

# **An Investigation of the Oral Mucosal and Periodontal diseases with Exfoliative Cytology in Tonga**

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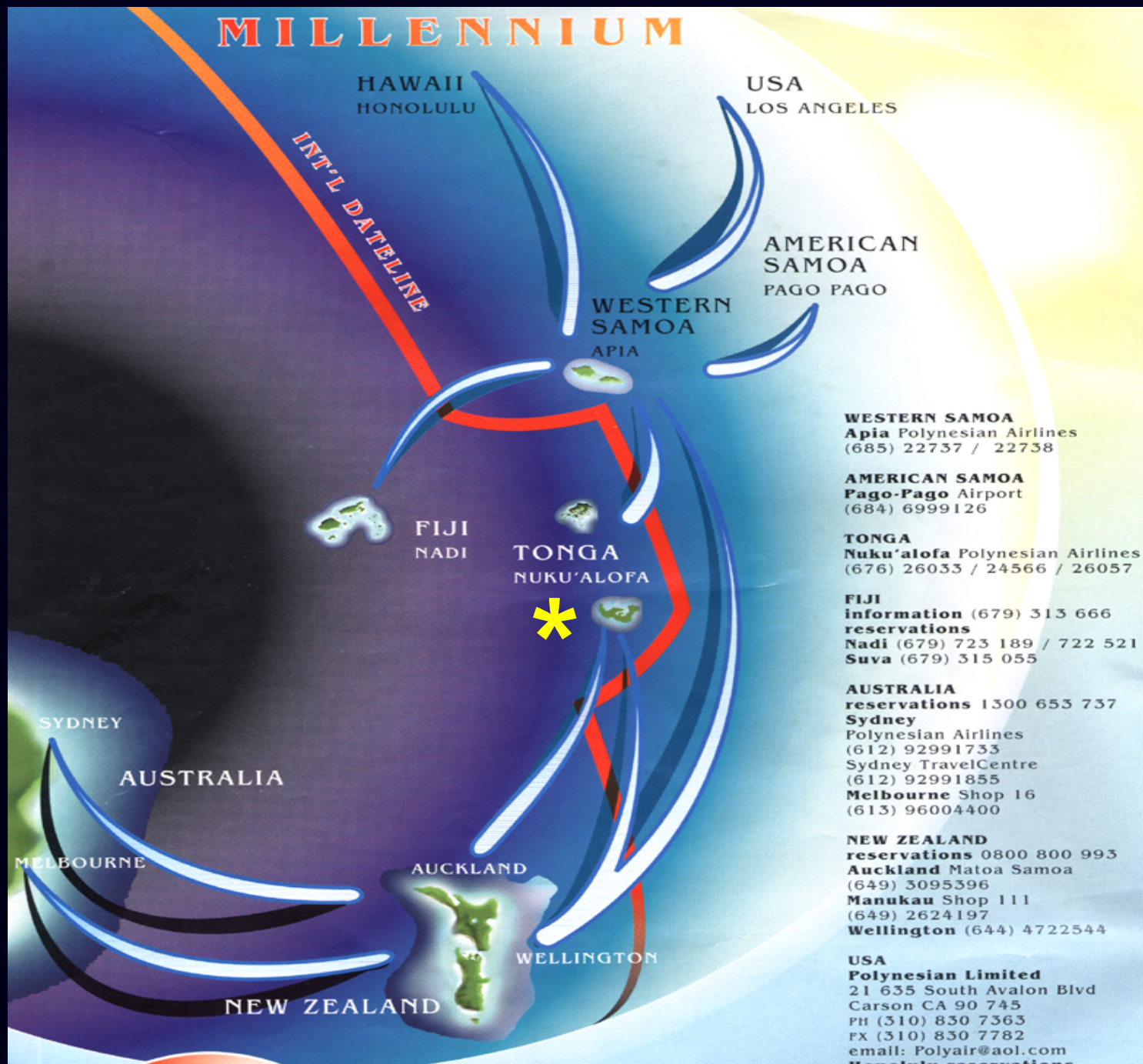
# Objective

