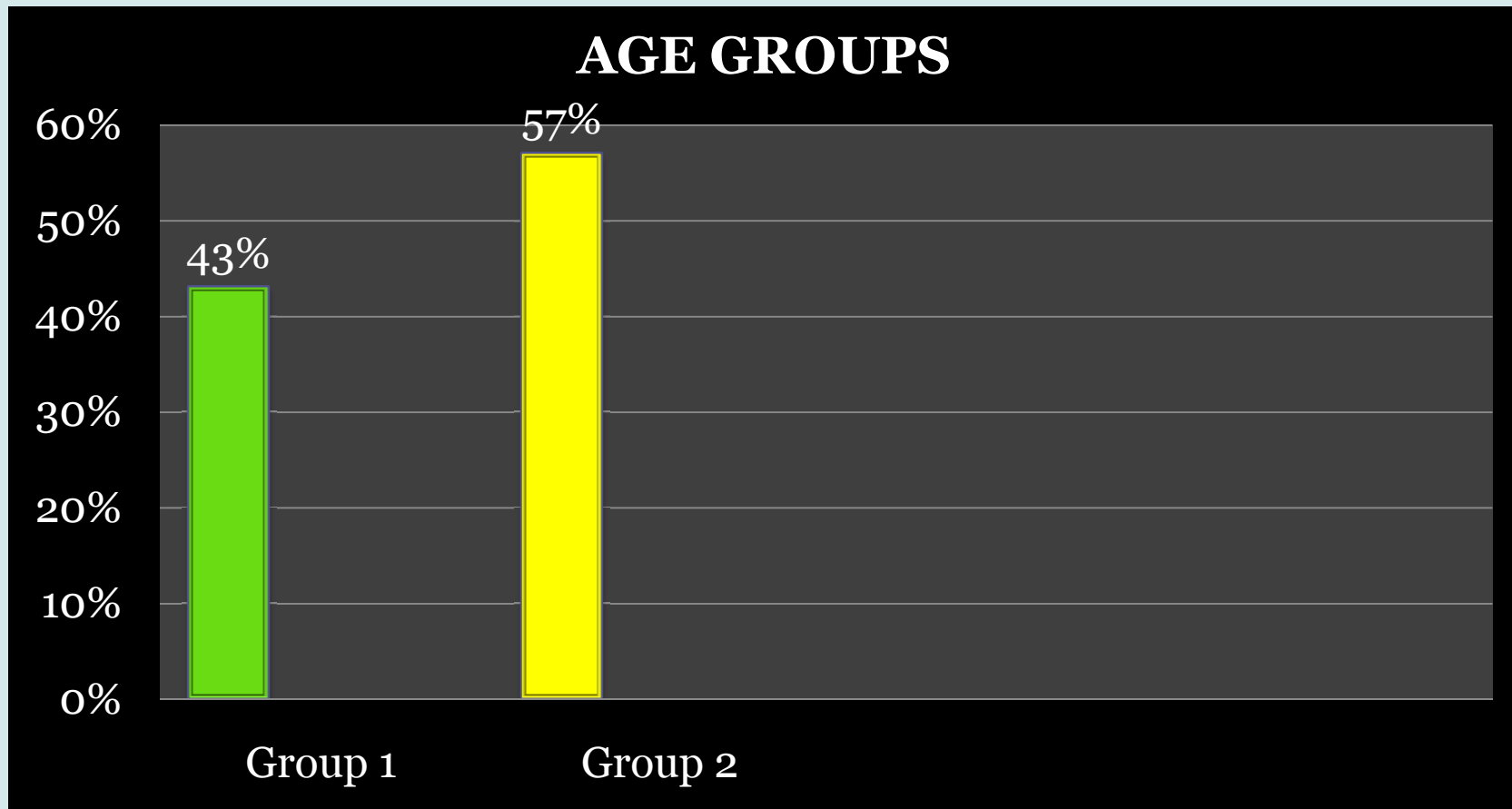




# RESULTS

- The mean age of the total n = 100 patients was  $42.5 \pm 13.8$  years
- Age range being 15 to 70 years
- Most of the cases (97 %) belonged to low socioeconomic status
- The oral hygiene was found to be poor in 91 %, fair in 8 % and good in 1 % only.
- The patients were classified into two groups according to age
  - Group 1 < 30 years (mean age = 20.2 years)
  - Group 2 > 30 years (mean age = 48.5 years)

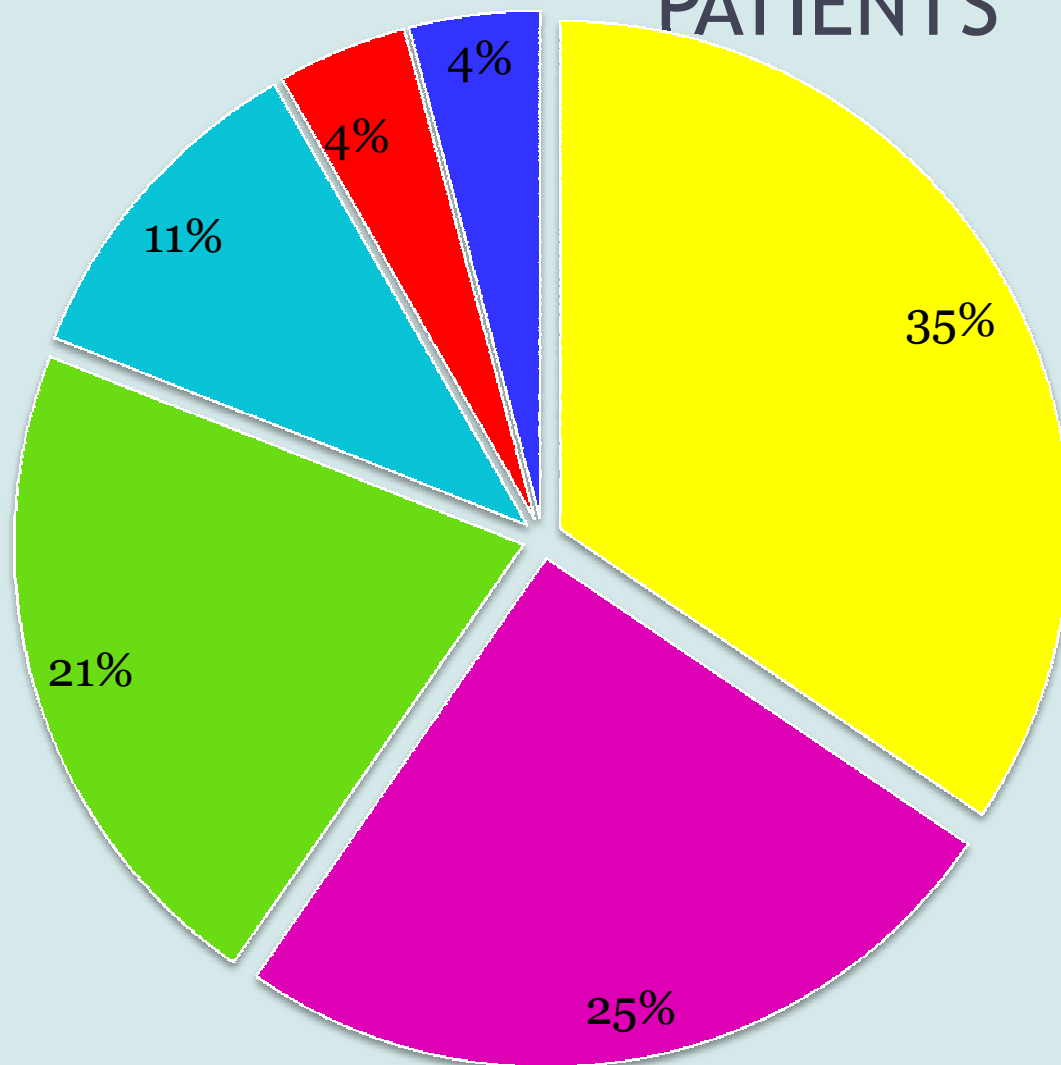
# AGE GROUPS



Group 1 < 30 years of age 43 %

Group 2 > 30 years of age 57 %

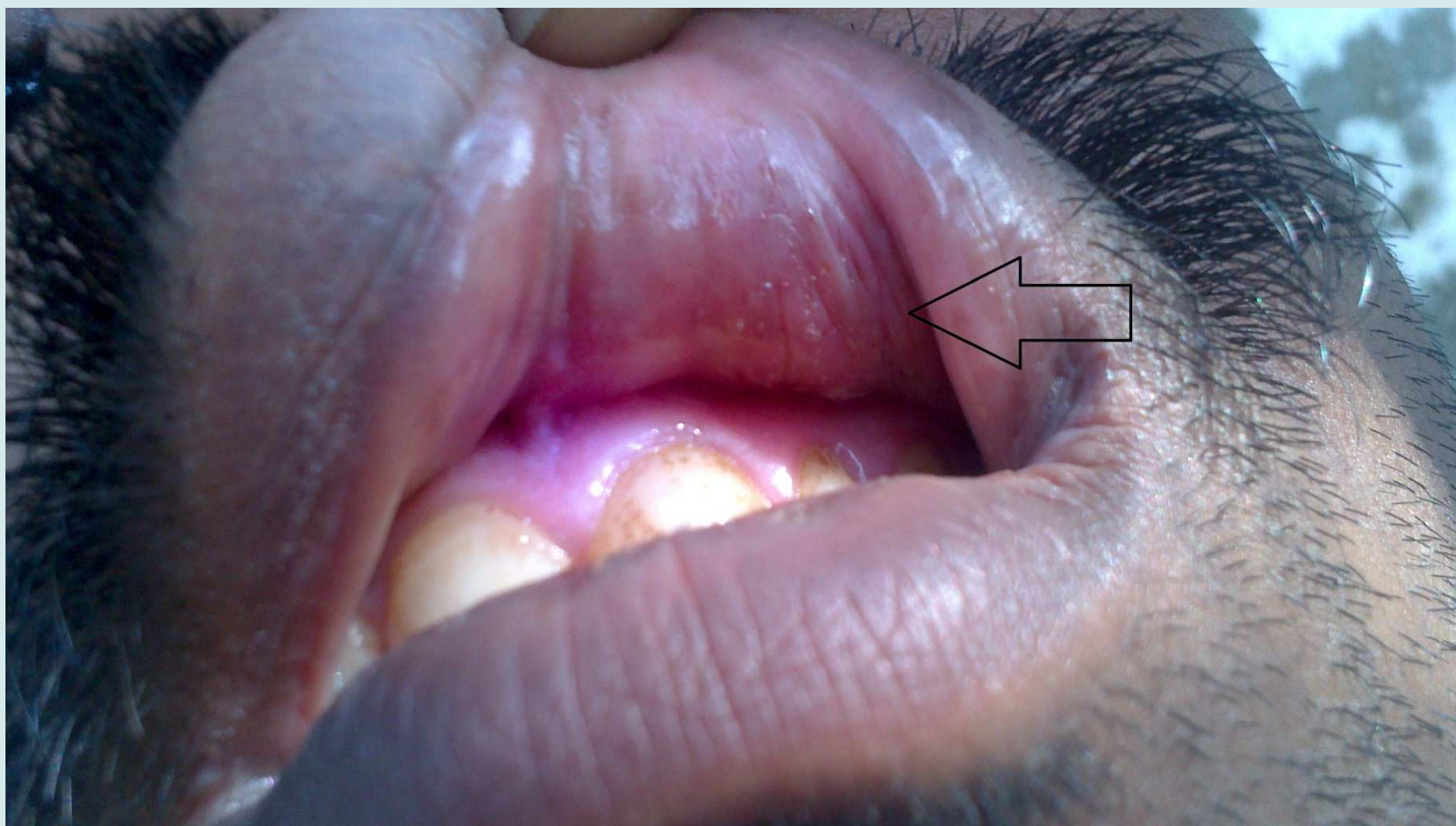
# CLINICAL FEATURES OBSERVED IN n = 100 PATIENTS



## percentages

- keratosis 100 %
- erythroplakia 73 %
- gingivitis 62 %
- white plaque 31 %
- oral pigmentation 12 %
- mucositis 12 %

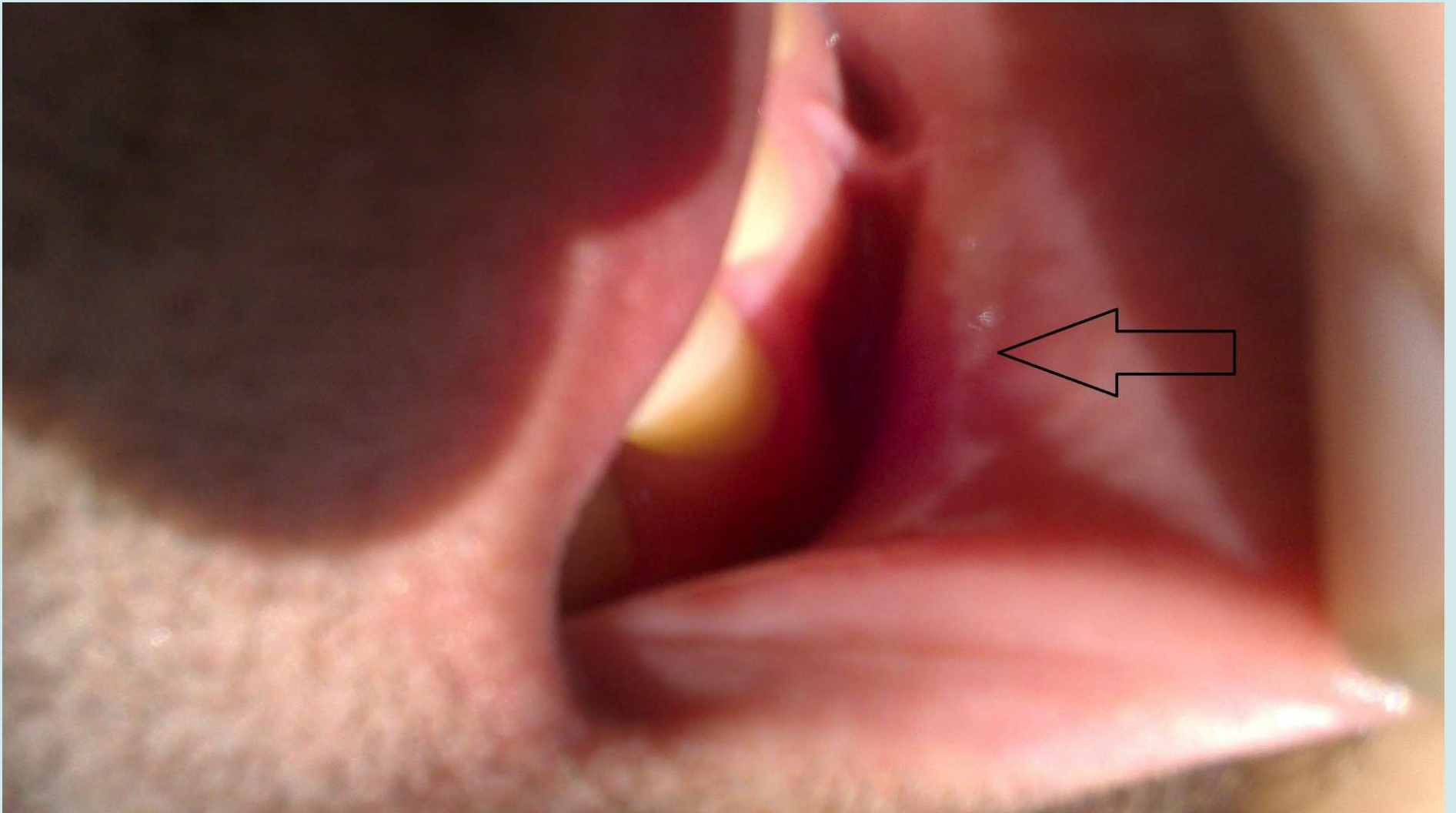
## WRINKLED MUCOSA



## GINGIVITIS AND PERIODONTITIS



# WHITE LESION



# TOBACCO POUCH KERATOSIS





# PIGMENTATION



## ASSOCIATIONS OF AGE GROUPS WITH CLINICAL FEATURES

- When both the age groups of patients were compared with the clinical features, the following associations were observed.

