ORAL LEUKOPLAKIA IN A SOUTH AFRICAN SAMPLE: A CLINICOPATHOLOGICAL STUDY

Rakesh Chandran

A research report submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry in the branch of Oral Pathology.

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DECLARATION

I, Rakesh Chandran declare that this report is my own work. It is being submitted for the degree of Master of Science in Dentistry in the branch of Oral pathology to the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature of Candidate
Date: 25 October 2012

DEDICATION

This thesis is dedicated to my parents for their belief in me, to my wife for her unconditional love and support and to my daughter.

ABSTRACT

Oral leukoplakia is the most common potentially malignant oral lesion. While the clinicopathological features in white patients are well characterised, this is not the case in black people. The aim of this study is to analyse the differences in the clinicopathological features of oral leukoplakia in different racial groups in the greater Johannesburg area of South Africa, with special emphasis on the black population. Only 14% of oral leukoplakia occurred in black persons compared to 80% in white persons. In contrast to white persons, black persons were diagnosed with oral leukoplakia at a younger age; there were more males affected than females; and the proportion of idiopathic leukoplakias was greater. There were significantly more black people (23%) with non-homogenous leukoplakia oral leukoplakia than white people with non-homogenous leukoplakia (13%), but there were significantly more white people (51%) than black people (23%) with dysplastic oral leukoplakia; and while in white people the floor of the mouth was the most frequently affected site, in black people it was the buccal mucosa. This study provides important differences in the clinicopathological features of oral leukoplakia between black persons and white persons.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

1. INTRODUCTION

Oral leukoplakia, the most common potentially malignant lesion of the oral cavity, is a white plaque that cannot be characterised clinically or histopathologically as any other specific entity and is not associated with any aetiological factor other than tobacco/areca nut use (1).

Older age, certain anatomical sites, high-grade epithelial dysplasia and lesional keratinocytes carrying DNA aneuploidy and/or loss of heterozygosity of certain chromosomal loci, are factors associated with a heightened risk of malignant transformation of leukoplakic lesions (2-4).

Based on the clinical appearance, oral leukoplakia can be classified into two main types, homogenous and non-homogenous. Non-homogenous leukoplakia can be further sub-classified into erythroleukoplakia, nodular and verrucous forms (1). Both homogenous leukoplakia and non-homogenous leukoplakia can clinically present as a single lesion or multiple lesions. Multiple lesions may affect a single oral-site or concurrently different oral sites.

Between 70% - 90% of oral leukoplakias are associated with tobacco use, and there is a close relationship between the amount and duration of cigarette, pipe and cigar smoking and the frequency of oral leukoplakia (5). Upon cessation of the habit, tobacco smoking-related oral leukoplakia may regress or resolve. Although there is a clear association between tobacco smoking and the development of oral leukoplakia and similarly between tobacco smoking and oral squamous cell carcinoma (SCC), tobacco smoking seems to be a poor predictor for the progression of oral leukoplakia to oral SCC (2).

The estimated prevalence of oral leukoplakia world-wide ranges from 0.5% to 3.46% (6). About 90% of all oral leukoplakia are tobacco related and the remainder are idiopathic (2). Oral leukoplakia more often affects males than females, is uncommon before middle-age and the prevalence is found to increase with age (5). With decreasing order of frequency, oral leukoplakia affects the buccal mucosa, the lower gingiva, the tongue and the floor of the mouth (2).

The overall risk of malignant transformation of oral leukoplakia is about 2%. Between 0.6% and 5% of homogenous leukoplakia and between 20-25% of non-homogenous leukoplakia unpredictably undergo malignant transformation (2, 7) and it is estimated that between 17% and 35% of oral SCC arise from pre-existing oral leukoplakia. The remaining oral SCC arise *de novo* from apparently normal oral epithelium (6).

It is becoming increasingly evident that most oral leukoplakias arise within precancerised oral epithelial fields of genetically altered keratinocytes. However, clinically it is impossible to distinguish oral leukoplakia evolving within a benign epithelial field from leukoplakia arising from precancerous epithelial fields. The concept of field cancerisation

explains why some oral leukoplakias may occur concurrently in the same subject, why some leukoplakias progress to oral SCC, and why some oral leukoplakias recur despite treatment (8).

Histopathologically oral leukoplakia may show hyperplasia with or without dysplasia, different degrees of dysplasia, or carcinoma *in situ*.

The epidemiology and demographics of oral leukoplakia in black persons is not well understood. One study from South Africa showed 86% of persons with oral leukoplakia were white, 9% were black and 5% were asian despite the fact that the vast majority of the South African population is black (9). This apparent low prevalence of oral leukoplakia in black South Africans is difficult to explain. This may be due to the fact that in the pre-1994 era, black people had limited access to government health facilities, that black persons in South Africa smoke less compared to other South African population groups, and that in general, black people seek medical treatment late in the course of their disease and by the time of diagnosis, the oral leukoplakia has already transformed into oral SCC (10, 11). It is however possible that for reasons unknown, the prevalence of oral leukoplakia in South African blacks is inherently low.

Therefore a study investigating the clinical and histopathological features of oral leukoplakia in different racial population groups in South Africa may shed some light to whether oral leukoplakia in black South Africans is comparable to other racial population groups.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Definition, epidemiology and aetiopathogenesis

The term ‘leukoplakia’ was coined in 1877 by Schwimmer to describe a white lesion of the tongue (12). The definition of leukoplakia has undergone several modifications since then with the intention of standardising diagnostic criteria in order to improve the communication between clinicians and pathologists, to standardise treatment modalities and to facilitate research (1). Currently, oral leukoplakia is defined as a white patch that cannot be characterised clinically or microscopically as any other specific entity and is not associated with any aetiological agent other than tobacco/ areca nut use (1, 5, 13).

Based on clinical examination, a diagnosis of leukoplakia is made when all known lesions of similar appearance are excluded (Table I) and after a histological examination could not identify any other known entities (1, 13).

Table I: Clinical differential diagnosis of leukoplakia (14)

Aspirin burn
Candidiasis, pseudomembranous
Candidiasis, hyperplastic
Frictional lesion
Hairy leukoplakia
Leukoedema
Linea alba
Lupus erythematosus
Morsicatio (habitual chewing or biting of the cheek, tongue, lips)
Papilloma and allied lesions
Syphilis, secondary (‘‘mucous patches”)
Tobacco-induced lesions(palatal lesions in reverse smokers)
Smoker’s palate (nicotinic stomatitis)
Snuff induced lesion
White sponge nevus

There are only few studies in the literature that report the incidence rate of oral leukoplakia. The first study conducted in a rural village setting in India reported an age-adjusted incidence rate for oral leukoplakia of 240 per 100,000 persons/year in males and 3 per 100,000 persons/year in females (15-17). The second study conducted in a city in Japan reported an incidence rate of 409 per 100,000 persons/year in males and 70 per 100,000 persons/year in females (16).