The diagnosis of a mucosal lesion cannot be based solely on clinical findings. So far, there is no reliable method applicable to the oral cavity that can replace a biopsy, but some may be used as a supplement. Cultures are considered the golden standard in microbiological evaluation of dental diseases but they are expensive and time consuming to perform especially in third world countries. Cytological investigations of the mucosal problems in Tonga have not been previously reported. Therefore, the purpose of this study was to assess the oral mucosal condition in Tonga, and to estimate the method “exfoliative cytology”. We will use this study’s finding to update the sparse information on oral mucosal lesion in Tonga and as an additional aid to formulating additional strategies to enhance better preventive oral health plan.

# Introduction

**Tonga**, a South Pacific Polynesian island, a member of the Commonwealth of Nations, and with a population of about 104,000, consists of 171 islands (Fig.1). For the past 40 years or more, **Tonga** had had a low caries rate but a high prevalence of periodontal diseases, and other mucosal conditions were relatively low. It was reported that the prevalence of microbial plaque, calculus and gingival inflammation was very high in all subjects at all ages particularly over 30 years in **Tonga**. A recent survey has shown that periodontal disease remains a concern in the adult population, and caries is gradually increasing among children. In **Tonga**, periodontal abscess related to periodontitis is also a common clinical finding, but all reports were done by macroscopic observation and no objective method including assessment of distribution of microflora had been used.





Where is  
Tonga?  
† Tonga is  
in the South  
Pacific  
Ocean, a  
Polynesian  
island near  
Fiji, Samoa  
and NZ †.

Fig.1



# Materials and methods

## **1. Subjects**

The study was conducted at Vaiola Hospital (main island), where 70 % of the population lives, during 2001 to 2003, with clinical evidence of mucosal (minor) and periodontal (major) lesions identified during clinical examination. The clinical oral health status was briefly assessed prior to the delivery of cytological investigation, and was expressed as satisfactory (good) or unsatisfactory (poor), depending on the clinical findings. A mouth with a lot of debris, calculus and gingival inflammation was considered unsatisfactory.

## **2. The characteristics of periodontitis, gingivitis and mucosal lesions**

The periodontal check points; 1) periodontal pocket over 5 mm, 2) plaque deposition, 3) existence of subgingival calculus, and 4) drainage from the pocket. The site chosen for smearing was severely involved and was performed on the basis of the following check points. One ~ four were confirmed as for periodontitis. Among the six points of pocket surroundings (WHO probe), the deepest one was selected for smearing.

The characteristics of gingivitis were the present or absent of gingival swelling without attachment loss and bleeding after probing. Oral mucosal lesions were assessed as any visible lesions that included; pain, swelling, redness or whiteness, nodular and rough surface were observed by an ocular inspection and palpation.