	<b>Group 1 n (%)</b>	<b>Group 2 n (%)</b>	<b>Total n (%)</b>	<b>P Value</b>
MUCOSITIS	43 (100)	57 (100)	100 (100)	--
ULCERATION	43 (100)	57 (100)	100 (100)	--
WHITE PLAQUE	10 (23.3)	21 (37)	31 (31)	0.146
XEROSTOMIA	43 (100)	57 (100)	100 (100)	--
ERYTHROPLAKIA	32 (74.4)	41 (72)	73 (73)	0.781
PERIODONTITIS	18 (42)	17 (30)	35 (35)	0.212
TOBACCO POUCH KERATOSIS	43 (100)	57 (100)	100 (100)	--
GINGIVITIS	26 (61)	36 (62)	62 (62)	0.784

\*Group 1 = age group less than 30 years

\*Group 2 = age group above 30 years

- When the patients were classified according to duration of naswar usage the maximum number  $n = 54$  was seen in the group 1 followed by  $n = 41$  and  $n = 5$  in group 2 and group 3 respectively.
- When the duration of usage was associated with the clinical observations, the following associations were recorded

- \*Group 1 = mild → less than 5 years of intake
- \*Group 2 = moderate → between 5 to 10 years of usage
- \*Group 3 = heavy → more than 10 years of usage

	<b>Group 1 n (%)</b>	<b>Group 2 n (%)</b>	<b>Group 3 n (%)</b>	<b>Total</b>	<b>P value</b>
MUCOSITIS	54 (100)	41 (100)	5 (100)	100 (100)	--
ULCERATION	54 (100)	41 (100)	5 (100)	100 (100)	--
WHITE PLAQUE	14 (26)	15 (37)	2 (40)	31 (31)	0.503
XEROSTOMIA	54 (100)	41 (100)	5 (100)	100 (100)	--
ERYTHROPLAKIA	40 (74.1)	31 (76)	2 (40)	73 (73)	0.254
PERIODONTITIS	23 (43)	12 (29.3)	0 (0)	35 (35)	0.121
TOBACCO POUCH KERATOSIS	54 (100)	41 (100)	5 (100)	100 (100)	--
GINGIVITIS	32 (59.3)	26 (63.4)	4 (80)	62 (62)	0.738

\*Group 1 = mild → less than 5 years of intake

\*Group 2 = moderate → between 5 to 10 years of usage

\*Group 3 = heavy → more than 10 years of usage

- When the patients were grouped according to dose of snuff (naswar) per day, it was observed that maximum number of patients (n = 45) fell in Group 2, followed by (n = 39) in Group 3, while the least number of cases (n = 16) were found in Group 1.
- Likewise when the dose of snuff per day was associated with the clinical features, we noted the following results

\* Group 1 = Mild ( less than 5 times a day )  
\* Group 2 = Moderate ( Between 5 to 10 times a day )  
\* Group 3 = Heavy ( More than 10 times a day )

	<b>Group 1 n (%)</b>	<b>Group 2 n (%)</b>	<b>Group 3 n (%)</b>	<b>Total</b>	<b>P Value</b>
MUCOSITIS	16 (100 )	45 (100 )	39 (100 )	100 (100)	--
ULCERATION	16 (100 )	45 (100 )	39 (100)	100 (100)	--
WHITE PLAQUE	6 (38)	10 (22.2)	15 (38.5)	31 (31 )	0.257
XEROSTOMIA	16 (100 )	45 (100 )	39 (100 )	100 (100 )	--
<b>ERYTHROPLAKIA</b>	<b>5 (31.2)</b>	<b>37 (82)</b>	<b>31 (80)</b>	<b>73 (73 )</b>	<b>0.001*</b>
<b>PERIODONTITIS</b>	<b>11 (69 )</b>	<b>17 (39)</b>	<b>7 (18)</b>	<b>35 (35 )</b>	<b>0.001**</b>
TOBACCO POUCH KERATOSIS	16 (100 )	45 (100 )	39 (100 )	100 (100 )	--
GINGIVITIS	10 (62.5)	28 (62.2)	24 (62 )	62 (62 )	0.997

\* Fishers exact test

\*\*Pearson Chi square test

\* Group 1 = Mild ( less then 5 times a day )

\* Group 2 = Moderate ( Between 5 to 10 times a day )

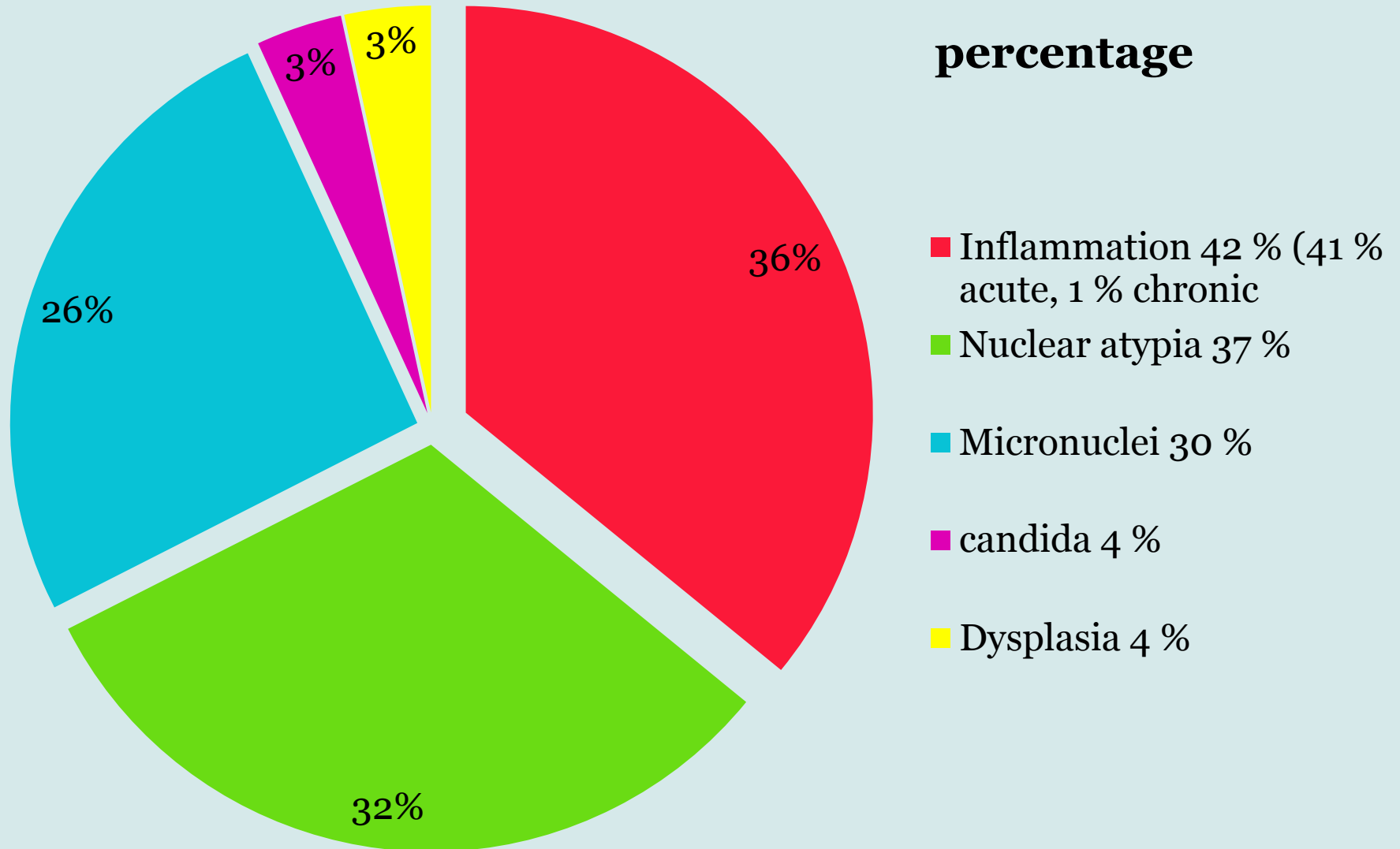
\* Group 3 = Heavy ( More then 10 times a day )

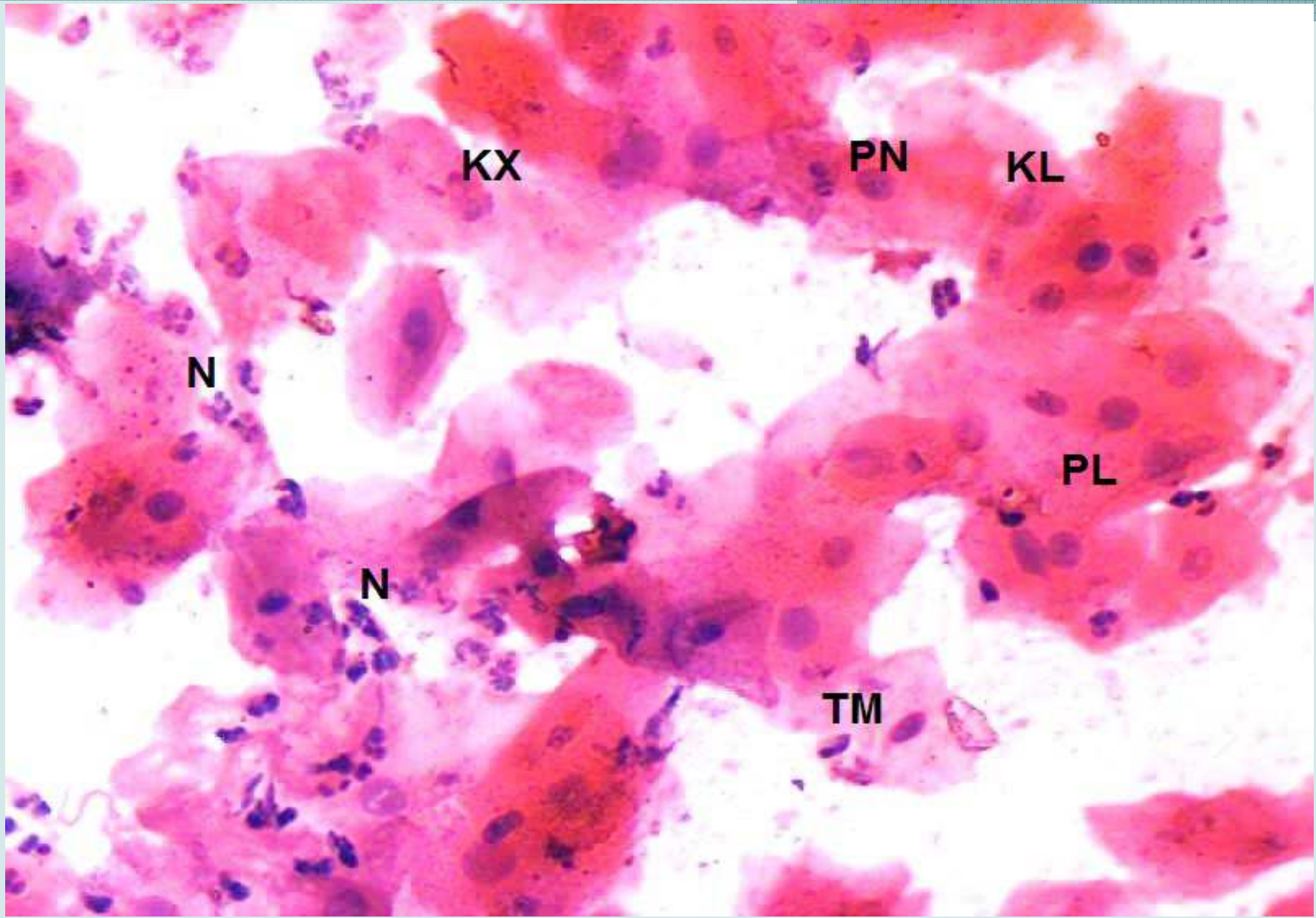
## CYTOPATHOLOGICAL OBSERVATIONS

- Microscopic examination revealed variety of oral lesions ranging from simple inflammation to atypical as well as dysplastic transformations.