The reported prevalence of leukoplakia in the world population varies from less than 1% to more than 5% (18, 19). Worldwide, the pooled prevalence obtained from systematic reviews was estimated between 1.49% and 4.27% (6). The prevalence of leukoplakia varies in different parts of the world, being higher in the Indian subcontinent than in Western countries. Scheifele et al (20) reviewed data from studies conducted during the

years 1985—2002 (number the individuals examined exceeding 500) and reported that the prevalence of oral leukoplakia ranges from 0.6 to 3.1% (Table II). In the United States, the prevalence of oral leukoplakia is 2.9 in white Americans over 35 years of age (21). A more recent study from the United States estimated the prevalence to be 0.4 which is considerably lower compared to previous studies (20). The prevalence and incidence of oral leukoplakia among black persons in South Africa is unknown.

Table II: Prevalence of oral leukoplakia in different countries

(studies published 1985—2002 with n>500) (20)

Country	Year	Participants	Prevalence of OL (%)
Saudi Arabia	1985	674	1.9
USA	1986	23,616	2.9
Netherlands	1988	1000	1.4
Sweden	1990	920	1.9
Hungary	1991	7820	1.3
Japan	1991	3131	2.5
Cambodia	1995	1319	1.1
Netherlands	1996	1000	0.6
Netherlands	1996	1000	0.2
Germany	1996	1000	0.9
Argentina (Cordoba)	1997	4183	6.8
Argentina (Buenos Aires)	1997	4838	2.8
Malaysia	1997	11,707	1.0
India	2000	49,179	2.3
Yugoslavia	2000	2385	2.2
Slovenia	2000	555	3.1

Oral leukoplakia may be idiopathic or may be associated with tobacco/areca nut use (1, 22). The role of tobacco in the pathogenesis of oral leukoplakia has been extensively reported (6, 12, 17, 23, 24). Although, tobacco smoke seems to be an important agent implicated in the pathogenesis of oral leukoplakia, its pathogenic role cannot be established in all cases. In certain geographic areas like the Indian subcontinent, tobacco and areca nut use, either alone or in combination, account for most cases of oral leukoplakia (2, 6, 15). Betel quid chewing (a combination of areca nut, betel leaves and slaked lime) is common practice in Asia and among migrated asian communities in Africa, Europe and North America (25). Smoking tobacco in the form of cigarettes is an important risk factor for leukoplakia in the developed world (19, 26). In Sweden, where snuff dipping was common practise, a survey found white oral patches in 24.8% of the population and reported that snuff dipping caused the lesions in 7.2% of cases (27). However, there are researchers who do not include snuff-induced lesions under the umbrella of oral leukoplakia since the malignant potential and the clinical course of snuff keratosis are different from those of oral leukoplakia (5, 14).

Human papilloma virus (HPV) infection has been implicated in the pathogenesis of HPV-cytopositive oral leukoplakia, specifically proliferative verrucous leukoplakia (28). The rate of HPV detection in oral potentially malignant lesions show extreme variations probably owing to differences in the geographic locations, ethnicity, sample size and in the methods of tissue detection (29-31). Miller and Johnstone (31) also reported an increased frequency of HPV in oral dysplastic epithelium in comparison with normal mucosa. However the exact role which HPV-genotypes play in the pathogenesis of oral leukoplakia is unknown.

2.2 Clinical characteristics

Leukoplakic lesions tend to occur in people over the age of 30 years (32, 33). The gender distribution of leukoplakia is 1:1 except in some geographical areas like the Indian subcontinent where males are more frequently affected, probably because they use tobacco/areca nut more than females (32, 33). Oral leukoplakia may occur as a single lesion, as multiple lesions or as diffusely widespread lesions (33, 34). The proposed classification and staging system for oral leukoplakia based on the size of the lesion is shown in Table III (34). Theoretically, the site affected should relate to the region of the oral cavity in contact with the mutagenic agent. The site affected thus varies in different parts of the world (15, 33). However, in many cases these agents are unknown. It is possible that in some cases, precancerous oral leukoplakia may arise from spontaneously induced cytogenetic alterations in oral keratinocytes (8, 35).

Table III: Proposed classification and staging system for oral leukoplakia (36)

L- Classification of size of oral leukoplakia	
L_1	Size of single or multiple leukoplakias together <2 cm
L_2	Size of single or multiple leukoplakias together 2-4 cm
L_3	Size of single or multiple leukoplakias together >4 cm
L_4	Size not specified
P-Pathology	
P0	No epithelial dysplasia (includes ‘‘no or perhaps mild epithelial dysplasia’’; equals OIN grade 0)
P1	Distinct epithelial dysplasia (includes ‘‘mild to moderate’’ and ‘‘moderate to possibly severe’’ epithelial dysplasia; equals OIN grades 1 and 2)
P‘x	Absence or presence of epithelial dysplasia not specified in the pathology report
OLEP staging system	
Stage I	L_1P_0
Stage II	L_2P_0
Stage III	L_3P_0 or $L_1L_2P_1$
Stage IV	L_3P_1

Clinically, oral leukoplakias are either homogenous or non-homogenous based on surface colour and/or morphological characteristics (1, 7). Homogeneous lesions are uniformly flat and may exhibit surface irregularities in the form of shallow cracks and are classified into flat, corrugated, wrinkled and pumice-like based on the surface appearance (1, 7, 37). The non-homogenous varieties include:

- speckled: mixed, white and red, but retaining predominantly white character;
- nodular: small polypoid outgrowths, rounded red or white excrescences;
- verrucous: wrinkled or corrugated surface appearance;
- proliferative verrucous leukoplakia (PVL): multiple, simultaneous leukoplakias, which tend to recur and have an aggressive course.

The clinical appearance of leukoplakic lesions is variable and may change over time (12). Some homogenous oral leukoplakia may become larger or change its clinical appearance to non-homogenous, but most of the lesions will remain stable or regress (2). Erythroleukoplakia should be distinguished clinically from oral erythroplakia, a precancerous lesion that carries the greatest risk of malignant transformation amongst all other precancerous oral lesions (5). Proliferative verrucous leukoplakia, which is considered by some as a distinct clinical sub-type of oral leukoplakia is characterised by multiple concurrent lesions that frequently affect wide areas of the oral mucosa (1).

