Abstract:

Many factors have been identified as important causative agents that responsible for the development of oral precancerous and cancerous lesions in Sudan.

Toombak have been identified as a major risk factor therefore the aim of this study was to assess the cellular proliferative activity that associated with Toombak use.

In this study 90 healthy individuals were selected for this study of whom76 were Currently Toombak users and 14were non Toombak users.

Result: cytological atypia was identified as:

Per nuclear hallo, Bactria infection, irregular, high N\C ratio, poly morph, keratinization, inflammation and nuclear atypia.

The mean count NORs count was 3.2 in cases and 1.2 in controls.

Conclusion: Toombak is a major risk factor for occurrence of cellular proliferative activity features that may progress in to oral precancerous or cancerous lesions.

الخلاصة:

تم تحديد العديد من العوامل المسببة المهمة والمسؤول عن تطور الاورام السرطانية . في السودان يعتبر التمباك من اكثر المخاطر لسرطان الفم . لذلك تهدف هذه الدراسة الي تقيم النشاط الخلوي لمستخدمي التمباك

في هذه الدراسة م اختيار ٩٠ شخصا منهم ١٤ شخص لا يتعاطون التمباك و ٧٦ شخص يتعاطون التمباك استخدمت طريقتي باب والسلفر لصبغ المسحات الخلوية

تم تحديد متوسط النمطية الخلوية للحالات عينات التي صبغت بال -Ag NORهي٣,٢للحالات ١,٢للضوابط.

1.1Introduction

Exfoliative cytology has been used for decades as simple, dependable and acceptable techniques to support clinical judgment in helping differentiate benign and malignant lesion, many of principles, concepts and technique used in Exfoliative cytology.

Papanicolaou stain is metachromatic staining cytological technique used to differentiate cells in smear preparation of various body secretion.

The incidence rates of oral cancer are 3.7% for men and 2.6% for women in the Sudan [11]. Several lifestyle risk factors for the development of oral cancer are familiar, including tobacco products, alcohol, infections, dietary factors, chemical irritants and frank carcinogens. Prevalence of oral cancer is 3.2% in Sudan and the disease is mainly attributed to N-nitrosamine rich oral snuff consumption [11]. There are mainly 4 smokeless tobacco products: loose leaf or chewing tobacco, snuff, plug tobacco and twist or roll tobacco. Chewing tobacco and snuff are by far the most widely distributed types of smokeless tobacco. Initially, snuff was used for nasal application (sniffing). However, snuff is now habitually used orally by insertion it between lower gum and cheek or lip (dipping) [12,13].The oral use of snuff in North America and Western Europe is causally associated with an increased risk for cancer of the oral cavity and pharynx. Snuff dipping has also been incriminated as being associated with cancer of the nasal cavity, esophagus, pancreas, kidney and urinary bladder [12,14], and other pre-neoplastic changes such as leukoplakia [2,3].

The main goal of this study was to review the studied risk factors that linked to etiology of oral cancer in Sudan.

Toombak is a major risk for occurrence of cellular proliferative activity features that may process in to oral precancerous or cancerous lesions.
Tobacco is mixed with natron or atron is added to the mixture and after period of about 2hour called saffa (idris et al 1995)
Tobacco is one of the major preventable causes of premature death and disease in the world .it is harmful plant to human health.
Tobacco products contain carcinogens which contribute to cancer of the oral cavity and the risk of other head and neck cancers.
Tobacco contain many kinds of heavy metals such as lead and cadmium mercury cobalt magnesium .Toombak has been presented to produce a variety of oral mucosal changes such as dysplasia and hyper keratosis

1:2) Rationale

The aim of this study was to assess the cellular proliferative activity which is the possible induced by the habit of Toombak dipping to enable early calculation carcinogenesis process using Exfoliative cytology as an easy noninvasive diagnostic and screening procedure .

Cancer in developed countries is second most common cause of death

1:3) objective:

3-1) General objective:١:

To: Assessment of Oral cellular proliferative activity among Toombak users in barber city, Sudan 2017

1:3-2)Specific objective:

To compare between AgNOR and pap stain

To detect the oral pre-cancerous or cancerous lesions

To determine the mean *Ag-NOR* among Toombak users.

To compare between mean *Ag-NOR* among Toombak and non Toombak users

To correlate between the mean *Ag-NOR* and the frequency of dipping per day.