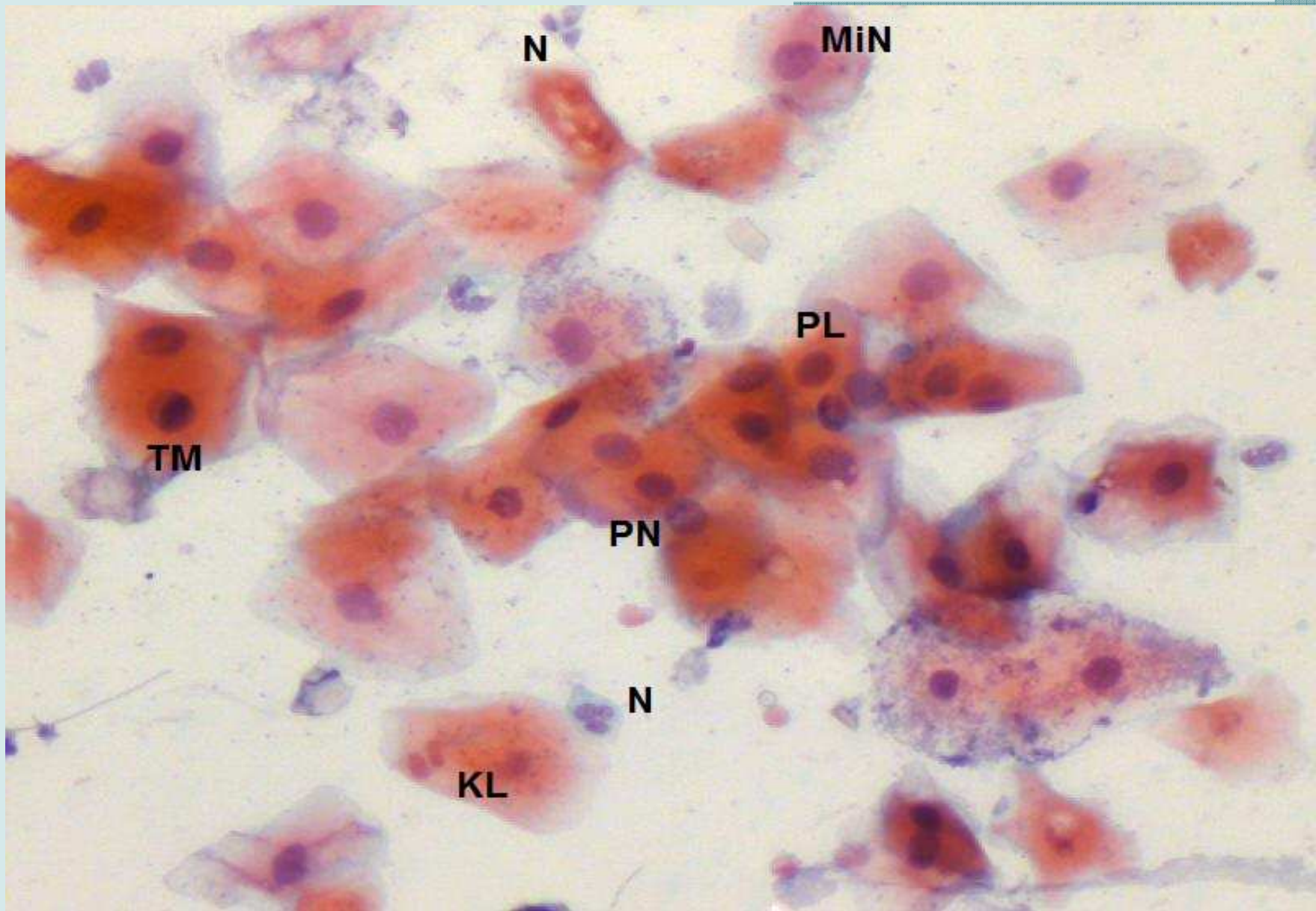


# CYTOLOGICAL CHANGES SEEN IN n = 100 PATIENTS

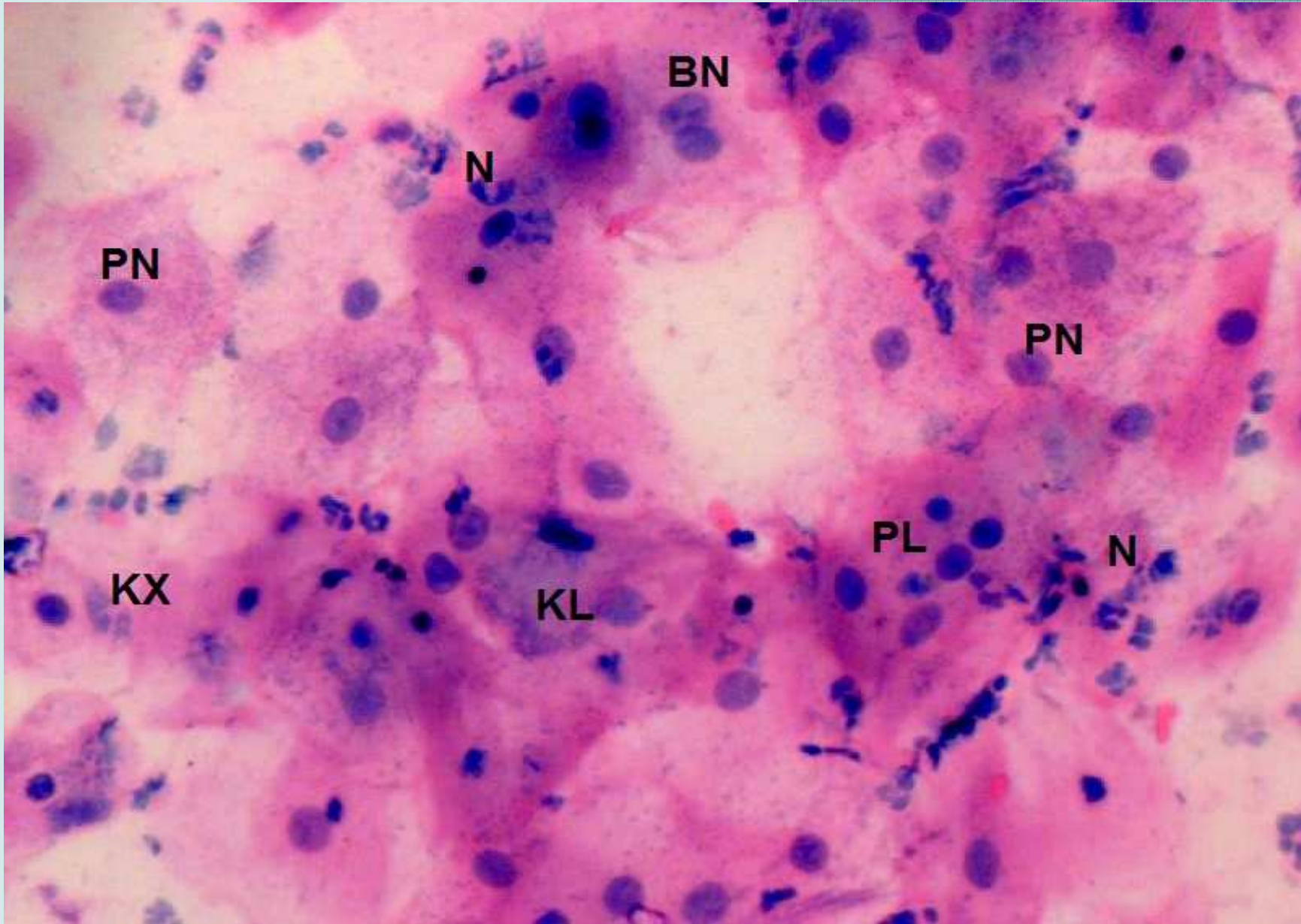




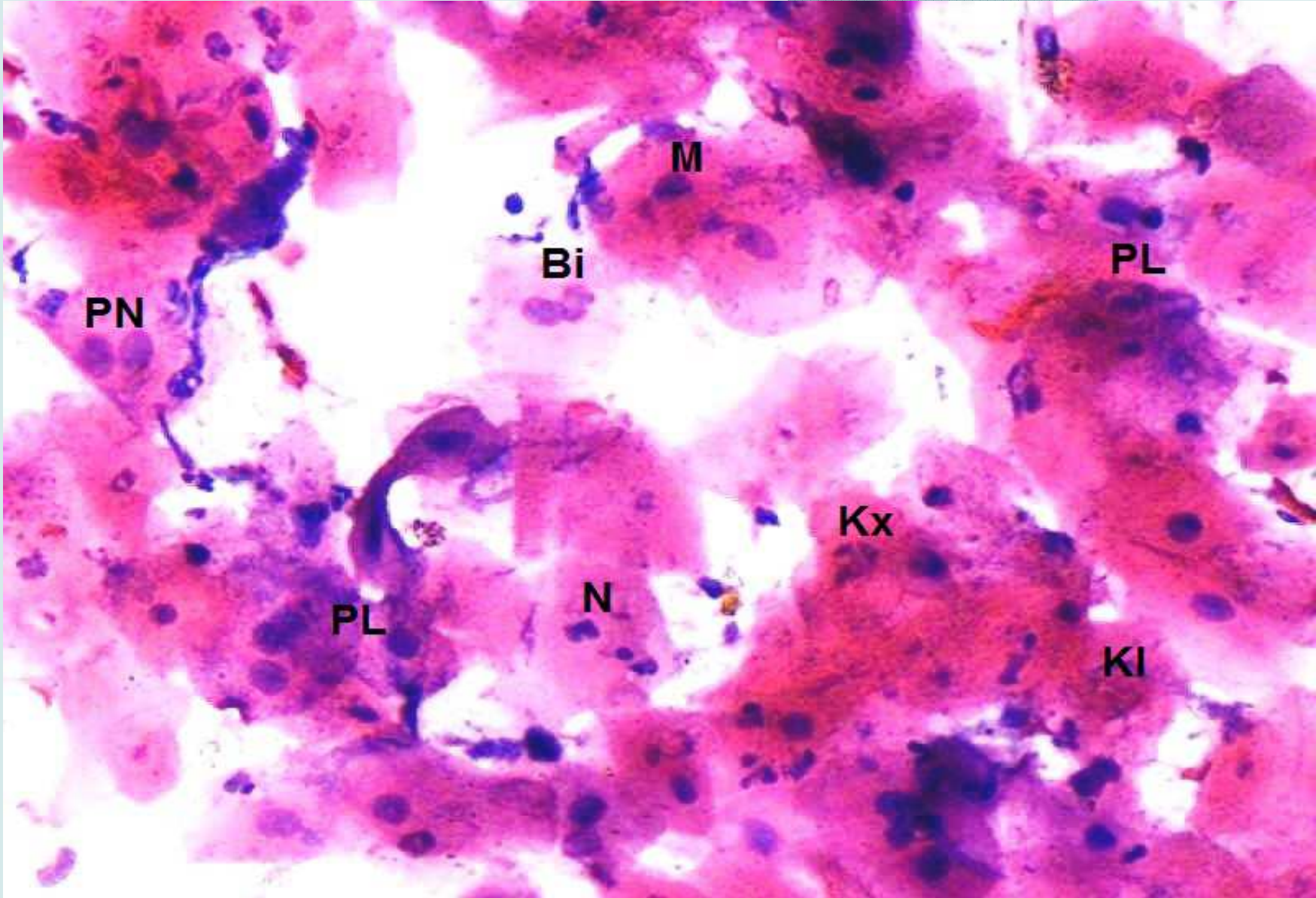
Inflammatory Atypia (H/E)



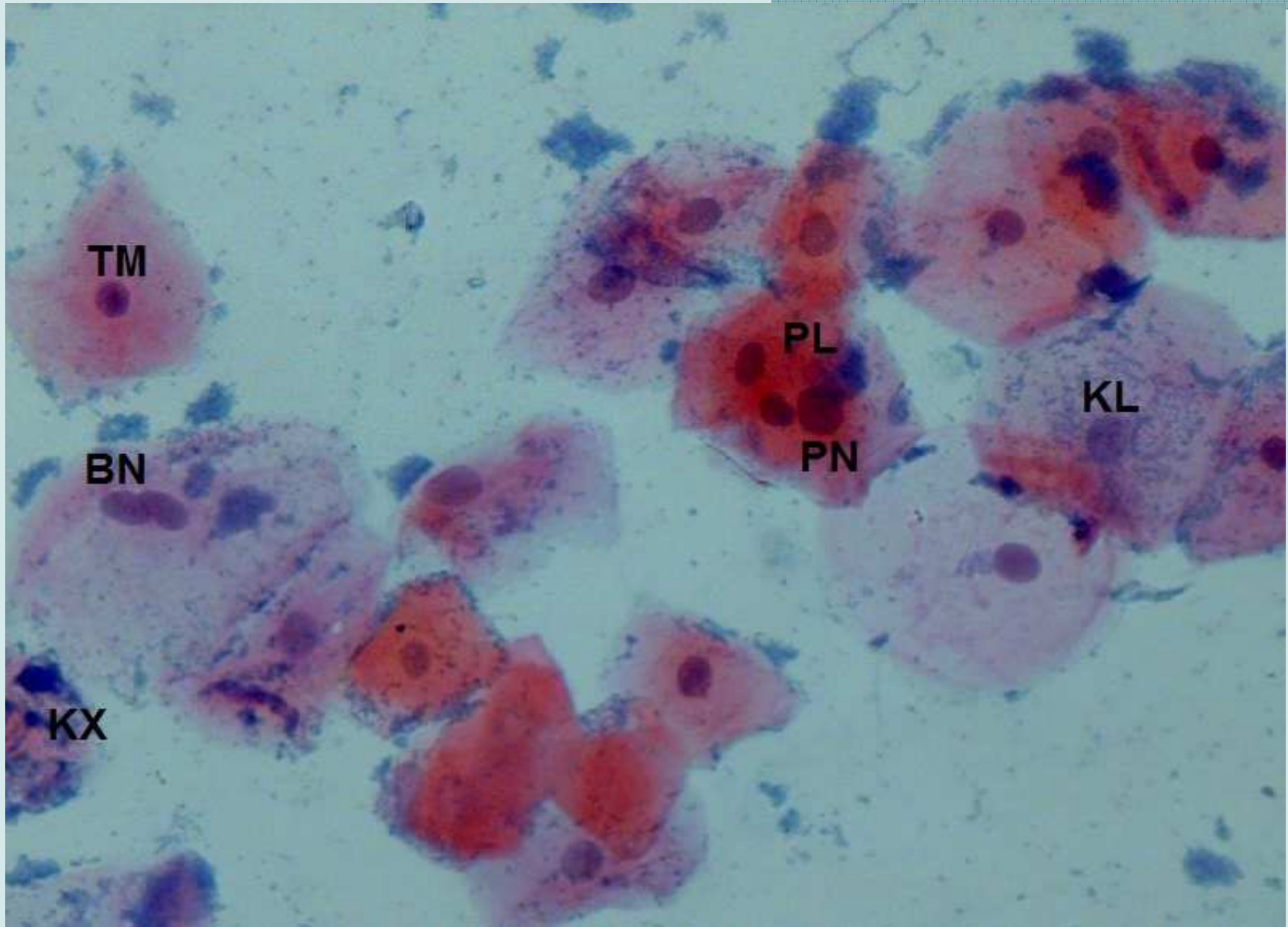
Inflammatory Atypia - (PAP)