2.3 Histopathological features

Squamous hyperplasia, hyperkeratosis and epithelial dysplasia, singularly or in combination are histopathological features commonly found in oral leukoplakia. Squamous hyperplasia refers to increased number of cells in the spinous layer or in the basal/parabasal cell layers and the architecture shows regular stratification without cellular atypia. Hyperkeratosis refers to the thickening of the stratum corneum and is often associated with a qualitative abnormality of the keratin. It may also be accompanied by an increase in the granular layer. Dysplasia refers to architectural disturbance accompanied by cytological atypia. A list of the dysplastic features is given in table IV.

Table IV: Histopathological features of epithelial dysplasia (38)

1	Loss of polarity
2	Presence of more than one layer of cells having a basaloid appearance
3	Increased nuclear cytoplasmic ratio
4	Drop shaped rete processes
5	Irregular epithelial stratification
6	Increased number of mitotic figures (few abnormal mitoses may be present)
7	Presence of mitotic figures in the superficial half of the epithelium
8	Cellular pleomorphism
9	Nuclear hyperchromatism
10	Enlarged nucleoli
11	Reduction of cellular cohesion
12	Keratinisation of single cells or cell groups in the prickle layer

Epithelial dysplasia can be further classified into mild, moderate and severe. The grades of epithelial dysplasia and the histological features of carcinoma *in situ* are given in table V.

Table V: Grades of dysplasia and features of carcinoma *in situ* (39)

Mild dysplasia
Architectural disturbance limited to the lower third of the epithelium, accompanied by minimal cytological atypia
Moderate dysplasia
Architectural disturbance extending into the middle third of the epithelium The presence of marked atypia may indicate that a lesion should be categorised as severe dysplasia despite not extending into the upper third of the epithelium.
Severe dysplasia
Recognition of severe dysplasia starts with greater than two thirds of the epithelium showing architectural disturbance with associated cytological atypia.
Carcinoma in situ
Recognition of severe dysplasia starts with full thickness or almost full thickness architectural abnormalities in the viable cellular layers accompanied by pronounced cytological atypia. Atypical mitotic figures and abnormally superficial mitoses are commonly seen in carcinoma in situ.

Epithelium with moderate and severe dysplasia is two times more likely to undergo malignant transformation than epithelium with mild dysplasia or hyperplasia (40, 41). Non-homogenous leukoplakia is more likely to be dysplastic than the epithelium of homogenous leukoplakia.

Grading of epithelial dysplasia is highly subjective and there is considerable inter-examiner and intra-examiner variability (39). Moreover an incisional biopsy of an affected site may not be representative of the whole lesion in terms of dysplastic changes (42).

Therefore, although an important and useful indicator to assess the malignant potential of a lesion, epithelial dysplasia is a poor predictor for cancerous transformation.

2.4 Diagnosis of oral leukoplakia

A provisional clinical diagnosis of oral leukoplakia is made when a white lesion cannot be characterised clinically as any other specific pathological entity and is not associated with any aetiological agent other than tobacco or areca nut use. A definitive diagnosis of oral leukoplakia is established after provisional clinical diagnosis of oral leukoplakia has been made and the lesion cannot be characterised microscopically as any other specific pathological entity. Thus a definitive diagnosis of oral leukoplakia is established by exclusion of other entities on clinical and histopathological grounds (8). A biopsy is mandatory not only to exclude other disease entities but also to ensure that the lesion is not squamous cell carcinoma, and to establish the presence or absence of epithelial dysplasia (22).

2.5 The malignant potential of oral leukoplakia

Leukoplakia is the most common premalignant lesion of the oral mucosa (1, 37, 43). The risk of malignant transformation of oral leukoplakia is relatively low, and in most cases unpredictable (5, 34). The reported rates of malignant transformation of oral leukoplakia vary among studies, ranging from less than 1% to about 20%, probably owing to differences between the studies with regard to case selection criteria (hospital-based patient population, house to house surveyors, etc.), to geographic areas, to ethnic groups investigated, to tobacco use, to the time of follow-up, and to diagnostic criteria (7, 18).

Moreover, the reported rates of malignant transformation of oral leukoplakia, do not distinguish between treated oral leukoplakia (and different treatment modalities) and untreated cases, thus affecting the reliability of the reported results (7).

Non-homogenous leukoplakia has a greater risk of progression to carcinoma than homogenous leukoplakia (2, 5). The estimated rate of malignant transformation of homogenous leukoplakia is 0.6 – 5% and of non-homogenous leukoplakia is 20 – 25% (2, 5); and it is estimated that the overall lifetime risk of malignant transformation of oral leukoplakia is less than 2% per year (2).

Additional clinical parameters associated with increased malignant potential of oral leukoplakia are the anatomical oral sub-sites in which they arise and the size of the lesion. In general, leukoplakia of the floor of the mouth, of the ventrolateral tongue and the soft palate are considered to show a higher-risk of malignant transformation than other anatomical sub-sites (2, 3); and it is suggested that large oral leukoplakic lesions carry a higher rate of malignant transformation than smaller size lesions (2, 7).

At a molecular level the development of oral leukoplakia and its carcinomatous transformation is associated with loss of heterozygosity (LoH) at certain specific chromosomal loci, and DNA aneuploidy (35). Leukoplakic lesions of the floor of the mouth, of the ventrolateral surface of the tongue and of the soft palate have a higher risk of carcinomatous transformation and it is at these sites that the keratinocytes show increased LoH (35, 44).

If the chromosomes in the DNA are not uniformly distributed to the daughter cells or if parts of chromosomes become detached, the chromosomal segregation during mitosis is unbalanced. This is termed aneuploidy (40). The presence of aneuploidy in leukoplakia is one of the most predictable indicators of malignant transformation (45). Leukoplakia with keratinocytes showing DNA aneuploidy have higher carcinomatous transformation potential than those with normal diploid DNA content (35).

The concept of field precancerization can explain why oral leukoplakia may develop at a single oral site or at multiple oral sites and why it may recur at the same site from which it was previously successfully excised. A field of precancerization in the oral cavity can be defined as an area of clinically normal-looking epithelium which is either microscopically normal or shows dysplasia; but in which some keratinocytes have undergone cytogenetic alterations. Leukoplakia and other premalignant lesions originating within the oral epithelium (i.e. erythroplakia) may be a clinical manifestation of such precancerised epithelial fields (8, 35).