### **3. Exfoliative cytology**

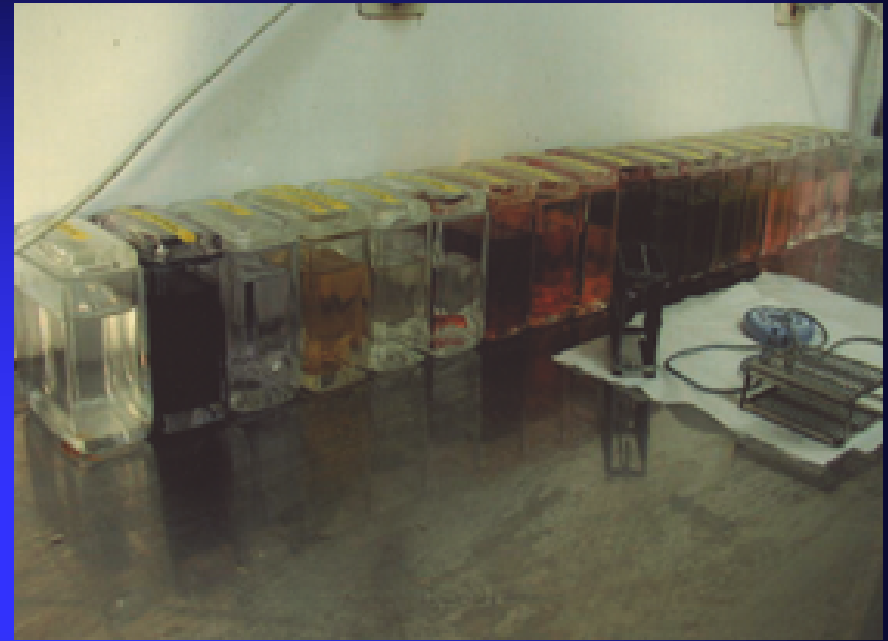
In all cases, the cells were exfoliated using Cytobrush (Teikokuzokiseiyaku K.K., Japan) as shown in Fig. 2 from the pocket epithelium (77.5 %) or the supragingival alveolar mucosa (22.5 %). The diameter of the cytobrush head was 6mm and its length without the handle was 2 cm (marked as 0.5, 1.0, 1.5 cm) from the tip. In order to collect subgingival smears, the head of the brush was introduced through the sulcus or pocket orifice as far apically as possible, then the size was approximated according to the WHO probe measurement. If it was impossible, supragingival mucosa was selected. The collected cells were smeared on labeled glass slides.

### **4. Papanicolaou's staining**

Two glass slides were made for each case. All cytological smears were immediately fixed in 95 % ethyl alcohol, and stained by the Papanicolaou method (Fig. 3). On the basis of the cytomorphological characteristics, the presence of microorganisms, debris, inflammatory cells and changes of squamous cells were microscopically analyzed by a cytologist and 2 dentists. The microorganisms were identified according to their Papanicolaou's characteristic morphology.



**Fig. 2: The demonstration of using the cytobrush in the oral cavity of a patient with a deep periodontal pocket (pocket depth=greater than 5 mm)**



**Fig. 3: The demonstration of sets of alcohol and xylene use in Papanicolau staining.**



# Results

## **1. Clinical findings**

Hundred and twenty patients (62 males and 58 females), ranging from 3 to 76 years old with a mean age of  $32.7 \pm 20.9$  years (Fig. 4). Regarding the patient's chief complaints; sore teeth and swollen gingiva were frequent and clinically found in all cases. The locations of cytological areas in the oral cavity are outlined in Table 1. Clinical diagnosis was summarized in Table 2. Overall, 73 cases (60.8 %) were diagnosed with acute periodontal abscesses arising from periodontitis, caries and pericoronitis, and 48.0 % of these (35 cases) had mild extra oral swelling (Fig. 5).

## **2. Children between the age of 3 and 14 years**

In this study, 38 children (23 females and 15 males) were participated and their age ranged from 3 to 14 years. The mean age was  $8.5 \pm 3.5$  years. Sixteen of the 38 children were identified as having one periodontal abscess related to the carious tooth involved while 18 were identified as having one or more periodontal abscesses related to many carious teeth, but no detection of any bony destruction. Among children, 13 presented with mild facial swelling. The percentage of children ( $n=35$ ) showing oral debris was 92.1 % and plaque was observed in almost all these children. In contrast, the percentages of children showing calculus with caries ( $n=6$ ), and gingivitis ( $n=4$ ) were low at 15.8 % and 6.0 % respectively.

### **3. Patients with gingivitis**

Twenty adults (16.7 %) had mild to moderate gingivitis, bleeding on probing and had fairly plaque accumulation. Regarding the distribution in the jaws, 8 (40 %) were found in the lower jaw, and 12 (60 %) in the maxillary region. No gingival abscesses were found (Fig. 6).