To correlate between the mean *Ag-NOR* and the duration .

1:4) Literature review

1:4:1)Definition of Cytology :

Study ofstructure and function of individual cell or small group of cells .

1.4.2) Definition of Exfoliative cytology:

It is the study of cells that have been shed or removed from the epithelial surface of various organs.[1]

1:4:.3) Fixation in cytology:-

The purpose of cytological fixatives is to maintain, as closely as possible, the cytomorphologic characteristics and diagnostically essential cytochemical elements of the cell.

A suitable fixative for cytodiagnostic purposes should perform the functions include Penetrate cells rapidly,Minimize cell shrinkage,Maintain morphology,

Facilitate diffusion of dyes across cell boundaries,Help cells adhere to a glass surface,Provide consistent results over time,Produce a permanent cell record and Stop cellular and microbial growth (anti-microbial).

Historically, Papanicolaou,like Raider before him,used ether and 95% ethanol (1:1) as the cytological fixative of choice. Currently, 95% ethanol, 80% isopropanol, 100% methanol, 95% denatured alcohol, and various commercially available spray fixatives have replaced the ether/ethanol mixture as the fixative of choice for safety reasons.

For laboratories using liquid-based techniques, the type of fixative depends on the method selected. Current liquid-based preparations enhance cellular preservation by using fixative solutions that are either ethanol or methanol-based. Cell alterations may be minimal among the various fixatives in use and may fall into one of five categories: wet

fixation, wet fixation with subsequent air drying, spray fixation, liquid-based fixation, and lysing fixation for bloody samples.[1]

1.1.4.Air Drying for Selected Cell Samples:-

Remains The air-dried sample is stained with methods other than Papanicolaou staining method, such as Wright–Giemsa, Diff- Quik, or May–Grunewald–Giemsa staining procedures. The air dried slide is immersed in a methanol fixative on arrival in the laboratory, where it until it is stained.(D)

1.1.5. Oral cytology:-

Oral Exfoliative cytology is not a substitute for biopsy. Rather, it is a useful although not essential adjunct in the diagnosis of oral surface lesions due to cancer, viral disease, vesiculobullous dermatomes or fungal infection. Al though this painless, a traumatic and simple technique for collecting a sample of superficial cells is used Extensively in the diagnosis of less visible and accessible lesions, such as those in the uterine cervix on the lung, emphasis is here placed on its role in detecting and monitoring premalignant lesions and squamous cell carcinoma of the mouth. [2]

1.1.6) Oral cancer:

It is **cancer** that occurs in any part of the mouth; on the tongue's surface, in the lips, inside the cheek, in the gums, in the roof and floor of the mouth, in the tonsils, and also the salivary gland**.** [3]

Most patients have no detectable symptoms during the early stages of oral cancerSmokers, heavy drinkers should have regular checkups at the dentists' - dentists are often able to identify signs of oral cancer. When signs and symptoms do appear, the typically include Patches on the lining of the mouth or tongue, usually red or red and white in color ,Mouth ulcers that do not go away , A sore that does not heal,A

swelling in the mouth that persists for over three weeks, A lump or thickening of the skin or lining of the mouth ,Pain when swallowing ,Loosening teeth (tooth) for no clear reason ,Dentures don't fit properly , Jaw pain , Jaw stiffness ,Sore throat ,A sensation that something is stuck in your throat ,Painful tongue , A hoarse voice , Pain in the neck that does not go away. [3]

1.1.6.1) The risk factors for mouth cancer:

A risk factor is anything that increases that likelihood of developing a disease or condition. For example, regular smoking increases the risk of developing lung cancer; therefore smoking is a risk factor for lung cancer. The risk factors for mouth cancer include:

Smoking - studies indicate that a 40-per-day smoker has a risk five times great than a lifetime non-smoker of developing oral cancer ,Chewing tobacco , Taking snuff (snorting tobacco).

Both heavy and regular alcohol consumption - somebody who consumes an average of 30 pints of beer per week has a risk five times greater than a teetotaler or somebody who drinks moderately.

Heavy smoking combined with heavy drinking - as tobacco and alcohol have a synergistic effect (their combined effect is greater than each one added together separately), people who drink and also smoke a lot have a significantly higher risk of developing oral cancer compared to others. Somebody who smokes 40 cigarettes per day and consumes an average of 30 pints of beer a week is 38 times more likely to develop oral cancer compared to other people.