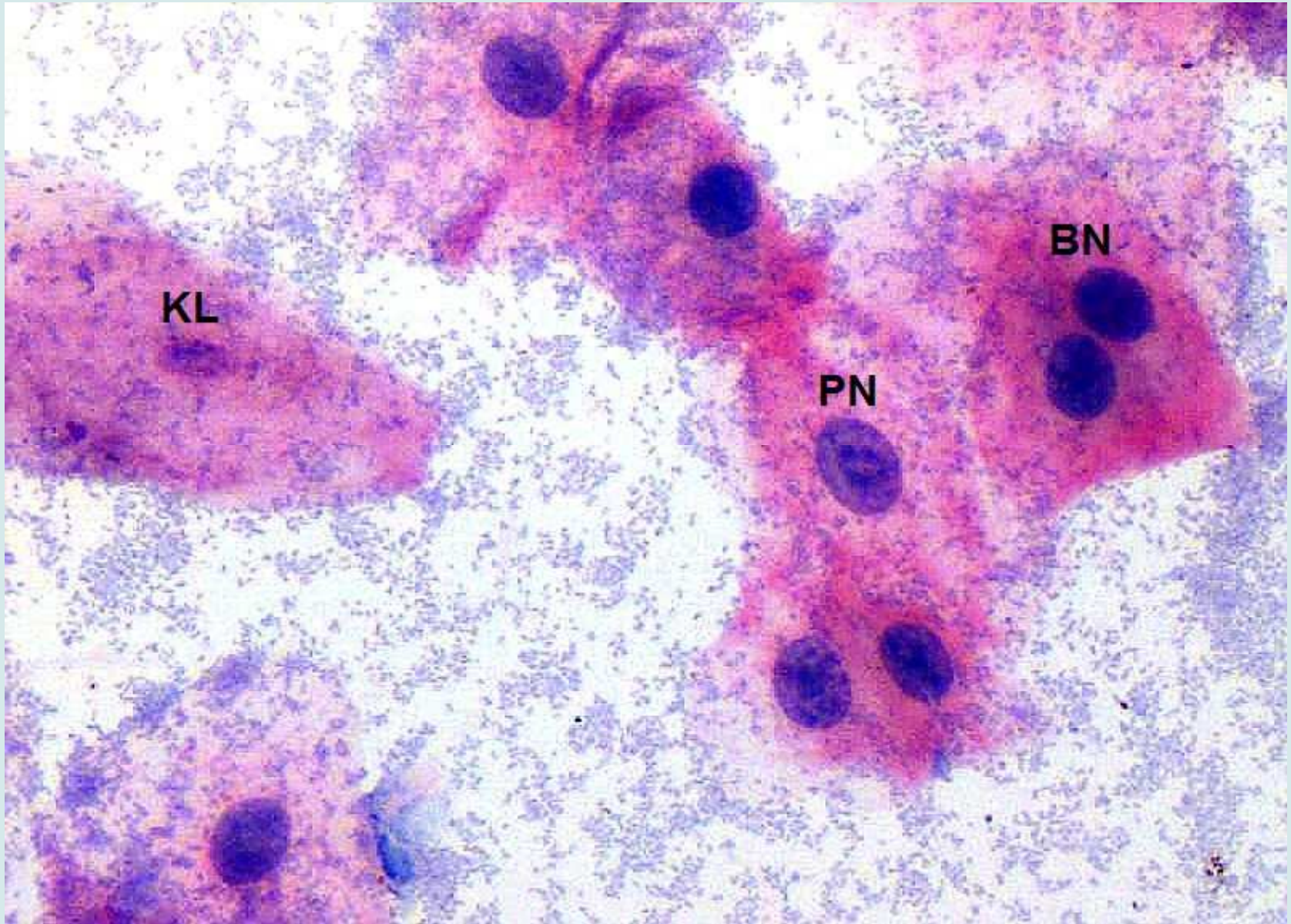
Inflammatory Atypia - moderate degree (H/E)



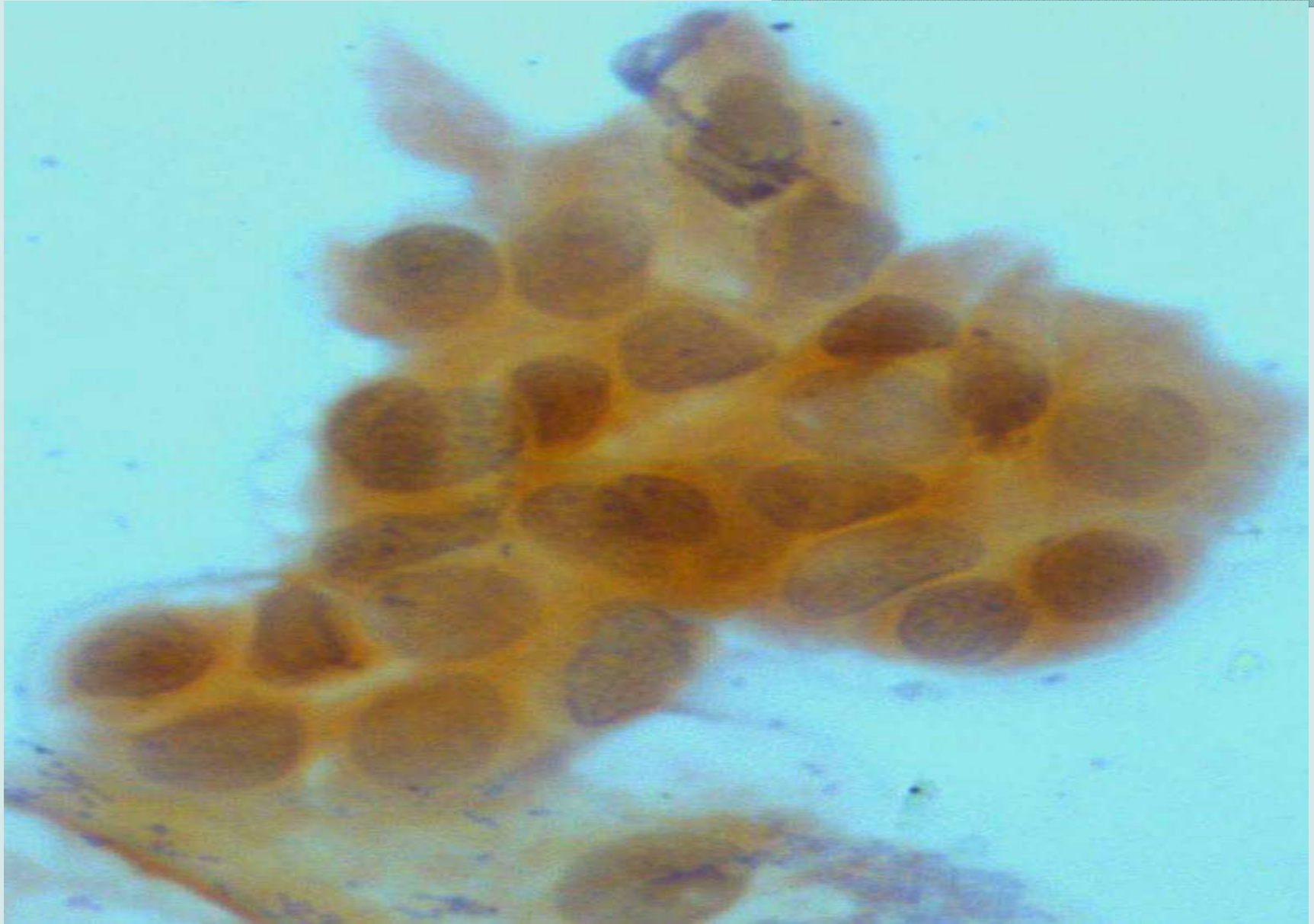
Epithelial Atypia (H/E)



Epithelial Atypia (PAP)

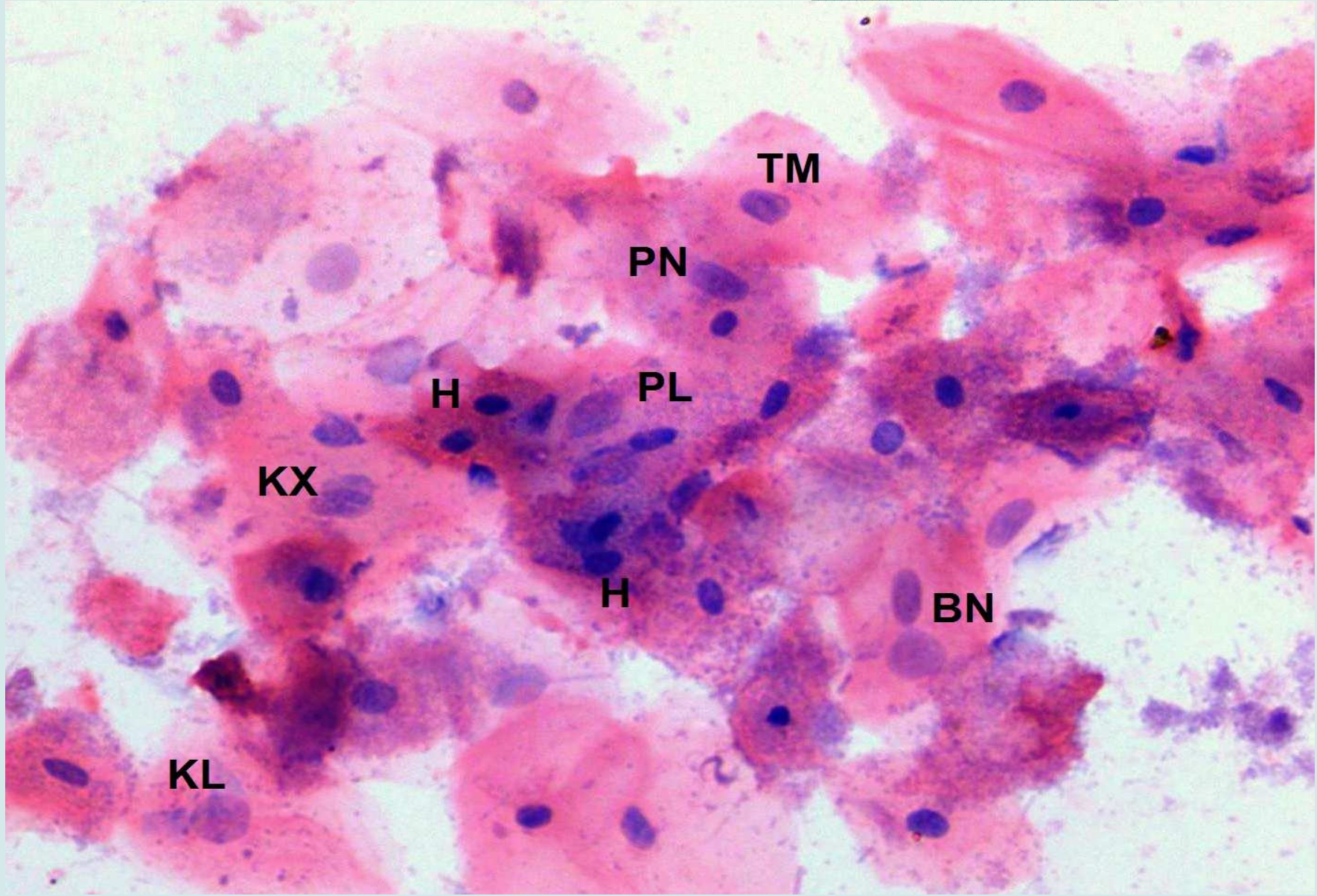


Epithelial Atypia (H/E)