Accumulation of additional cytogenic alterations to initially transformed keratinocytes in such a field of precancerised oral epithelium could bring about progression of the existing premalignant leukoplakia to squamous cell carcinoma, or the *de novo* development of squamous cell carcinoma (8, 35). Depending on whether the transformed keratinocytes evolved from one progenitor basal cell that underwent clonal expansion, or evolved from several progenitor basal cells that underwent independent initial transformation and subsequent clonal expansion, the leukoplakia can be of a monoclonal or a polyclonal molecular profile (35). The time for the progression of leukoplakia within a field of

precancerised epithelial fields to squamous cell carcinoma if it ever does, may on average be 5 to 8 years (46).

However, not all oral leukoplakias have malignant potential. In fact the majority of leukoplakia arises within benign fields of oral epithelium and others regress or remain stable. However, it is impossible to distinguish benign oral leukoplakia from premalignant leukoplakia (35).

2.6 Treatment

Smoking is a major risk factor for oral leukoplakia and hence managing leukoplakia would include cessation of smoking and avoidance of other possible associated risk factors (alcohol, high-risk sexual behaviour associated with HPV infection). There are different treatment options for leukoplakia, including observation and monitoring, surgical excision, cryotherapy and laser therapy (Table VI). Complete excision of the lesion is recommended if the lesion occurs on the ventral/lateral tongue, floor of the mouth, soft palate and oropharynx especially in the presence of dysplasia (47). However, the risk of malignant transformation is not completely eliminated by any of the current therapies.

Table VI: Treatment options for leukoplakia (47)

Surgical excision
Laser surgery Electrocautery
Retinoids Vitamin A Vitamin E fi-carotene
Topical bleomycin
Cryosurgery
Photodynamic therapy

The treatment guidelines are given in table VII.

Table VII: Treatment guidelines for oral leukoplakia (47)

Eliminate all contributing factors
Absence of dysplasia or presence of mild dysplasia
Surgical excision/laser surgery of lesions on the ventral/lateral tongue, floor of the mouth, soft palate, oropharynx Close observation and follow-up for all other anatomic locations
Presence of moderate or severe dysplasia
Surgical excision or laser therapy are preferred treatments
Red lesions (erythroplakia or leukoerythroplakia)
Surgical excision
Proliferative verrucous leukoplakia
Surgical excision/laser surgery if possible
Regular follow-up is highly recommended

CHAPTER 3

3. AIMS AND OBJECTIVES

Aim

The aim of this study is to analyse the differences in the clinicopathological features of oral leukoplakia among different racial groups in the greater Johannesburg area of South Africa, with special emphasis on the black population.

The objectives of the study are

1. To determine the age, gender, ethnicity, site of the lesion, habits, clinical types and histopathological diagnosis of leukoplakia cases.
2. To determine the association between the age, gender, site of the lesion, habits, clinical types, histopathological diagnosis and the ethnicity of the patient.

CHAPTER 4

4. MATERIALS AND METHODS

4.1 Sample size and selection of participants

The histopathological reports of all cases diagnosed clinically as oral leukoplakia and histologically as hyperkeratosis without dysplasia, hyperkeratosis with mild, moderate or severe dysplasia, and carcinoma *in situ* over a 21 year period (1990 – 2010) were retrieved from the archives of the Division of Oral Pathology at the University of Witwatersrand, Johannesburg.

4.2 Clinical evaluation

Clinical data with regard to age, gender, race, clinical appearance (homogenous or non-homogenous leukoplakia), number of leukoplakic lesions, site of the lesion in the mouth, and tobacco use were recorded. When the information was incomplete in the histopathological reports, the required information was obtained from the patients clinical records. The retrieved information was entered onto a data collection form (Appendix 1).

4.3 Histological evaluation

The haematoxylin and eosin (H&E) stained sections of each case diagnosed with oral leukoplakia was reviewed by the researcher in conjunction with an oral pathologist using a dual headed microscope [Nikon Eclipse 80*i* (Nikon Corp, Tokyo, Japan)] fitted with 10x oculars and a 40x objective to confirm the diagnosis.

4.4 Inclusion criteria

- Cases that were included in the study were those diagnosed clinically with oral leukoplakia and histologically as hyperkeratosis without dysplasia, hyperkeratosis with mild, moderate or severe epithelial dysplasia and carcinoma *in situ.*
- In cases with multiple biopsies for recurrent leukoplakic lesions, only the first biopsy report was included.
- In cases with multiple lesions, the lesion that was biopsied first was included.

4.5 Exclusion criteria

- Cases with insufficient clinical data to confirm a clinical diagnosis of oral leukoplakia.
- Cases diagnosed clinically as oral leukoplakia but on histopathological examination were found to be squamous cell carcinoma.
- Cases in which a cause for the hyperkeratosis was apparent, for example friction, candidiasis and snuff use.

4.6 Ethical considerations

The ethics code M080850 used for this research adheres to international ethical criteria for research. This is a blanket code for use on archival block material obtained from human tissues allocated to the Division of Oral Pathology, Department of Anatomical Pathology and covers the review of the histological sections.

An ethics clearance certificate No: M110614 (Appendix 2) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg to view patient's hospital files in order to obtain clinical data needed for the study. Permission to access the clinical records of the patients was obtained from the CEO of Charlotte Maxeke Johannesburg Academic Hospital (Appendix 3). The identity of the patient was kept confidential.

4.7 Statistical analysis

The data obtained was entered into Microsoft® Excel® spreadsheets and then transferred to the statistical software programme IBM® SPSS® Statistics, version 20.0 (IBM Corporation, New York2011) for statistical analysis under the guidance of the statistician Mr Charles Chimedza. Chi-squared tests, t-tests, binomial-distribution tests and binary logistical regression was computed to conduct statistical hypothesis tests and to explore differences among histopathological and clinical parameters of oral leukoplakia between blacks, coloureds, asians and whites. *p*-values of 0.05 or less was considered as statistically significant.

When performing binary logistical regression analysis, the categorical variables namely: age, gender, site, ethnicity, and smoking habit were computed to the outcome (dependent) variables namely: the clinical classification of leukoplakia and the presence of epithelial dysplasia. Hosmer and Lemeshow test was done to check 'goodness-of-fit' of the model.

There were only few asians and coloureds in the study and the comparisons are thus between the white and black patients. When computing chi-squared tests and logistical

regression analysis, the data was filtered to include only white and black patients even though the asians and coloureds are represented in the results section.