Too much sun exposure on the lips, as well as sunlamps or sunbeds.

Diet - people, who consume lots of red meat, processed meat and fried foods are more likely to develop oral cancer than others.

GERD (gastro-esophageal reflux disease) - people with this digestive condition where acid from the stomach leaks back up through the gullet (esophagus) have a higher risk of oral cancer.

HPV (human papillomavirus) infection.

Prior radiation treatment (radiotherapy) in the head and/or neck area.

Regularly chewing betel nuts - these nuts, from the betel palm tree, are popular in some parts of south East Asia. They are slightly addictive and are also carcinogenic. [3]

- Exposure to certain chemicals - especially asbestos, sulphuric acid and formaldehyde. [3]

1.1.6.2) Causes of oral cancer:

Cancer starts when the structure of the DNA (deoxyribonucleic acid) alters - a genetic mutation. DNA provides the cells with a basic set of instructions, much like a computer program for life. The instructions tell cells when to grow, reproduce, and die, among other things. When there is a genetic mutation cells grow in an uncontrollable manner, eventually producing a lump(tumor). If the cancer is left untreated it grows and eventually spreads to other parts of the body, usually through the lymphatic system - a series of nodes (glands) that exist throughout the body. The lymph glands produce many of the cells of our immune system. As soon as the cancer reaches the lymphatic system it can spread anywhere in the body and invade bones, blood and organs. The cancer cells continue reproducing uncontrollably, gradually occupying more and more space. Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division, and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not experience programmatic death and instead continue to grow and divide. This leads

to a mass of abnormal cells that grows out of control. With time, oral cancer may spread firstly to other parts of the mouth, then the head and neck, and eventually to other parts of the body. Mouth cancers typically start in the squamous cells (flat, thin cells) than line the lips and the inside of the mouth - they are referred to assquamous cell carcinomas. [3]

1.1.6.3)Stages of cancer of the lip and oral cavity:

Stages of mouth cancer and lip cancer are indicated using Roman numerals from I to IV, with I being the smallest and IV the largest or most advanced.

- Stage I - the tumor is less than 1 inch in diameter (2 cm) and has not reached nearby lymph nodes. [3]
- Stage II - the tumor is over 1 inch in diameter (2cm) but less than 2 inches (4 cm) and has not reached nearby lymph nodes.
- Stage III - any of the three possibilities below:

The tumor is over 2 inches (4 cm) in diameter.

The tumor has spread to just one nearby lymph node on the same side of the neck as the tumor.

The cancer in the lymph node is no more than 3cm.

- Stage IV - any of the possibilities below:

The cancer has reached tissues around the oral cavity and lip. Nearby lymph nodes may or may not contain cancer.

The cancer has spread to 2 or more lymph nodes on the same side of the neck as the tumor.

The cancer has spread to lymph nodes on the other side of the neck.

Lymph nodes on either side have a tumor that measures over 6cm.

The cancer has spread further, to other parts of the body. [3]

1.2.2. papanicolaou stain :

Is multichromatic staining cytological technique .used to differentiate cells in smear prepration of various body secreation the spacemen can be gynecological smears (pap smears),sputum brush ,washing urine, cerebrospinal fluid ,synovial fluid, abdominal fluid, seminal fluid and fine needle spiration.pap staining is reliable technique and use for cervical cancer screening.[3]

1.2.3. papanicolaou stain for cytological preparations:

The universal stain for cytological preparations is the Papanicolaou stain. Harris's hematoxylin is the pink hues to the cell cytoplasm result, however,should retain the transparent quality of the cytoplasmicstain, and the nuclear chromatin should beeasily
distinguished. [1]

1.2.4 Component of papanicolaou:

1.2.4.1Eosin:

Eosin is the most suitable stain to combine with an alum hematoxylin to demonstrate the general histological architecture of a tissue. Its particular value is its ability, with proper differentiation, to distinguish between the cytoplasm of different types of cell, and between the different types of connective tissue fibers and matrices, by staining them differing shades of red and pink.

We used eosin Y is much the most widely used, and despite its synonym it is also satisfactorily soluble in alcohol; it is sometimes sold as 'water and alcohol soluble'. As a cytoplasmic stain, it is usually used as a 0.5 or 1.0% solution in distilled water, with a crystal of thymol added to inhibit the growth of fungi. The addition of a little acetic acid (0.5 ml to 1000 ml stain) is said to sharpen the staining.