### **4. Patients with periodontitis**

Abscess formation was readily identified clinically in these patients due to concurrent signs such as redness, edema and swelling. Concerning about pocket depth, there were more cases with greater than 5 mm (65.0 %) than less than 5 mm (12.5 %). The mean smear pocket depth was 7 mm, ranging from 2 mm to 11 mm. When the abscess condition was linked to the patients periodontal condition, 22 (18.3 %) were diagnosed suffering from moderate to severe periodontitis and were also untreated, and 12 of them showed mild facial swelling of the affected side (Fig. 7).

### **5. Patients with pericoronitis**

Other affected teeth were the lower third molars of adult patients (8.3 %). They had localized gingival swelling, and pericoronal abscess developed around the crown of partially overlaid (n=5) and the malposed (n=5) wisdom tooth. All the patients with mild to moderate facial swelling had surgical intervention and amoxicillin was the principal antibiotic.

## 6. Cytological findings

Light microscopical findings showed the abundance of microorganisms that resembled *Actinomyces* and *Entamoeba* surrounded by varying amounts of debris based on the cytological characteristic morphology (60.8 %). Of this 60.8 %, 28.3 % were children. In addition, the smears were infiltrated mainly with neutrophils and lymphocytes in all cases, and to a lesser extent with macrophages (Fig. 8a).

The smears taken from one of deepest periodontal pockets identified the large number of *Actinomyces* associated with *Entamoeba* like microorganisms. At higher magnification, the *Actinomyces* like microorganisms exhibited fine filamentous structures (Fig. 8b), and the majority of the *Entamoeba* like microorganisms contained leukocytes within their digestive vacuoles and other debris could be observed within the cytoplasm, and nuclei were eccentrically located (Fig. 8c). Smears taken from inflamed alveolar mucosa of gingivitis cases and from superficial pockets also revealed large bacterial mass of *Actinomyces* and debris but unable to find *Entamoeba* like microorganisms.



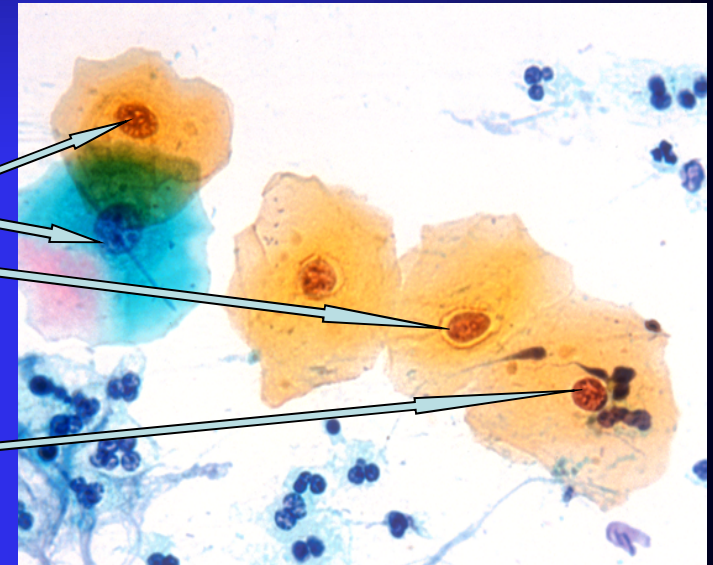
## 7. Changes in squamous epithelial cells

Cytological characteristics of epithelial cells were summarized in Table 3. The cells showed reactive changes due to inflammation. Nuclear swelling of squamous epithelial cells was frequently visible. Other cytomorphological changes were observed in the squamous cells such as: perinuclear halo, and pyknotic chromatin.

Nuclear swelling

Halo

Pyknosis



## 8. Clinical and cytological findings

Concerning patients associated with microorganisms resemble to *Actinomyces* and *Entamoeba* (45.3 %), they were mainly observed in periodontitis and carious patients with 30.0 % and 23.1 %, respectively. Characteristics of *Actinomyces* and *Entamoeba* like microorganisms were identified in 60.8 % cases. Clinically, these patients had unsatisfactory and poor dental hygiene.