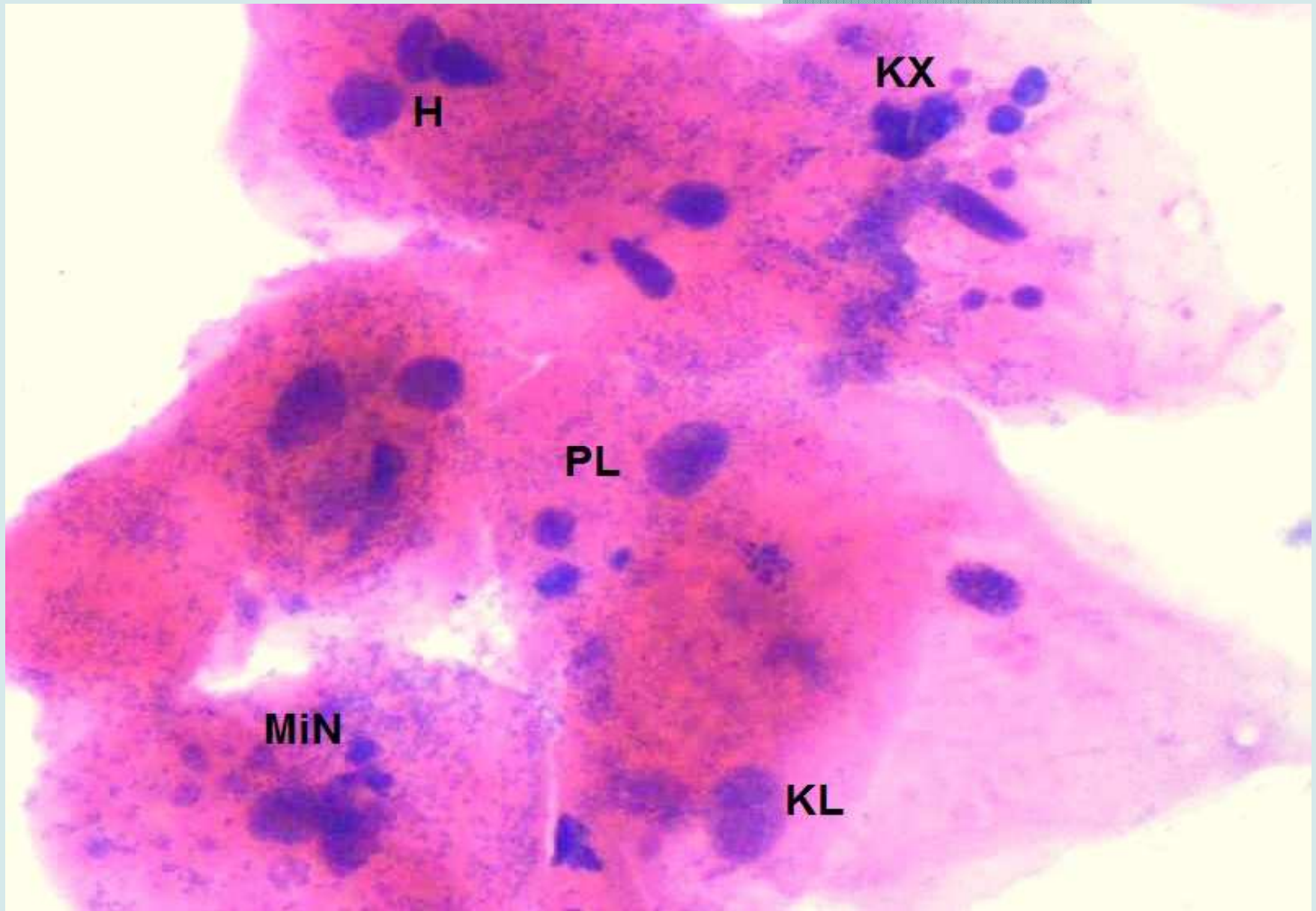


Pleomorphic clump of epithelial cells (PAP)