CHAPTER 5

5. RESULTS

There were a total of 21,300 biopsy specimens submitted for histopathological examination to the Oral Pathology Department at the University of Witwatersrand for the 21 year period from 1990-2010 (Table VIII). One hundred and forty-three biopsy reports were found to have a histopathological diagnosis of hyperkeratosis with or without dysplasia and a clinical diagnosis of oral leukoplakia. The histopathological findings of these cases were re-examined by a specialist in oral pathology (Prof Shabnum Meer) and the researcher. Two reports were excluded owing to the presence of candida infection. Thirty-three cases were excluded as there was insufficient clinical data to confirm a clinical diagnosis of oral leukoplakia. Furthermore, data with regard to family history of cancer was incomplete or unreliable and was therefore not used for statistical analysis.

Nine of the 143 biopsy reports were repeat biopsies of recurrent lesions. In these cases only the first biopsy report was included in the statistical analysis. Five of the 95 patients had multiple oral lesions but only one lesion was biopsied and sent for microscopical examination. Another five cases had multiple oral lesions sent for microscopical examination of which only the first lesion examined was included in the study. After re-examination and exclusion of the above mentioned cases, a total of **95** cases were included in the study (Table VIII).

Table VIII Case distribution per year

Year	Total cases biopsied	**Cases selected**	Percent
1990	670	**14**	14.7
1991	764	**12**	12.6
1992	756	**6**	6.3
1993	725	**13**	13.7
1994	602	**4**	4.2
1995	824	**4**	4.2
1996	661	**5**	5.3
1997	636	**10**	10.5
1998	442	**3**	3.2
1999	1066	**2**	2.1
2000	1134	**3**	3.2
2001	1184	**1**	1.2
2002	1382	**2**	2.2
2003	1477	**2**	2.2
2004	1380	**3**	3.2
2005	1294	**0**	0.0
2006	1086	**2**	2.1
2007	1004	**3**	3.2
2008	1124	**3**	3.2
2009	1618	**0**	0.0
2010	1471	**3**	3.2
	21300	**95**	**100**

5.1 Age, gender and ethnicity

There was a wide age distribution of cases occurring from the 3rd to the 9th decades with most cases occuring in the 5th and the 6th decade (Table IX). The mean age of the patients at the time of diagnosis was **50 years** (standard deviation of 12.94, range 21-80). The mean age at the time of diagnosis was **48 years for men** (standard deviation: 13.57) and **52 years for women** (standard deviation: 12.13). This difference was not statistically significant (*p*-value=0.288). There was an equal gender distribution (M:F=48:47) (Table IX).

Table IX: Age in relation to gender

Age group	Male (n=48)		Female (n=47)		**Total** (n=95)	
20-29	4	(8.3%)	1	(2.1%)	**5**	(5.3%)
30-39	9	(18.8%)	7	(14.9%)	**16**	(16.8%)
40-49	13	(27.1%)	11	(23.4%)	**24**	(25.3%)
50-59	11	(22.9%)	14	(29.8%)	**25**	(26.3%)
60-69	8	(16.7%)	9	(19.1%)	**17**	(17.9%)
70-79	2	(4.2%)	5	(10.6%)	**7**	(7.4%)
80-89	1	(2.1%)	0	(0.0%)	**1**	(1.1%)

Most of the patients (76) were white (80%). Thirteen patients (13.7%) were black, five patients were asian (5.3%) and one patient (1%) was coloured (Table X). When relating the gender to the ethnicity of the patients, there were **more white females than white males** with oral leukoplakia (F: M=10:9), but there were more black males than black females with oral leukoplakia (F: M= 5:8) (Table X). **Black patients were diagnosed with oral leukoplakia at a younger age** (47 years, standard deviation: 12.39) than white patients (51 years, standard deviation: 13.05), however this was not statistically significant (*p*-value=0.508).

Table X: Gender and age in relation to ethnicity

		White (n=76)	Black (n=13)	Asian (n=5)	Coloured (n=1)	**Total** (n=95)
Age (years)	20-29	4 (5.3%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	**5** (5.3%)
	30-39	12 (15.8%)	2 (15.4%)	2 (40.0%)	0 (0.0%)	**16** (16.8%)
	40-49	18 (23.7%)	5 (38.5%)	1 (20.0%)	0 (0.0%)	**24** (25.3%)
	50-59	21 (27.6%)	3 (23.3%)	1 (20.0%)	0 (0.0%)	**25** (26.3%)
	60-69	14 (18.4%)	1 (7.7%)	1 (20.0%)	1 (100%)	**17** (17.9%)
	70-79	6 (7.9%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	**7** (7.4%)
	80-89	1 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**1** (1.1%)
Gender	Male	36 (47.9%)	8 (61.5%)	3 (60.0%)	1 (100%)	**48** (50.5%)
	Female	40 (52.6%)	5 (38.5%)	2 (40.0%)	0 (0.0%)	**47** (49.5%)

5.2 Oral site distribution

The most commonly affected site was the **floor of the mouth**, followed by the buccal mucosa and the lateral aspect of the tongue (Table XI). There was no difference with regard to the site affected by leukoplakia in men and women.

Table XI: Site in relation to gender

	Male (n=48)		Female (n=47)		**Total** (n=95)	
Buccal mucosa	8	(16.7%)	10	(21.3%)	**18**	(18.9%)
Alveolar ridge	3	(6.2%)	6	(12.8%)	**9**	(9.5%)
Floor of the mouth	12	(25.0%)	13	(27.7%)	**25**	(26.3%)
Retro molar pad	3	(6.2%)	1	(2.1%)	**4**	(4.2%)
Lateral tongue	7	(14.6%)	7	(14.9%)	**14**	(14.7%)
Ventral tongue	2	(4.2%)	2	(4.3%)	**4**	(4.2%)
Gingiva	5	(10.4%)	3	(6.4%)	**8**	(8.4%)
Soft palate	3	(6.2%)	3	(6.4%)	**6**	(6.3%
Hard palate	3	(6.2%)	0	(0.0%)	**3**	(3.2%)
Lower lip	2	(4.2%)	1	(2.1%)	**3**	(3.2%)
Commissure of the lip	0	(0.0%)	1	(2.1%)	**1**	(1.1%)

When comparing white to black patients, in white patients with oral leukoplakia, the most frequently affected site was the floor of the mouth followed by the lateral aspect of the tongue, while in black patients with oral leukoplakia; the buccal mucosa was the most affected site (Table XII).