1.2.4.2Hematoxylin:

Hematoxylin is natural dye extracted from the heartwood (‘logwood’) of the tree *Hematoxylon campechianum* that originated in the Mexican State of Campeche, but which is now mainly cultivated in the West Indies. The hematoxylin is extracted from logwood with hot water, and then precipitated out from the aqueous solution using urea (see prior editions).

Hematoxylin itself is not a stain. The major oxidization product is hematein, a natural dye that is responsible for the color properties. Hematein can be produced from hematoxylin in two ways. (1)

Natural oxidation (‘ripening’) by exposure to light and air

This is a slow process, sometimes taking as long as 3–4 months, but the resultant solution seems to retain its staining ability for a long time. Ehrlich’s and Delafield’s hematoxylin solutions are examples of naturally ripened hematoxylins. (1)

Harris’s hematoxylin:

This alum hematoxylin was traditionally chemically ripened with mercuric oxide. As mercuric oxide is highly toxic, environmentally unfriendly, and has detrimental and corrosive long-term effects on some automated staining machines, sodium or potassium iodate is frequently used as a substitute for oxidation. Harris is a useful general-purpose hematoxylin and gives particularly clear nuclear staining, and for this reason has been used, as a progressive stain, in diagnostic exfoliative cytology. In routine histological practice, it is generally used regressively, but can be useful when used progressively. When using Harris’s hematoxylin as a progressive stain, an acetic acid-alcohol rinse provides a more controllable method in removing excess stain from tissue components and the glass slide. The traditional hydrochloric acid-alcohol acts quickly and indiscriminately, is more difficult to

control, and can result in a light nuclear stain. A 5–10% solution of acetic acid, in 70–95% alcohol, detaches dye molecules from the cytoplasm/nucleoplasm while keeping nucleic acid complexes intact. [4]

2.2.4.3.orang G:-

Orange G is an acidic dye.43 It stains keratin a bright, intense orange. The granules in eosinophilic, superficial cells (possibly those containing eleidin) are also stained. Because keratin, not normally present in squamous epithelial cells, may be found in the presence of keratinizing squamous cancers, the presence of intense orangeophilia is important to the diagnostician. In the modified OG formula, the dye content is quantitatively controlled. OG is slightly soluble in 95% ethanol and even more soluble in water. In the modified formulas, the OG content is reduced to a level of its solubility in 95% ethanol.

When glacial acetic acid is added to the formula, the solution stains rapidly and intensely. Positive hydrogen ions are added to the amino acids of cellular proteins, shifting the balance of charges to the acidic side of the proteins' isolectric point and thus increasing the bonding sites to which the negatively charged OG can attach. The staining time must be limited or the subsequent uptake of eosin Y is inhibited. The addition of phosphotungstic acid, a mordant that strongly binds to proteins, helps intensify the color achieved [1]

2 Nuclear organization regions:

The nucleolus organizer region (*NOR*) or nucleolar organizer is a chromosomal region around which the nucleolus forms. This region is the particular part of a chromosome that is associated with a nucleolus after the nucleusdivides. The region contains several tandem copies of ribosomal *DNA* genes. In humans, the *NOR* contains genes for 5.8S, 18S, and 28S *rRNA*clustered on the short arms of chromosomes 13, 14, 15, 21 and 22 (the *acrocentric* chromosomes).Nucleolus organizer