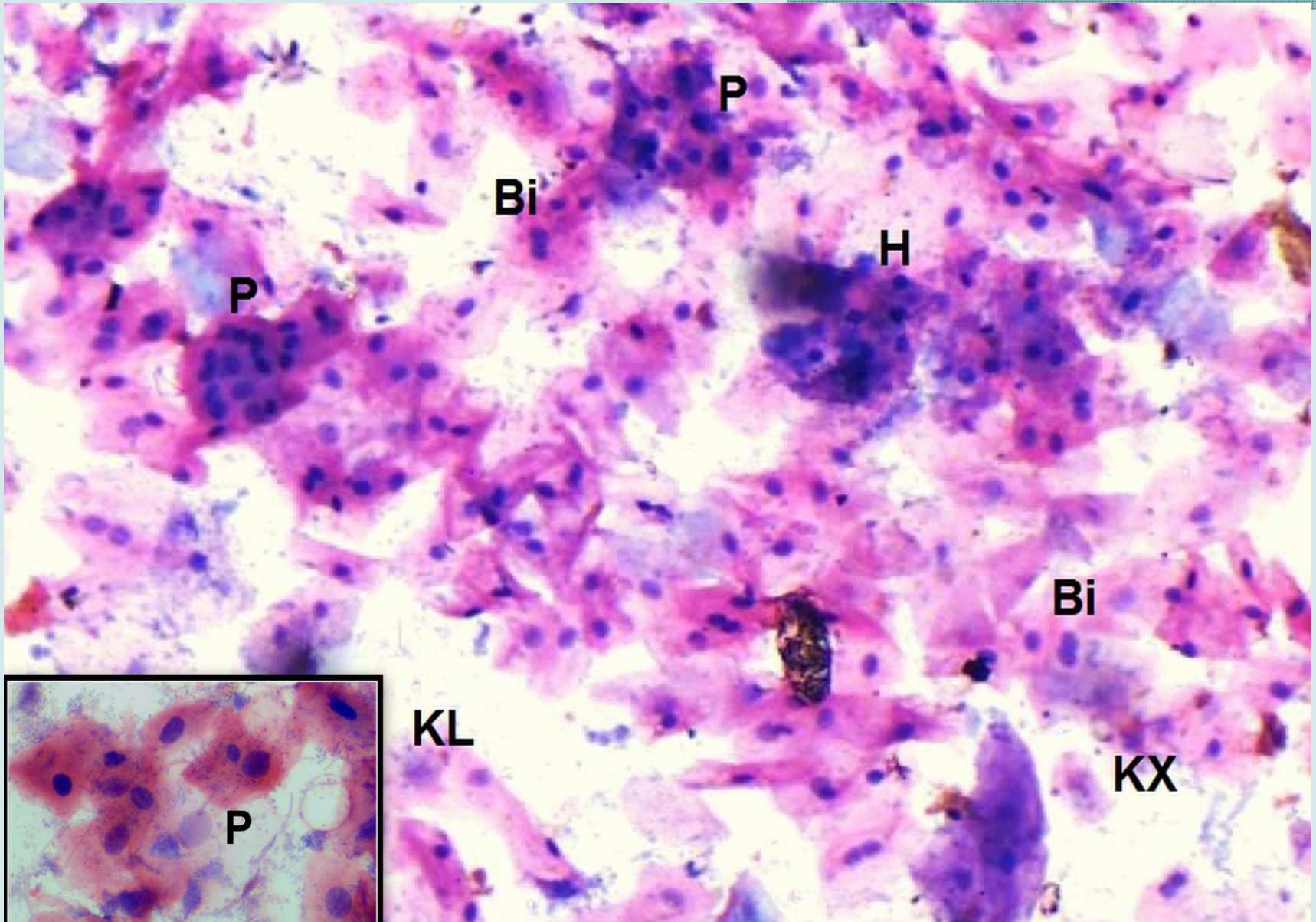




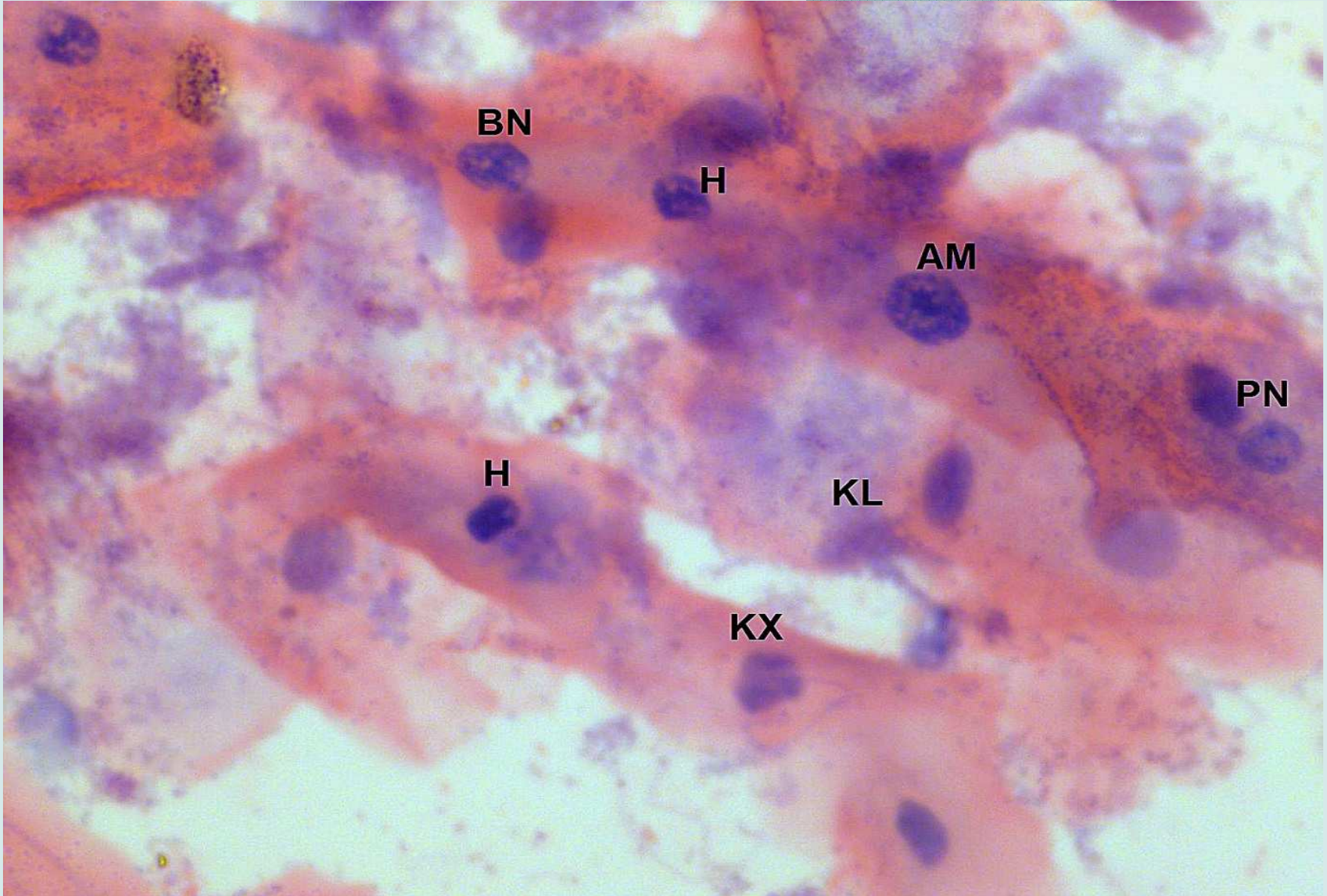
Grade 1 dysplasia



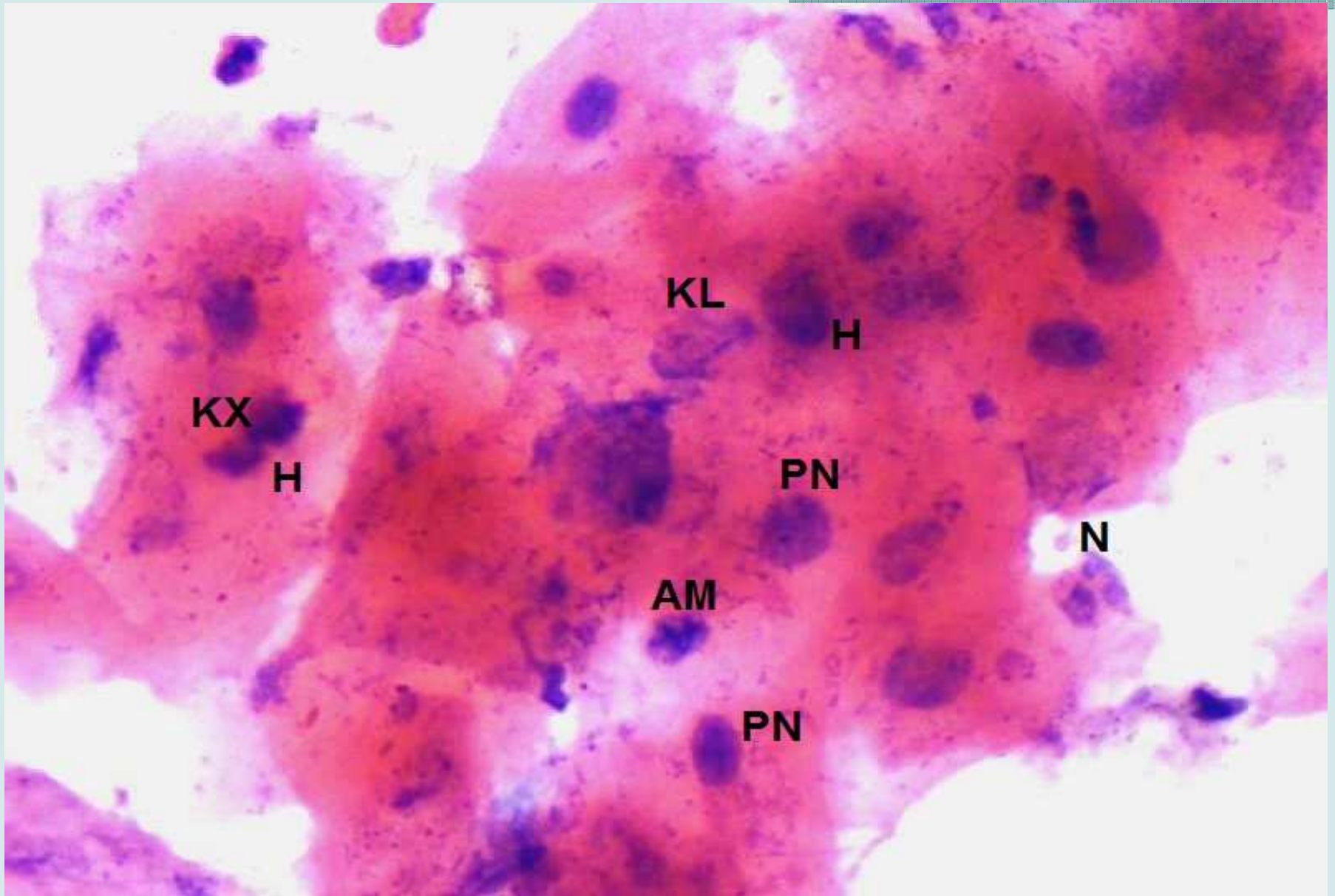
Grade 1 dysplasia



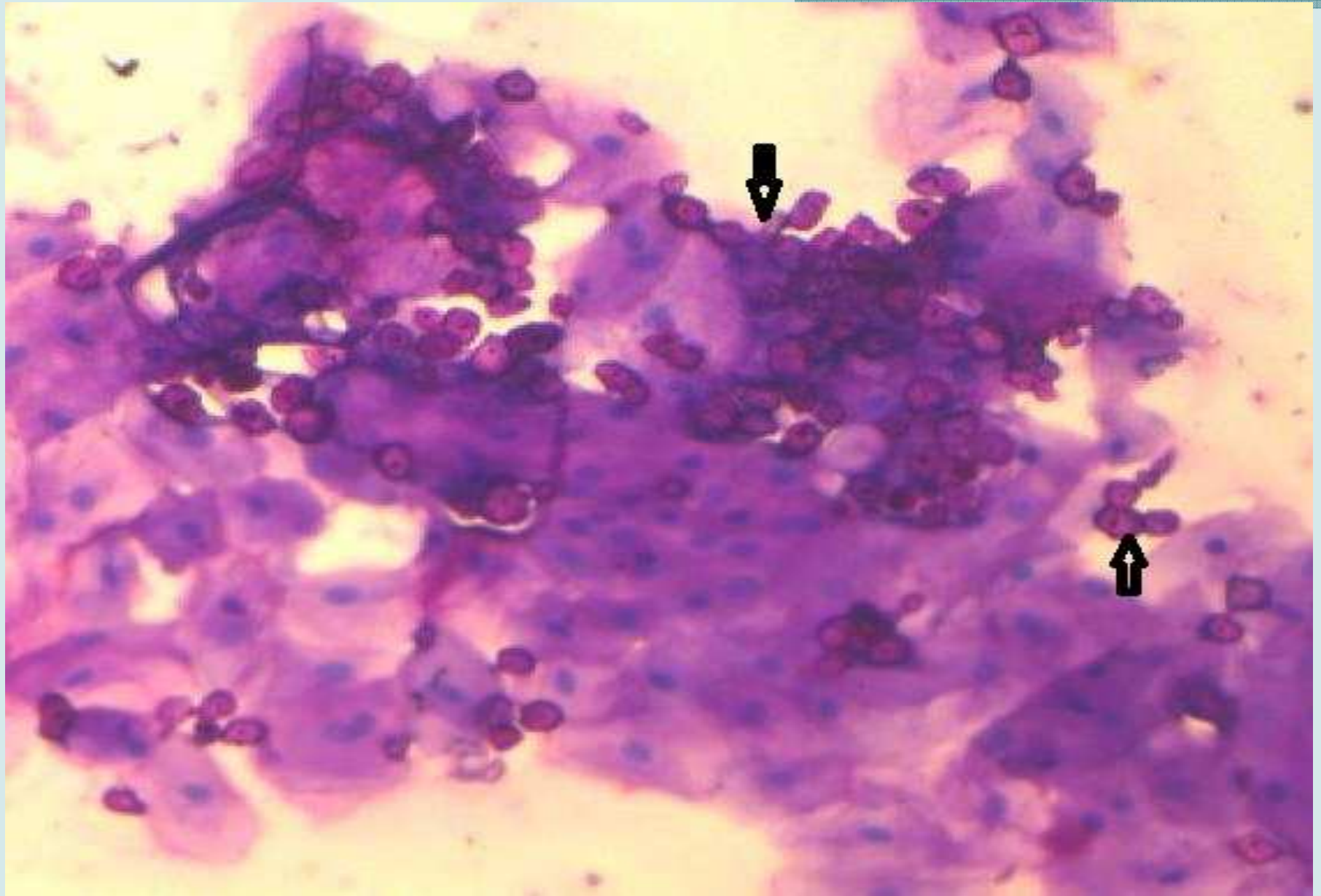
Grade 1 dysplasia



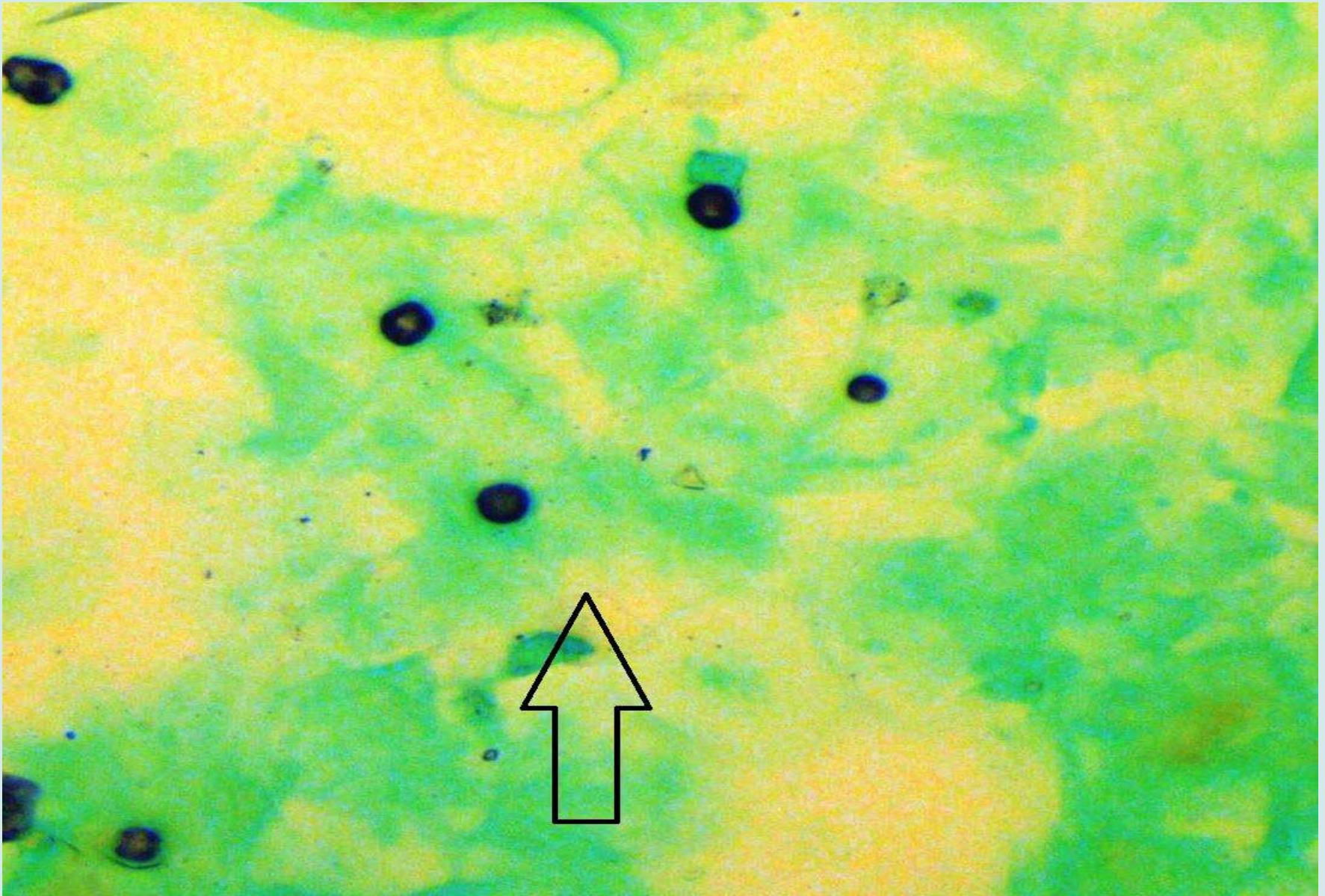
Grade 2 dysplasia (H/E)



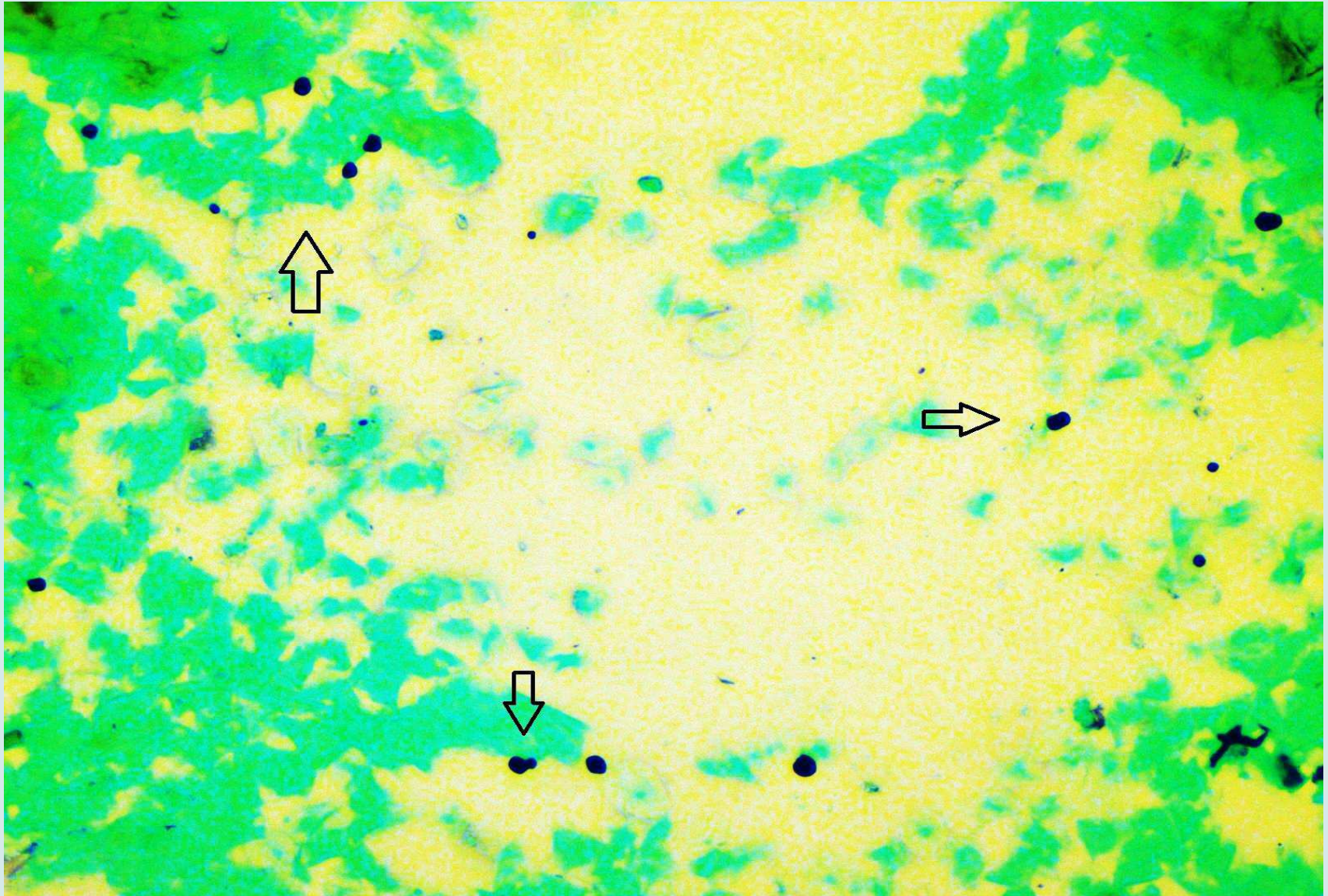
Grade 2 Dysplasia (H/E)



Spores of Candida (PAS)

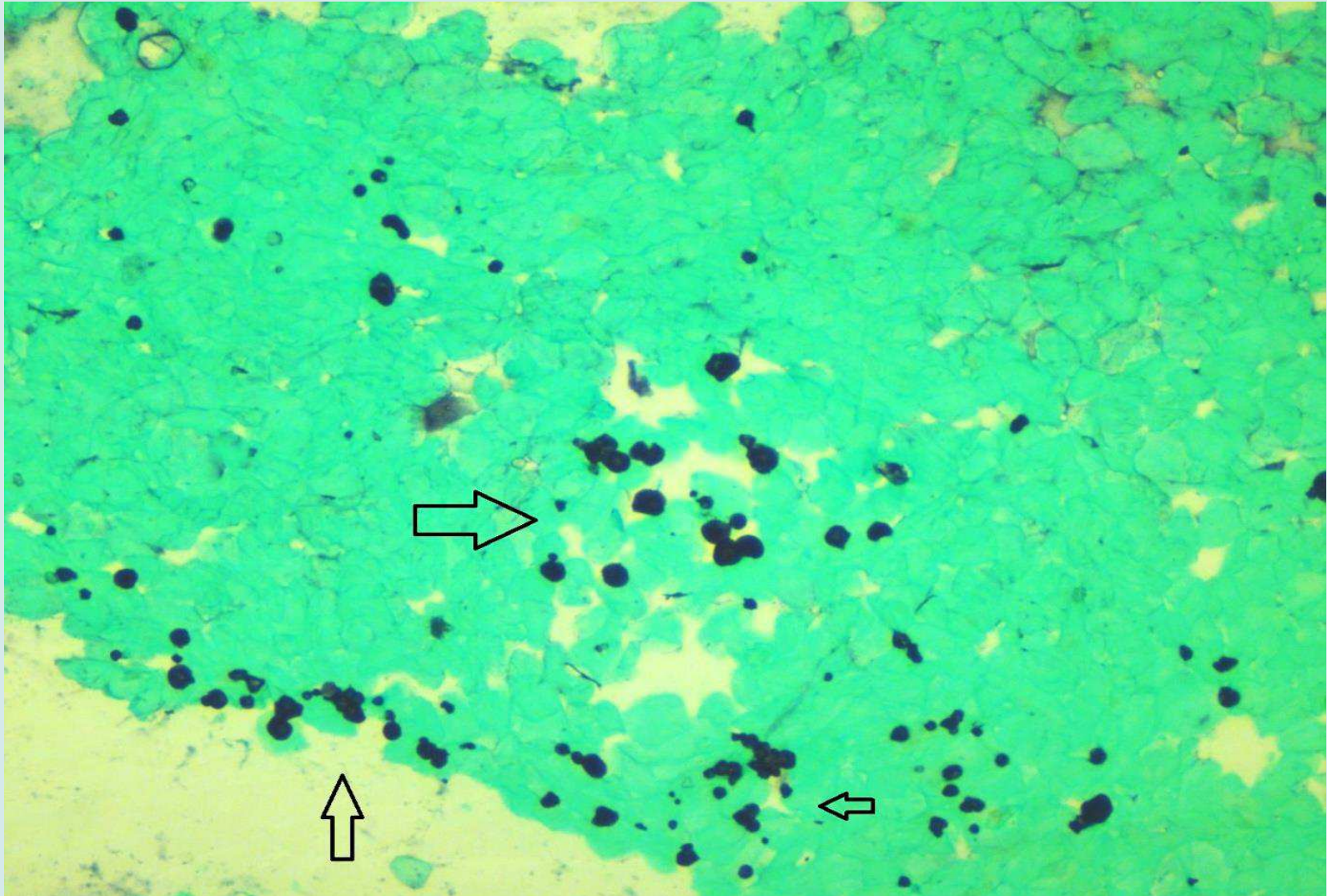


Spores of Candida (GMS)