Table XII: Site in relation to ethnicity

	White (n=76)		Black (n=13)		Asian (n=5)		Coloured (n=1)		**Total** (n=95)	
Buccal mucosa	10	(13.2%)	6	(46.2%)	2	(40.0%)	0	(0.0%)	**18**	(18.9%)
Alveolar ridge	8	(10.5%)	1	(7.7%)	0	(0.0%)	0	(0.0%)	**9**	(9.5%)
Floor of the mouth	23	(30.3%)	1	(7.7%)	0	(0.0%)	1	(100.0%)	**25**	(26.3%)
Retro molar pad	2	(2.6%)	2	(15.4%)	0	(0.0%)	0	(0.0%)	**4**	(4.2%)
Lateral tongue	13	(17.1%)	0	(0.0%)	1	(20.0%)	0	(0.0%)	**14**	(14.7%)
Ventral tongue	2	(2.6%)	1	(7.7%)	1	(20.0%)	0	(0.0%)	**4**	(4.2%)
Gingiva	8	(10.5%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	**8**	(8.4%)
Soft palate	5	(6.6%)	1	(7.7%)	0	(0.0%)	0	(0.0%)	**6**	(6.3%)
Hard palate	3	(3.9%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	**3**	(3.2%)
Lower lip	1	(1.3%)	1	(6.7%)	1	(16.7%)	0	(0.0%)	**3**	(3.2%)
Commissure of the lip	1	(1.3%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	**1**	(1.1%)

The data with regard to the extent of the leukoplakic lesions was incomplete or unreliable and was therefore not used for statistical analysis.

5.3 Habits

The habits that were investigated were tobacco smoking, alcohol consumption and betel nut use. The data with regard to alcohol consumption was incomplete or unreliable and was therefore not used for statistical analysis. The data with regard to betel nut use (2 patients) and duration of smoking was excluded as it was incomplete.

The relation of tobacco smoking to the different variables investigated is shown in table XIII. Seventy-eight percent (74/95) of the patients were tobacco smokers. The mean age of smokers at the time of diagnosis of oral leukoplakia (49 years, standard deviation: 13.05) was lower than the non-smokers (54 years, standard deviation: 12.02), but this was not statistically significant (*p*-value=0.815). More males (85.4%, 41/48) with oral leukoplakia smoked than females (70.2%, 33/47) with oral leukoplakia. In smokers the most frequently affected site was the floor of the mouth followed by the buccal mucosa, while in non-smokers the most commonly affected site was the lateral aspect of the tongue followed by the buccal mucosa.

Table XIII: Cross tabulation of smoking habit

		Smokers (n=74)		Non-smokers (n=14)		**Total** (n=95)	
Age	20-29	5	(6.8%)	0	(0.0%)	**5**	(5.3%)
(years)	30-39	13	(17.6%)	3	(14.3%)	**16**	(16.8%)
	40-49	19	(25.7%)	5	(23.8%)	**24**	(25.3%)
	50-59	21	(28.4%)	4	(19.0%)	**25**	(26.3%)
	60-69	10	(13.5%)	7	(33.3%)	**17**	(17.9%)
	70-79	5	(6.8%)	2	(9.5%)	**7**	(7.4%)
	80-89	1	(1.4%)	0	(0.0%)	**1**	(1.0%)
Gender	Male	41	(55.4%)	7	(33.3%)	**48**	(50.5%)
	Female	33	(44.6%)	14	(66.7%)	**47**	(49.5%)
Site	Buccal mucosa	14	(18.9%)	4	(19.0%)	**18**	(18.9%)
	Alveolar ridge	9	(12.2%)	0	(0.0%)	**9**	(9.5%)
	Floor of the mouth	24	(32.4%)	1	(4.8%)	**25**	(26.3%)
	Retro molar pad	4	(5.4%)	0	(0.0%)	**4**	(4.2%)
	Lateral tongue	6	(8.1%)	8	(38.1%)	**14**	(14.7%)
	Ventral tongue	2	(2.7%)	2	(9.5%)	**4**	(4.2%)
	Gingiva	6	(9.5%)	2	(8.1%)	**8**	(8.4%)
	Soft palate	4	(5.4%)	2	(9.5%)	**6**	(6.3%)
	Hard palate	3	(4.1%)	0	(0.0%)	**3**	(3.2%)
	Lower lip	1	(1.4%)	2	(9.5%)	**3**	(3.2%)
	Commissure of the lip	1	(1.4%)	0	(0.0%)	**1**	(1.1%)

In white patients, 82% (62/76) of the leukoplakic lesions were associated with smoking tobacco, while in black patients only 62% (8/13) of the leukoplakic lesions were associated with smoking tobacco (Table XIV).

Table XIV: Smoking in relation to ethnicity

	White (n=76)		Black (n=13)		Asian (n=5)		Coloured (n=1)		**Total** (n=95)	
Smokers	62	(81.6%)	8	(61.5%)	3	(60.0%)	1	(100%)	**74**	(77.9%)
Non-smokers	14	(18.4%)	5	(38.5%)	2	(40.0%)	0	(0.0%)	**21**	(22.1%)

5.4 Clinical types of oral leukoplakia

The frequencies of the different clinical types of oral leukoplakia in the study population are shown in table XV. Eighty-five percent (81/95) of the cases of oral leukoplakia were of the homogenous type and 15% (14/99) were of the non-homogenous type. Of the non-homogenous leukoplakia, 50% (7/14) were erythroleukoplakia, 43% (6/14) were verrucous leukoplakia and 7% (1/14) were proliferative verrucous leukoplakia.

Table XV: Clinical classification of leukoplakia

	Total (n=95)	Percent
Homogenous leukoplakia	**81**	85.3
Erythroleukoplakia	**7**	7.4
Nodular leukoplakia	**0**	0
Verrucous leukoplakia	**6**	6.3
Proliferative verrucous leukoplakia	**1**	1.1

The relation of homogenous and non-homogenous leukoplakia to the different variables investigated is shown in table XVI. The mean age at the time of diagnosis of homogenous and non-homogenous oral leukoplakia was 50 years (standard deviation: 12.53 and 15.62). Eighty-eight percent of males (42/48) and 83% of females (39/47) had homogenous leukoplakia. However there were more females (57%, 8/14) with non-homogenous leukoplakia than males (43%, 6/14) with non-homogenous leukoplakia. The floor of the mouth was the most frequently affected site in patients with homogenous and non-homogenous leukoplakia. Eighty-eight percent (65/74) of the smokers had homogenous leukoplakia compared to 76% (16/21) of the non-smokers; and 12% (9/74) of the smokers had non-homogenous leukoplakia compared to 24% (5/21) of the non-smokers.