# Table 1 Location of cytological smears

Locations	No. of cases (%)
Pocket depth (mucosa) > 5 mm on the brush marking* Mean pocket depth~7mm	78 (65.0)
Pocket depth (mucosa) < 5 mm on the brush marking** Mean pocket depth~3mm	15 (12.5)
Inflamed alveolar gingival surface	24 (20.0)
Leukoplakia (buccal mucosa)	3 (2.5)

\*: periodontitis with no pocket abscess: n=20

: periodontal abscess associated to periodontitis: n=22

multiple caries: n=14

single caries: n=17

pericoronitis: n=5

\*\* : periodontal abscess associated to multiple decay: n=4, single decay: n=6,  
pericoronitis: n=5

## Table 2: Clinical diagnosis and cytological findings

Clinical diagnosis	Total No.	Mean Age (yrs)	% *	Swelling case	% **	Act+Enta .	
				No.		No ***	% *
Periodontitis with no pocket abscess	20	50.9	16.7	0	0.0	19	15.8
Periodontal abscess associated to							
Periodontitis	22	59.8	18.3	12	54.5	19	15.8
Dental caries							
Multiple decay <sup>a</sup>	18	8.0	15.0	6	33.3	12	10.0
Single decay <sup>b</sup>	23	10.0	19.2	7	30.4	18	15.0
Pericoronitis	10	21.7	8.3	10	100.0	5	4.2
Marginal gingivitis <sup>c</sup>	24	27.9	20.0	0	0.0	0	0.0
Leukoplakia	3	42.0	2.5	0	0.0	0	0.0
<b>Total</b>	<b>120</b>		<b>100.0</b>	<b>35</b>		<b>73</b>	<b>60.8</b>

%\*: Percentage of number/all cases (120)

%\*\*: Percentage of number/total cases

Act.+ Enta\*\*\*.No.:Number of cases with *Actinomyces* + *Entamoeba* like microorganisms