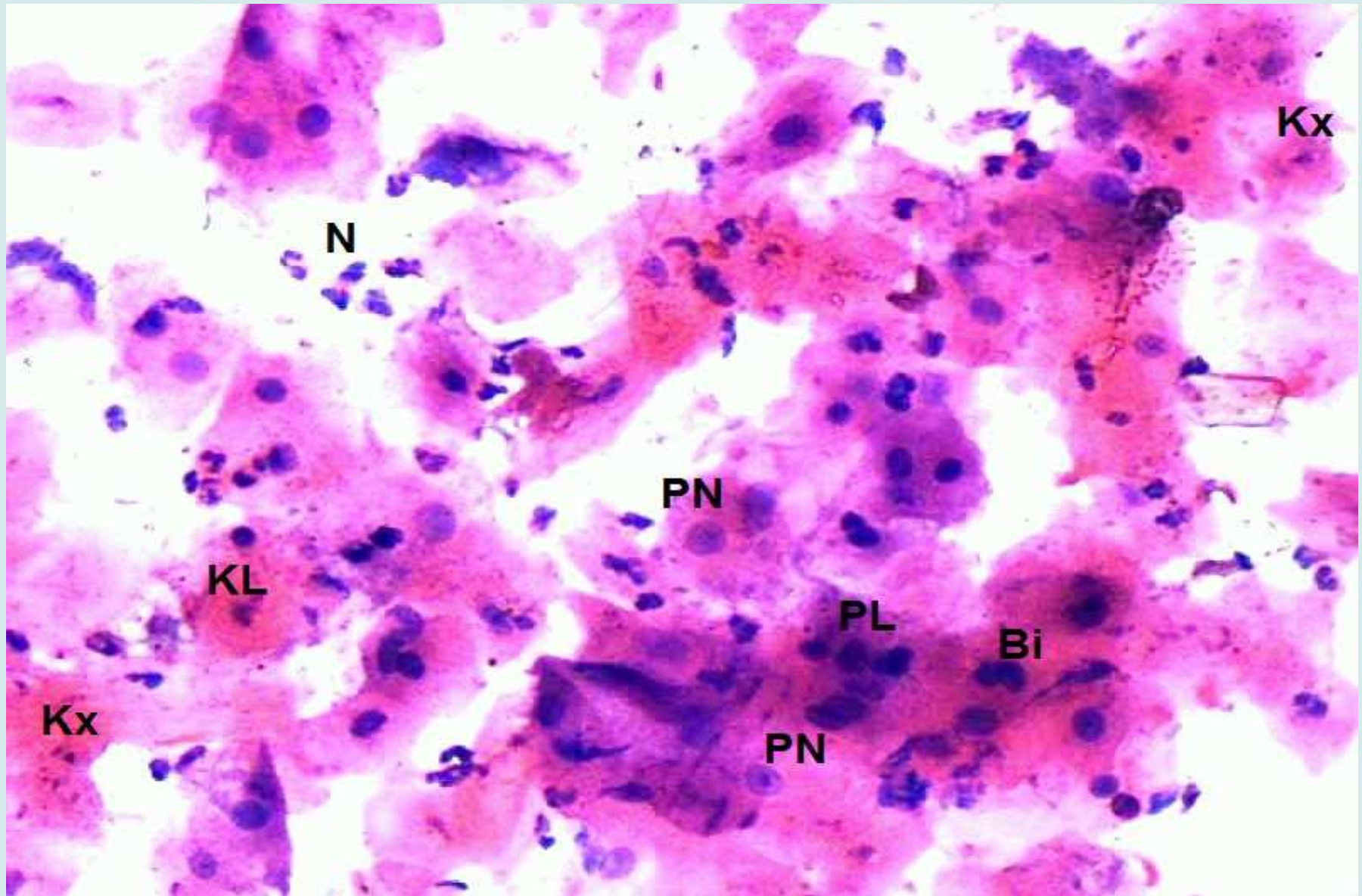
Spores of Candida (GMS)



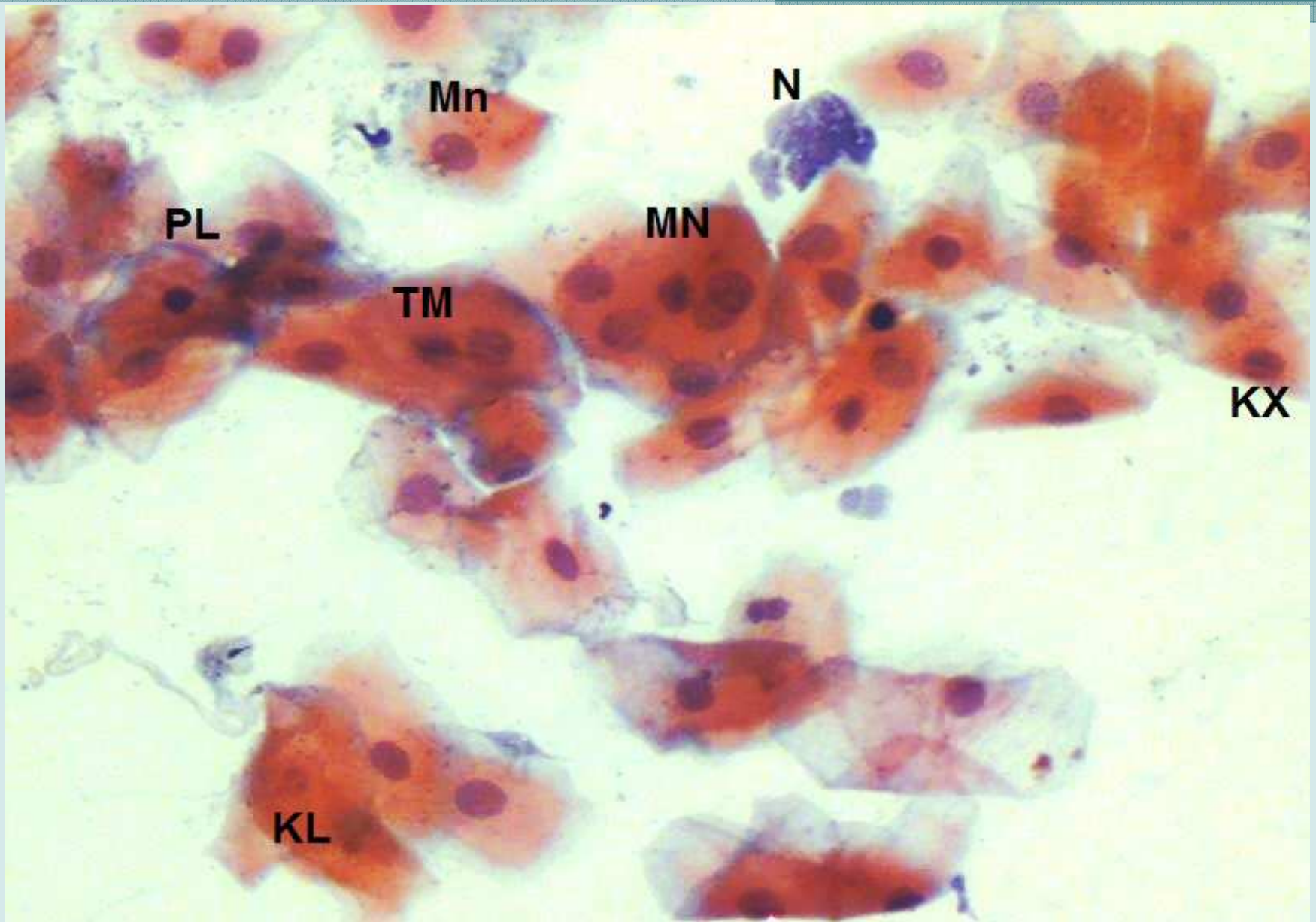


Spores of Candida (GMS)

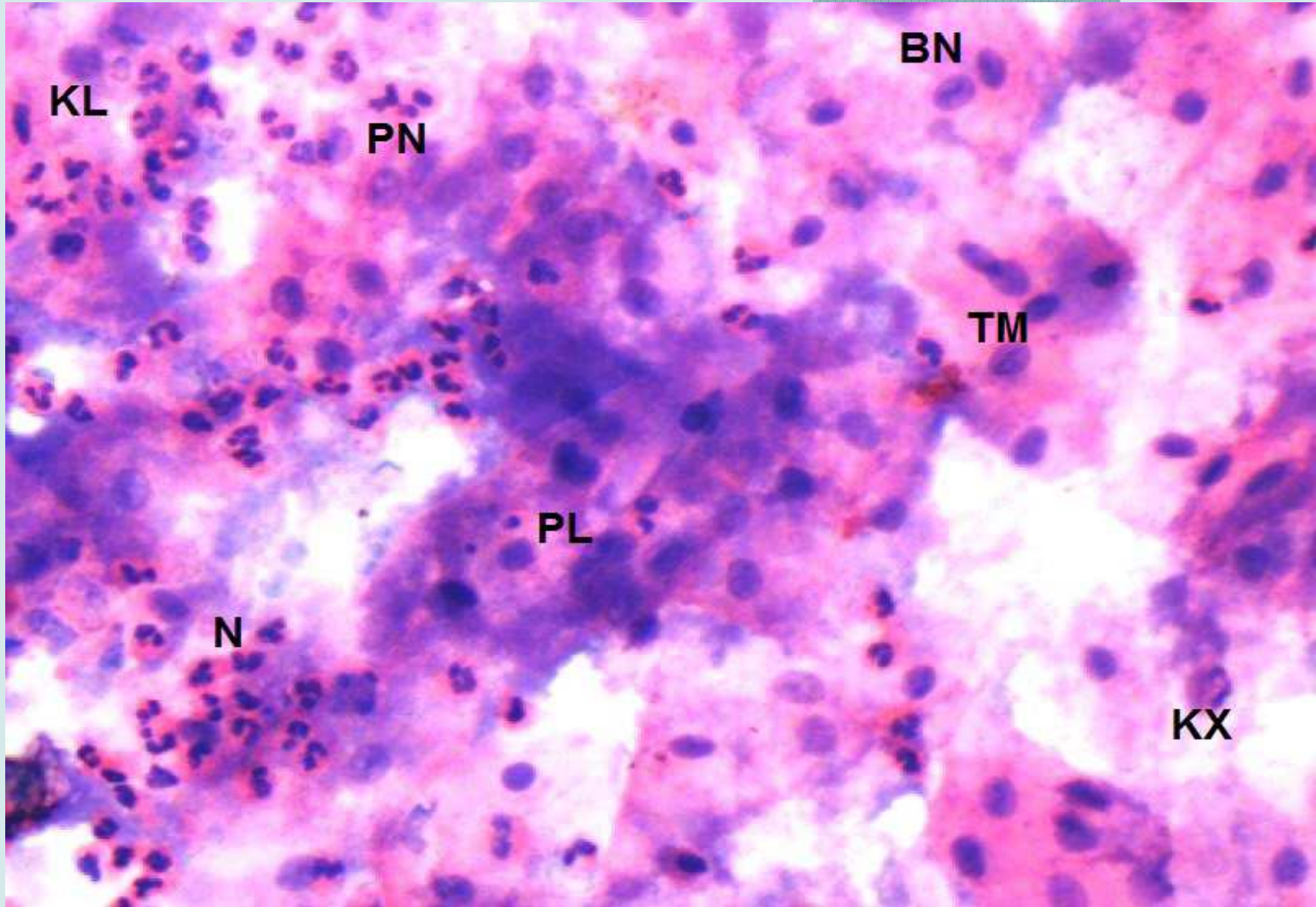
- As regards inflammation n = 26 (62 %) smears with mild, n = 7 (16 %) with moderate while n = 9 (22 %) smears with severe degree of inflammatory infiltrate were seen



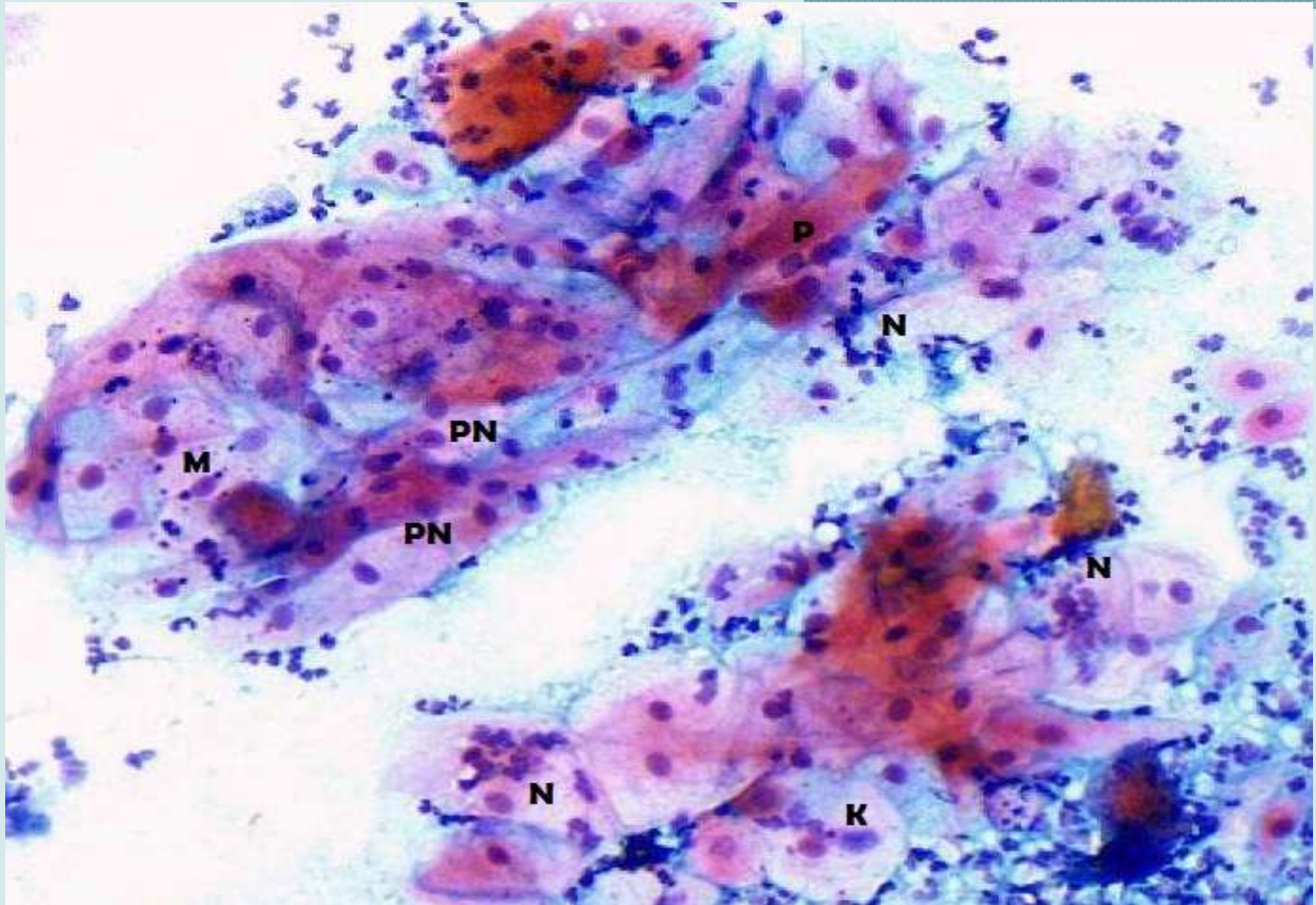
Inflammatory Atypia - mild degree (H/E)