Table XVI: Cross tabulation of homogenous and non-homogenous leukoplakia

		Homogenous (n=81)		Non-homogenous (n=14)		**Total** (n=95)	
Age (years)	Age: 20-29	4	(4.9%)	1	(7.1%)	**5**	(5.3%)
	Age: 30-39	13	(16.0%)	3	(21.4%)	**16**	(16.8%)
	Age: 40-49	22	(27.2%)	2	(14.3%)	**24**	(25.3%)
	Age: 50-59	20	(24.7%)	5	(35.7%)	**25**	(26.3%)
	Age: 60-69	17	(21.0%)	0	(0.0%)	**17**	(17.9%)
	Age: 70-79	4	(4.9%)	3	(21.4%)	**7**	(7.4%)
	Age: 80-89	1	(1.2%)	0	(0.0%)	**1**	(1.1%)
Gender	Male	42	(51.9%)	6	(42.9%)	**48**	(50.5%)
	Female	39	(48.1%)	8	(57.1%)	**47**	(49.5%)
Site	Buccal mucosa	14	(17.3%)	4	(28.6%)	**18**	(18.9%)
	Alveolar ridge	9	(11.1%)	0	(0.0%)	**9**	(9.5%)
	Floor of the mouth	20	(24.7%)	5	(35.7%)	**25**	(26.3%)
	Retro molar pad	4	(4.9%)	0	(0.0%)	**4**	(4.2%)
	Lateral tongue	13	(16.0%)	1	(7.1%)	**14**	(14.7%)
	Ventral tongue	2	(2.5%)	2	(14.3%)	**4**	(4.2%)
	Gingiva	7	(8.6%)	1	(7.1%)	**8**	(8.4%)
	Soft palate	5	(6.2%)	1	(7.1%)	**6**	(6.3%)
	Hard palate	3	(3.7%)	0	(0.0%)	**3**	(3.2%)
	Lower lip	3	(3.7%)	0	(0.0%)	**3**	(3.2%)
	Commissure of the lip	1	(1.2%)	0	(0.0%)	**1**	(1.1%)
Habit	Smokers	65	(80.2%)	9	(64.3%)	**74**	(77.9%)
	Non-smokers	16	(19.8%)	5	(35.7%)	**21**	(22.1%)

There were more black people (23%) with non-homogenous leukoplakia than white people with non-homogenous leukoplakia (13%) (Table XVII). This association however was not significant (*p*-value=0.349).

Table XVII: Relation of clinical classification and ethnicity

	White (n=76)	Black (n=13)	Asian (n=5)	Coloured (n=1)	**Total** (n=95)
Homogenous	66 (81.6%)	10 (61.5%)	4 (60.0%)	1 (100%)	**81** (85.3%)
Non-homogenous	10 (13.2%)	3 (23.1%)	1 (20.0%)	0 (0.0%)	**14** (14.7%)

The site affected in relation to the clinical subtypes of leukoplakia is shown in table XVIII. There were only few cases of the various type of non-homogenous leukoplakia for statistical analysis.

Table XVIII: Site in relation to the clinical subtypes

	Homogenous leukoplakia (n=81)	Erythroleukoplakia (n=7)	Verrucous leukoplakia (n=6)	Proliferative verrucous leukoplakia (n=1)	**Total** (n=95)
Buccal mucosa	14 (17.3%)	1 (14.3%)	2 (33.3%)	1 (100%)	**18** (18.9%)
Alveolar ridge	9 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**9** (9.5%)
Floor of the mouth	20 (24.7%)	2 (28.6%)	3 (50.0%)	0 (0.0%)	**25** (26.3%)
Retro molar pad	4 (4.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**4** (4.2%)
Lateral tongue	13 (16.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	**14** (14.7%)
Ventral tongue	2 (2.5%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	**4** (4.2%)
Gingiva	7 (8.6%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	**8** (8.4%)
Soft palate	5 (6.2%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	**6** (6.3%)
Hard palate	3 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**3** (3.2%)
Lower lip	3 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**3** (3.2%)
Commissure of the lip	1 (1.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**1** (1.1%)

5.5 Histopathological diagnosis

The presence of epithelial dysplasia in relation to the different variables investigated is shown in table XIX. Forty-seven percent (45/95) of the patients had dysplastic oral leukoplakia and 53% (50/95) had non-dysplastic oral leukoplakia. The mean age of the patients at the time of diagnosis of dysplastic oral leukoplakia (52 years, standard deviation: 13.69) was higher than for those diagnosed with non-dysplastic oral leukoplakia

(49 years, standard deviation: 12.21), but this was not statistically significant. Females (53.3%, 24/45) had more frequently than males (46.7%, 21/45) dysplastic oral leukoplakia.

Dysplastic oral leukoplakia most frequently affected the floor of the mouth followed by the lateral aspect of the tongue while non-dysplastic oral leukoplakia affected most frequently the buccal mucosa followed by the floor of the mouth. Chi-squared tests revealed a **significant** relationship (*p*-value= 0.000) between the site affected and the presence of epithelial dysplasia. Binary logistic regression analysis revealed a **significantly** higher likelihood of the lateral aspect of the tongue (*p*-value =0.001), the floor of the mouth (*p*-value= 0.003) and the buccal mucosa (*p*-value =0.017) to show presence of epithelial dysplasia.

In smokers, forty-three percent (32/74) of the leukoplakic lesions had epithelial dysplasia while in non-smokers 62% (13/21) had epithelial dysplasia. This difference was not statistically significant (*p*-value=0.292). Forty-eight percent (39/81) of homogenous leukoplakia were dysplastic while only 42.8% (6/14) of non-homogenous leukoplakia were dysplastic. Fifty-two percent (42/81) of homogenous leukoplakia had no epithelial dysplasia while 57.1% (8/14) of non-homogenous leukoplakia had no epithelial dysplasia.