<sup>a</sup>.: all children

<sup>b</sup>.: 16 children, 7 adults

<sup>c</sup>.: 4 children , 20 adults

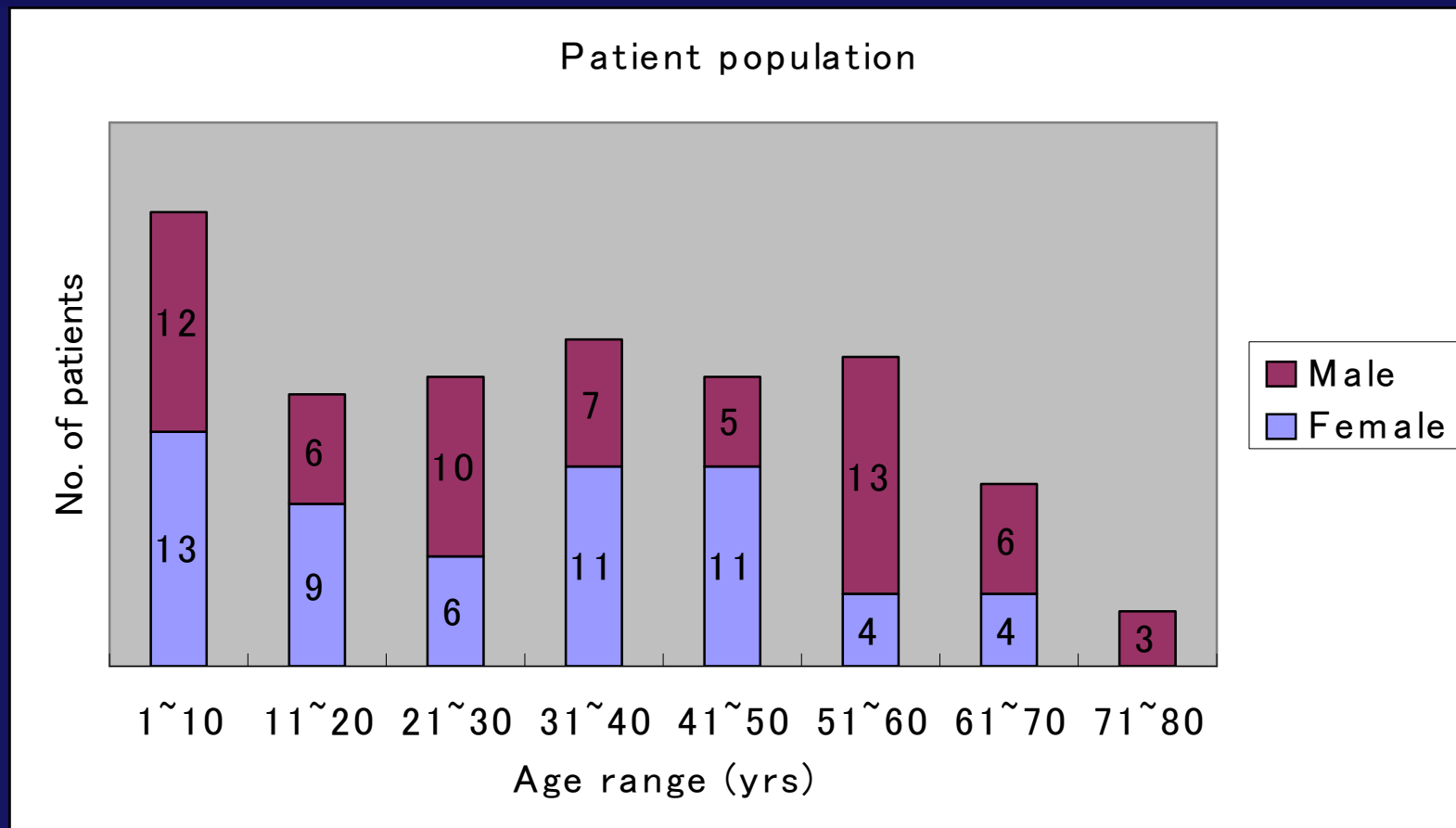


**Table 3 : Summary of cytological characteristics**

<b>Characteristics</b>	<b>No. of cases(%)</b>			
	<b>Severe</b>	<b>Moderate</b>	<b>Mild</b>	<b>Negative</b>
<b>Nuclear Swelling (NS)</b>	-	112 (93.3)	8 (6.67)	-
<b>Pyknosis (P)</b>	-	100 (83.3)	20 (16.7)	-
<b>Halo (H)</b>	-	90 (75.0)	30 (25.0)	-
<b>Karyorrhexis (K)</b>	-	75 (62.5)	45 (37.5)	-
<b>N/C ratio</b>	-	110 (91.7)	10 (8.3)	-
<b>Multinucleation (M)</b>	Presence in small amounts			
<b>Vacuolation (V)</b>	Presence in small amounts			
<b>Have all*</b>				
<b>(NS,P,H,K,N/C,M,V)</b>	120 (100%)			
<b>Cell size and shape</b>	Variable within physiological limits			