regions (*NORs*) are head-to-tail arrays of genes encoding the precursor of the three largest ribosomal *RNAs* (18S, 5.8S and 25S in plants). *NORs* include active *rRNA* genes, which give rise to secondary constrictions of metaphase chromosomes, and silent *rRNA* genes, which are often highly compacted in dense heterochromatin. At metaphase, a proteinaceous remnant of the nucleolus often remains associated with the secondary constriction. Each*rRNA* gene at a *NOR* is nearly identical in sequence, although variation in size due to differences in the number of repeated *DNA* elements in the intergenic spacer region is common.[5]In complete chromosome complements there were always 6 chromosomes with a terminal Nucleolus organizing region (*NOR*). In most cases part of the *NOR* was decondensed, and several of this decondensed areas formed together a big collecting nucleolus (ger.Sammlenukleolus). This collecting nucleolus was easily visible in phase contrast even without pretreatment due to its large size and particular structure. The shape of the collecting nucleolus ranged from nearly circular to irregular. Instead of just one collecting nucleolus of 6 *NOR* there were also several smaller collecting nucleoli made up from the *NORs* of only 2 to 5 nucleolus organizing chromosomes. Evaluation of nucleolar organizer region-associated proteins in breast malignancy. Nucleolus organizer regions (*NORs)* have been identified by means of an argyrophilic technique (*Ag-NOR*) in routinely processed. This method reveals *NORs* as black dots in the nuclei of cells, by virtue of the argyrophilia of *NOR*-associated proteins. The number of *Ag-NORs* has been thought to be related to cellular activation and has recently been applied to non-Hodgkin’s lymphomas and melanocytic skin lesions. It was found in the present study that the total number of *Ag-NORs* in malignant breast lesions significantly exceeded those of normal breast and benign lesions.

The number of clumps of *Ag-NORs*, however, were not useful discriminators. Neither numbers of total *Ag-NORs*nor of clumps of *Ag-NORs* correlate with mitotic counts and it may be that their numbers relate to ploidy. It is suggested that the *Ag-NOR* technique ill find increasing application as an adjunct to diagnostic histopathology. In karyotype analysis, a silver stain can be used to identify the *NOR*.[6][7]Silver nitrate inserts into the *NOR*-associated protein in the stalks and satellites, staining the proteins dark black. The amount of rstain deposited and the number of *NORs* differs among the population, although the cell should normally have a maximum of 10 *NORs* per cell.Several genes are located at the *NOR*, including *RUNX2*,[8]*UBTF*,[9] and *APC*[10] genes.

2)Material and Methods

2.1)Study area:

The study will be conducted Barber town in rival Nile state in Sudan during period between April 2017 to Agust2017

2-2)Study design:

This is cross sectional descriptive study, will be conducted in period from April to July to detect of oral cellular proliferative activity among toombak

2:3) Study population:

User toombak for 4year

User toombak 7year

User toombak more than 10

2:4)Data collection tools:

Data was collected by using questionnaires and laboratory base results.

2:5) Sampling and processing:

The study included smears that obtained from participant s using a Slids. The obtained material were prepared in clean grease free frosted glass slide. All smear were immediately fixed while they were wet in 95% ethyl alcohol for 15 minutes.

2:6)Inclusion and exclusion criteria

Include Toombak user, smoker and exclude drugs, Alcoholism

2:7 Sample size

180 smear samples collected from oral mucosa [Toombakdeposits] to screen and conforming the proliferative of cellular the age of all samples up to 18 years

Sample technique:

The study based on convenience sampling during attendance of Toombak users and non Toombak users in Berber city.

2:9 Data Analysis:

The gathered data is analyzed with SPSS (statistical package of social science) , The test used for calculating , degree of variation,
Personal and clinical data is collected by direct interviewing questionnaire from each subject .

2:10)Ethical consideration:

Permission to carry out study is obtained from the College of health Science Sheikh Abdullah Elbadri University . All samples examined are informed for the purpose of the study before collection of the samples and verbal consent taken from them .

2:11)Methodology:-

2:11:2) method:

Papanicolaou formula:

Harris's hematoxylin

Orange G 6

10% aqueous Orange G 50 ml

Alcohol 950 ml

Phosphotungstic acid 0–15 g

EA 50

0.04 M light green SF 10 ml

0.3 M eosin Y 20 ml

Phosphotungstic acid 2 g

Alcohol 750 ml

Methanol 250 ml

Glacial acetic acid 20 ml

Filter all stain.

Papanicolaou staining method:-

Hydrate in 95% alcohol, 2 minutes, and 70%alcohol, 2 minutes, Rinse in water, 1 minute ,Stain in Harris's hematoxylin, 8 minutes , Rinse in water, 2 minutes , Differentiate in 0.1% acid alcohol 10 seconds approx , Rinse in water, 2 minutes , 'Blue' in Scott's tap water substitute, 2 minutes , Rinse in water, 2 minutes , Dehydrate, 70% alcohol for 2 minutes , Dehydrate, 95% alcohol, 2 minutes , . Dehydrate, 95% alcohol, 2 minutes , Stain in OG 6, 2 minutes , Rinse in 95% alcohol, 2 minutes , . Rinse in 95% alcohol, 2 minutes , Stain in EA 50, 3 minutes , Rinse in 95% alcohol, 1 minute .