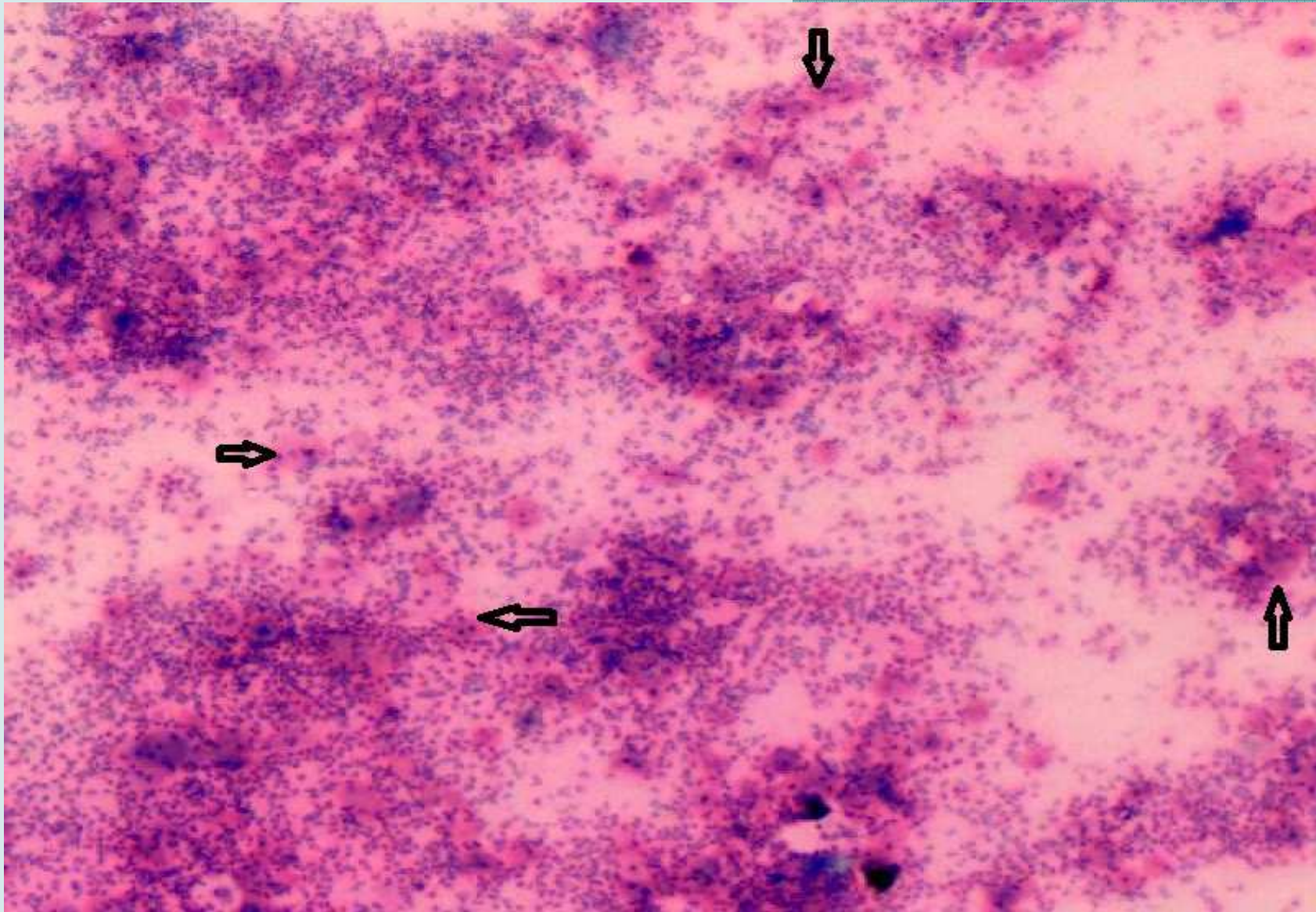
Inflammatory Atypia - mild degree (PAP)



Inflammatory Atypia - moderate degree (H/E)



Inflammatory Atypia - moderate degree (PAP)



Smear showing severe acute inflammatory infiltrate with degenerating sq. epithelial cells (arrows)

- When these cytopathological changes were associated with the age groups following associations were observed.



	<b>Group 1 n (%)</b>	<b>Group 2 n (%)</b>	<b>Total</b>	<b>P value</b>
INFLAMMATION	18 (42)	23 (40.4)	41 (41)	0.879
NUCLEAR ATYPIA	18 (42)	19 (33.3)	37 (37)	0.382
MICRONUCLEI	5 (11.6)	4 (7.0)	9 (9)	0.493
CANDIDA	1	3	4 (4)	
DYSPLASIA	1 (2.3)	3 (5.3)	4 (4)	0.632
CYTOPLASMIC VACUOLIZATION	13 (30.2)	24 (42.1)	37 (37)	0.223

Group 1 = age group less than 30 years

Group 2 = age group above 30 years

- When these cytopathological changes were associated with duration of snuff, the following associations were seen

	<b>Group 1 n (%)</b>	<b>Group 2 n (%)</b>	<b>Group 3 n (%)</b>	<b>Total</b>	<b>P Value</b>
INFLAMMATION	18 (33.3 )	21 (51.2)	2 (40 )	41 (41 )	0.247
NUCLEAR ATYPIA	<b>9 (17)</b>	<b>25 (61 )</b>	<b>3 (60)</b>	<b>37 (37 )</b>	<b>0.000*</b>
MICRONUCLEI	5 (9.3)	3 (7.3)	1 (20 )	9 (9)	0.562
CANDIDA	1	2	1	4 (4)	
DYSPLASIA	1 (2)	2 (5 )	1 (20)	4 (4)	0.139
CYTOPLASMIC VACUOLIZATION	<b>12 (22.2)</b>	<b>21 (51.2 )</b>	<b>4 (80 )</b>	<b>37 (37)</b>	<b>0.001**</b>

\* FISHERS EXACT TEST

\*\* FISHERS EXACT TEST

Group 1 = mild → less than 5 years of intake

Group 2 = moderate → between 5 to 10 years of usage

Group 3 = heavy → more than 10 years of usage

- When these cytopathological changes were associated with dosage of snuff per day the following observations were made

	<b>Group 1 n (%)</b>	<b>Group 2 n (%)</b>	<b>Group 3 n (%)</b>	<b>Total</b>	<b>P Value</b>
INFLAMMATION	5 (31.2)	16 (36)	20 ( 51.3 )	41 (41 )	0.236
NUCLEAR ATYPIA	<b>1 (6.2)</b>	<b>13 (29)</b>	<b>23 (59)</b>	<b>37 (37)</b>	<b>0.000*</b>
MICRONUCLEI	0 (0)	3 (7)	6 (15.4)	9 (9)	0.196
CANDIDA	1	1	2	4 (4)	
DYSPLASIA	<b>0 (0)</b>	<b>0 (0)</b>	<b>4 (10.3)</b>	<b>4 (4)</b>	<b>0.033**</b>
CYTOPLASMIC VACUOLIZATION	<b>0 (0)</b>	<b>12 (27)</b>	<b>25 (64.1)</b>	<b>37 (37)</b>	<b>0.000***</b>

\* PEARSON CHI SQUARE TEST

\*\* FISHERS EXACT TEST

\*\*\* PEARSON CHI SQUARE TEST

\*Group 1 = Mild ( less then 5 times a day )

\*Group 2 = Moderate ( Between 5 to 10 times a day )

\*Group 3 = Heavy ( More then 10 times a day )

# HIGHLIGHTS OF SIGNIFICANT ASSOCIATIONS

- Erythropakia when associated with dose of snuff per day ( $p=0.001$ )
- Periodontitis when associated with dose of snuff per day ( $p=0.001$ )
  - $P=0.021$  (Yong Chu et al, 2010; Columbia)
  - $P=0.002$  (Bergstrong, 2006; Sweden)
  - $P=0.001$  (Anand et al, 2013; India)
  - Monten et. al, 2006; Sweden. No. significant association of dose and periodontitis.

- Similarly when duration of snuff usage was associated with nuclear atypia the value of p came out significant  $p = 0.000$
- Cytoplasmic vacuolization associated with duration of snuff usage also showed a significant p value of 0.001

- Cytoplasmic vacuolization, nuclear atypia and dysplasia associated with dosage of snuff per day came out significant with p values 0.000, 0.000 and 0.033 respectively.

- Hirsch et. al., 1982; Sweden and Peterson et al, 1983; Denmark, associated dysplasia with duration of snuff usage
- Greer et. al., ( USA ) reported 23 % Atypia and 2 % Dysplasia
- Peterson et. al., ( Danish study ) reported 3% Dysplasia



## INCREASING TRENDS OF CLINICAL LESIONS WITH AGE, DURATION AND DOSAGE OF NASWAR

- White plaque, erythroplakia, xerostomia, tobacco pouch keratosis and gingivitis were found to be an increasing trend in patients who were of age group 2.
- Similarly mucositis, ulceration, white plaque, erythroplakia, xerostomia, periodontitis, gingivitis and tobacco pouch keratosis, all were found to be increasing with the increase in the dose of snuff per day ranging (Group 2 to Group 3)

## INCREASING TRENDS OF CYTOPATHOLOGICAL CHANGES WITH AGE, DOSE AND DURATION OF SNUFF

- Inflammation, nuclear atypia, vacuolization and dysplasia were seen to be more prominent cytopathological features in patients above the age of 30 (Group 2).
- Similarly all the above cytopathological features were seen to be gradually increasing when the dosage per day was increased (Group 2 to Group 3).

# DISCUSSION

Clinical Variables	Total	Studies in Pakistan	International studies
Age	42.5 ± 13.8	39.2 mean age (Zahoor rana et al, 2009)	16 to 24 years (Rolandson et al, 2006; sweden) 13.4 years (offenbacher et al, 1985; Southafrica) 36.5 years (Wickholm, 2003; Switzerland)
Socioeconomic status	Low 97 %	Low (Zahoor rana et al , 2009)	Mostly Low (Frithiof et al, 1983; Sweden) (Christensen et al, 1979; USA)
Mucositis	12 %	--	46% (Robertson, 1990; Canada) 75% (Rolandson et al, 2006; sweden)

Clinical Variables	Total	Studies in Pakistan	International studies
Gingivitis	62 %	--	47% (Monten et al, 2006; Sweden) 12 % (Rolandson et al, 2006; Sweden) Insignificant Offenbacher, 1985; Southafrica
Periodontitis	35 %	--	32% (malagi et al, 2013; India) 19% (rolandson, 2006; Sweden) 36 % (yong chu, 2010; Columbia)
Erythroplakia	73 %	-----	
White lesion	31 %	50% (Zahoor rana et al, 2009)	59% (monten et al, 2006; Sweden) 36% (Malagi, 2013; India)

Cytopathological Variables	Total	Studies in Pakistan	International studies
Inflammation Mild Moderate Severe	42 % 62 % 16 % 22 %	-----	76 % mild 14 % mod 9 % severe (Axel et al, 1976; Sweden) 46% (Robertson, 1990; Canada)
Nuclear atypia	37 %	-----	23% (Greer et al, 1986; USA) 18% (erenmemisoqlu, 2007; Turkey)
Dysplasia	4 % (G1=50 % G2=50 %)	12 % (Zahoor rana, 2009)	1% (Peterson et al, 1973; Denmark) 2.2% (Greer et al, 1986; USA) 4% (G1=75 %. G2=25%) (Erenmemisoqlu, 2007; Turkey)

Cytopathological changes	Total	Studies in Pakistan	International studies
Candida	4 %	--	-----
Cytoplasmic vacuolization	37 %		26 % (Axet et al, 1976; Sweden)
Micronuclei		--	60% (palaskar, 2009; India) 74% (roberts et al, 2014; USA)

## CONCLUSION

- This study is likely to help in screening habitual dipping tobacco users for pathological changes in oral cavity ranging from benign to precancerous and cancerous lesions.
- This study helped in creating awareness among this indigent population regarding oral and dental health in order to provide adequate and timely preventive as well as curative measures by the dental practitioners. As early detection of such mucosal changes will improve the clinical outcome.



- To validate the findings of this study, similar studies on larger scale should be carried out.
- There is a dire need to draft and enforce national policy and regulations for snuff usage and its manufacturing creating provisions of adequately tested material.

Folkman J. Tumor  
angiogenesis. In: Cancer  
Medicine 2000;5th  
ed:13-5.

**Thank You**