\*: all cases showed at least all characteristics

: leukoplakia cases mainly showed P with lesser degree of other characteristics



Mean age=32.7, SD=20.8

**Fig. 4: Characteristics of total patient population**

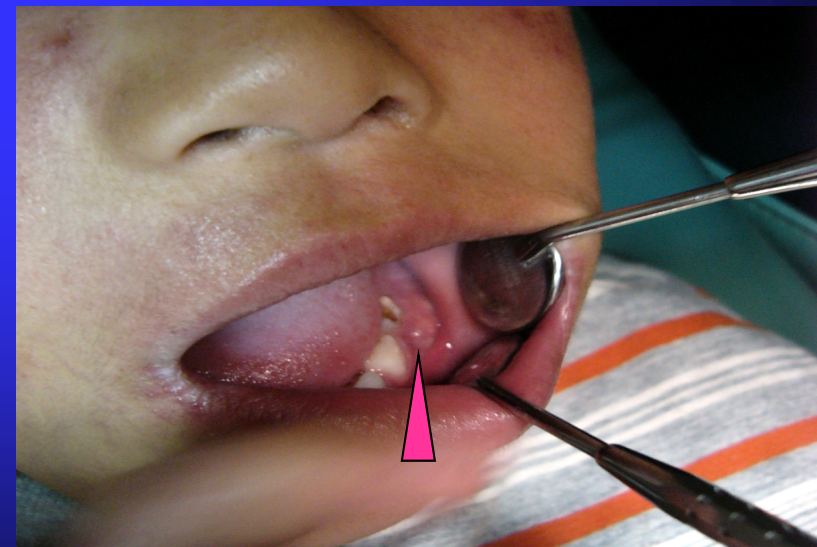


Fig. 5: Extra- and intra- oral swellings associated with dental caries (arrowheads).





**Tongan dentists at work**



**Fig. 6: Gingivitis and calculus**



**Fig. 7: Periodontitis**



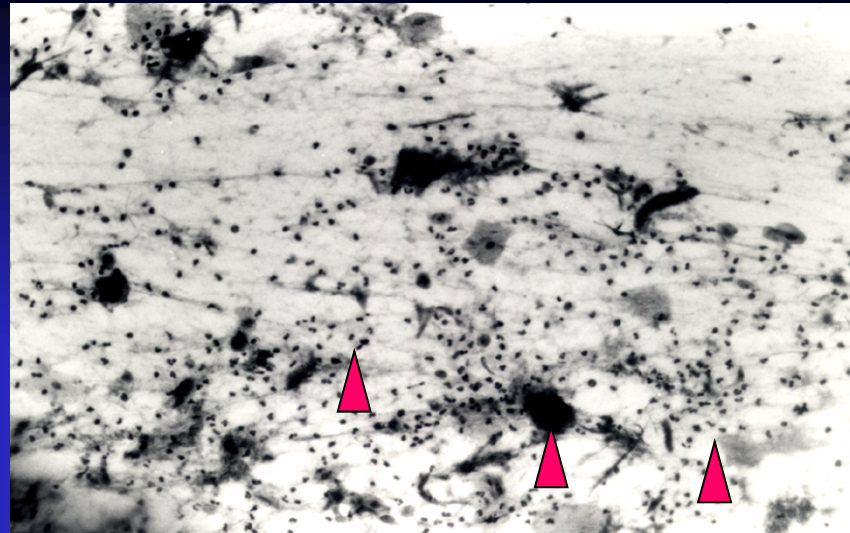


Fig. 8 (a): Papanicolaou stained oral smear with the background consisting of many inflammatory cells and debris (arrowheads) ( $\times 100$ ).

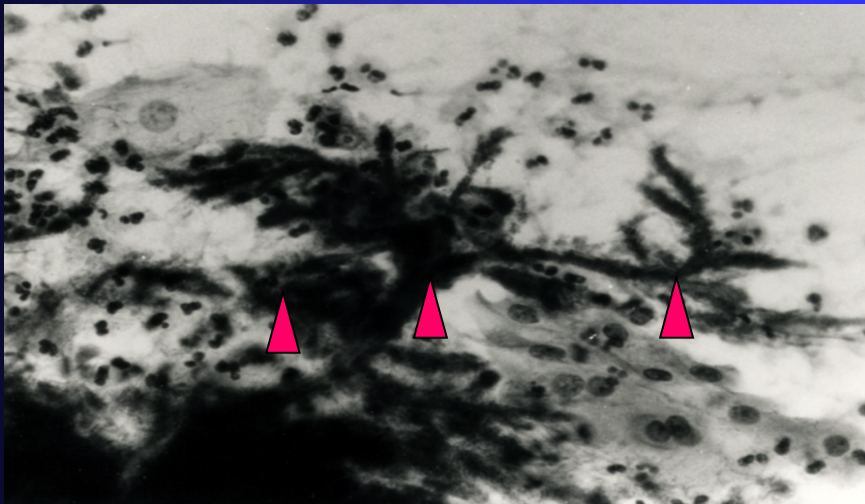


Fig. 8 (b): Well-formed branching of *Actinomyces* like microorganism (arrows) with some inflammatory cells at the periphery ( $\times 1000$ ).