2:11:2:2)AgNOR staining =POUYTRTmethod:

The smears will be stained by AgNOR staining method working solution will be freshly prepared

By mixing one volume of 2% gelatin in 1% formic acid solution and two volumes silver nitrate solution

All smears will be treated with silver stain to detect AgNOR.

Ag- NORs method:

All sample were done following the below protocol ,hydrate in 95% alcohol for 2 min , hydrate in 80% alcohol for 2 min , hydrate in 70%alcohol for 2 min ,Rinse in water for 2 min , stain in working solution of silver nitrate 45 min , Rinse in water for 1 min , Dehydrate 70 % alcohol , Dehydrate in 80% alcohol , Dehydrate in 95% alcohol , Dehydrate in Abs alcohol clear and mount

Results : NOR were counted as a black dot inside the nucleus by using time 100X

Result

Distribution of age by cases and control :

age	controls	Cases	Total
15-25	13	28	41
26-35	1	1	2
36-45	-	1	1
46-55	-	1	1
More than 56	-	1	1
Total	14	76	90

Table (1): The Correlations between the period and Ag- Nor:

Correlations			
		Ag- Nor	period
Ag- Nor	Pearson Correlation	1	0.272**
	Sig. (2-tailed)		0.010
	N	90	90
Period	Pearson Correlation	.272**	1
	Sig. (2-tailed)	.010	
	N	90	90
**. Correlation is significant at the 0.01 level (2-tailed).			

The table and the figure above showing the relationship between Ag-Nor and Period have a statistically significant relationship with a value of 0.000 which .is below the level of 0.05.

Table (2): The Correlations between the use/day and Ag- Nor:

Correlations			
		Ag- Nor	use/day
Ag- Nor	Pearson Correlation	1	.245*
	Sig. (2-tailed)		.020
	N	90	90
use/day	Pearson Correlation	.245*	1
	Sig. (2-tailed)	.020	
	N	90	90
*. Correlation is significant at the 0.05 level (2-tailed).			

The cases that staining with pap stain are:

56% pernuclear hallow

17% bactriainfaction

2% irregular

2% high ratio

2% pol keratosis

77%keratosis

65% inflammation

17% Nucler atypia

Dissection

More than 95% of the carcinomas of the oral cavity are of squamous cell type; in nature they constitute major health problem in developing countries, representing leading cause of death. The survival index continues to be small (50%), as compared to the progress in diagnosis and treatment of the other malignant tumors (Mehrotra and Yadav2006). In the Sudan, oral cancer is one of the major health problems, due to the habit of Toombak using (Idriset al. 1995). These facts explain the findings of the current study, particularly, the significant (P <0.0001) variation in the meanNORscount(3.1 among cases compared to 1.2 in controls). NORs have been shown to be the site of rDNAwhich are transcribed to rRNA.They can routinely highlight by virtueofargyrophilia of their associated proteins(Derenziniet al. 1998). It has been reported that, particularly human somatic cells could contain 10 demonstrableNORsin nuclei, but many resting cells contain only one particle(Crocker and Egan 1988). Due to increased proliferative activity of neoplastic cells, higher number of NOR particles in cancerous cells might be expected (Boldyet al. 1989).This in addition to the presence of are asonable of cytological atypia among cases compared to controls. These findings incriminate the role of Toombak use as a risk for development of cellular proliferative activity which may progress to oral cancer. In the Sudan, snuff, locally known asToombak, it is wide-spread in the country. The results of this study agreed with the study by (Ahmed and Mahgoub, 2007), who found that, the risk among Toombak user was high. Furthermore, study by (Idriset al.1994).

Recommendation

From this study AgNOR dye is better than pap stain in detecting cellular changes

And the period of using interaction has a greater impact than using\day.

Toombak is harm full of oral cell And the continuous of using can be development to precancerous and oral cancerous

And the period of using directly proportional with the proliferative of cell .

We recommend by don’t use the Toombak and user must reducing the dose.

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Aecessories:

Slide,bruchsteeth,coverglass,microscope, DPX,AgNOR stain and papanicolaou stain

Age

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Gender……

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Using

Day……

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Period

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Other……

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