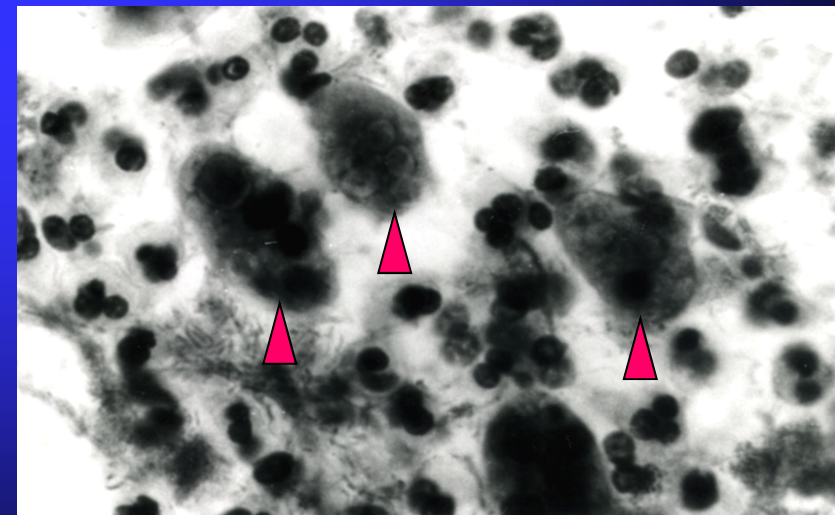


Fig. 8 (c): *Entamoeba* like microorganism (arrows) with digestive vacuoles containing leukocytes ( $\times 1000$ ).

# Discussion and conclusion

## **Our study showed that:**

- \* The clinicocytological results will provide knowledge of the periodontal diseases and oral mucosal lesions in Tonga.
- \* The clinical findings showed that poor oral hygiene was a main feature.
- \* That periodontal abscesses have a high prevalence in periodontitis patients and stems from pre-existing periodontal pocket (60.8 %).
- \* In younger population, periodontal abscesses developed in the absence of periodontitis due to impaction of foreign bodies as observed in dental caries and acute pericoronitis.
- \* In adult population, abscesses occurred as an acute exacerbation of an untreated periodontitis.
- \* The method highlighted the presence of large numbers of oral microorganisms in patients with poor oral conditions ( *Actinomyces* and *Entamoeba* like microorganisms) and debris in the smears, giving the background the dirty appearance.
- \* Only inflammatory atypia was found in the gingival mucosa. Polymorpholeukocytes was observed in all clinically inflamed cases, and was clearly suggestive of the hosts immediate response to infection.

- \* Exfoliative oral cytology might be effective assessment method in developing countries as a simple and less expensive examination for the screening of oral mucosal and periodontal diseases.
- \* It is worth integrating into the oral health programme planning, not only for further descriptive purposes, but also to enable the dental professional to tailor practical oral health instruction.
- \* Ascertaining the bacterial population using this simple technique may signal an important opportunity for dentists to improve their understanding of the nature of oral mucosal and periodontal diseases whereupon further preventive plans, reassurances and advice could be formulated.
- \* Since, the findings appear to be promoted by poor oral health, primary prevention should be emphasized through hygiene educational programmes ideally introduced in childhood.
- \* Because this is a pioneering study, further oral mucosal and periodontal studies and investigations in Tonga based on it, are needed, especially to assess and review the oral microflora and the health of the periodontal tissues using this simple method. Hence, worsening of the mucosal problems may be prevented.
- \* The study showed the clinical diagnoses were correctly matched with the cytological diagnoses. Only the presence of oral microorganisms, inflammatory cells and debris in varying quantities were revealed.