Table XIX: Cross tabulation of epithelial dysplasia

Dysplasia		No dysplasia (n=50)		Dysplasia (n=45)		**Total** (n=95)	
Age	20-29	3	(6.0%)	2	(4.4%)	**5**	(5.3%)
(years)	30-39	8	(16.0%)	8	(17.8%)	**16**	(16.8%)
	40-49	15	(30.0%)	9	(20.0%)	**24**	(25.3%)
	50-59	15	(30.0%)	10	(22.2%)	**25**	(26.3%)
	60-69	4	(8.0%)	13	(28.9%)	**17**	(17.9%)
	70-79	5	(10.0%)	2	(4.4%)	**7**	(7.4%)
	80-89	0	(0.0%)	1	(2.2%)	**1**	(1.1%)
Gender	Male	27	(54.0%)	21	(46.7%)	**48**	(50.5%)
	Female	23	(46.0%)	24	(53.3%)	**47**	(49.5%)
Site	Buccal mucosa	16	(32.0%)	2	(4.4%)	**18**	(18.9%)
	Alveolar ridge	5	(10.0%)	4	(8.9%)	**9**	(9.5%)
	Floor of the mouth	9	(18.0%)	16	(35.6%)	**25**	(26.3%)
	Retro molar pad	3	(6.0%)	1	(2.2%)	**4**	(4.2%)
	Lateral tongue	1	(2.0%)	13	(28.9%)	**14**	(14.7%)
	Ventral tongue	1	(2.0%)	3	(6.7%)	**4**	(4.2%)
	Gingiva	7	(14.0%)	1	(2.2%)	**8**	(8.4%)
	Soft palate	5	(10.0%)	1	(2.2%)	**6**	(6.3%)
	Hard palate	1	(2.0%)	2	(4.4%)	**3**	(3.2%)
	Lower lip	1	(2.0%)	2	(4.4%)	**3**	(3.2%)
	Commissure of the lip	1	(2.0%)	0	(0.0%)	**1**	(1.1%)
Habit	Smokers	42	(84.0%)	32	(71.1%)	**74**	(77.9%)
	Non-smokers	8	(16.0%)	13	(28.9%)	**21**	(21.1%)
Leukoplakia	Homogenous leukoplakia	42	(84.0%)	39	(86.7%)	**81**	(85.3%)
	Non-homogenous leukoplakia	8	(16.0%)	6	(13.3%)	**14**	(14.7%)
Clinical	Homogenous leukoplakia	42	(84.0%)	39	(86.7%)	**81**	(85.3%)
types	Erythroleukoplakia	3	(6.0%)	4	(8.9%)	**7**	(7.4%)
	Nodular leukoplakia	0	(0.0%)	0	(0.0%)	**0**	(0.0%)
	Verrucous leukoplakia	4	(8.0%)	2	(4.4%)	**6**	(6.3%)
	Proliferative verrucous	1	(2.0%)	0	(0.0%)	**1**	(1.1%)

Fifty-one percent (39/76) of the white patients with oral leukoplakia had epithelial dysplasia while only 23% (3/13) of the black patients with oral leukoplakia had epithelial dysplasia (Table XX). Chi-squared tests revealed a **significant** relationship (*p*-value= 0.059) between ethnicity and the presence of epithelial dysplasia.

Table XX: Relation of presence of epithelial dysplasia and ethnicity

	White (n=76)	Black (n=13)	Asian (n=5)	Coloured (n=1)	**Total** (n=95)
No dysplasia	37 (48.7%)	10 (76.9%)	2 (40.0%)	1 (100%)	**50** (52.6%)
Dysplasia	39 (51.3%)	3 (23.1%)	3 (60.0%)	0 (0.0%)	**45** (47.4%)

The different grades of epithelial dysplasia in relation to the different variables investigated, is shown in table XXI. Thirty-two percent (30/95) of the leukoplakic lesions had mild epithelial dysplasia, 14.7% (14/95) had moderate epithelial dysplasia and 1% (1/95) had severe epithelial dysplasia. Of the lesions with epithelial dysplasia, 66.7% (30/45) had mild epithelial dysplasia, 31.1% (14/45%) had moderate epithelial dysplasia and 2.2% (1/45) had severe dysplasia. There were no cases with a clinical diagnosis of leukoplakia that histopathologically showed features of carcinoma *in situ.*

Table XXI: Cross tabulation of the grades of epithelial dysplasia

		No dysplasia (n=50)		Mild dysplasia (n=30)		Moderate dysplasia (n=14)		Severe dysplasia (n=1)		**Total** (n=95)	
Age (years)	20-29	3	(6.0%)	2	(6.7%)	0	(0.0%)	0	(0.0%)	**5**	(5.3%)
	30-39	8	(16.0%)	5	(16.7%)	3	(21.4%)	0	(0.0%)	**16**	(16.8%)
	40-49	15	(30.0%)	6	(20.0%)	3	(21.4%)	0	(0.0%)	**24**	(25.3%)
	50-59	15	(30.0%)	7	(23.3%)	2	(14.3%)	1	(100%)	**25**	(26.3%)
	60-69	4	(8.0%)	8	(26.7%)	5	(35.7%)	0	(0.0%)	**17**	(17.9%)
	70-79	5	(10.0%)	1	(3.3%)	1	(7.1%)	0	(0.0%)	**7**	(7.4%)
	80-89	0	(0.0%)	1	(3.3%)	0	(0.0%)	0	(0.0%)	**1**	(1.1%)
Gender	Male	27	(54.0%)	14	(46.7%)	7	(50.0%)	0	(0.0%)	**48**	(50.5%)
	Female	23	(46.0%)	16	(53.3%)	7	(50.0%)	1	(100%)	**47**	(49.5%)
Site	Buccal mucosa	16	(32.0%)	2	(6.7%)	0	(0.0%)	0	(0.0%)	**18**	(18.9%)
	Alveolar ridge	5	(10.0%)	4	(13.3%)	0	(0.0%)	0	(0.0%)	**9**	(9.5%)
	Floor of the mouth	9	(18.0%)	13	(43.3%)	2	(14.3%)	1	(100%)	**25**	(26.3%)
	Retro molar pad	3	(6.0%)	1	(3.3%)	0	(0.0%)	0	(0.0%)	**4**	(4.2%)
	Lateral tongue	1	(2.0%)	5	(16.7%)	8	(57.1%)	0	(0.0%)	**14**	(14.7%)
	Ventral tongue	1	(2.0%)	0	(0.0%)	3	(21.4%)	0	(0.0%)	**4**	(4.2%)
	Gingiva	7	(14.0%)	1	(3.0%)	0	(0.0%)	0	(0.0%)	**8**	(8.4%)
	Soft palate	5	(10.0%)	1	(3.0%)	0	(0.0%)	0	(0.0%)	**6**	(6.3%)
	Hard palate	1	(2.0%)	1	(3.0%)	1	(7.1%)	0	(0.0%)	**3**	(3.2%)
	Lower lip	1	(2.0%)	2	(6.7%)	0	(0.0%)	0	(0.0%)	**3**	(3.2%)
	Commissure of the lip	1	(2.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	**1**	(1.1%)
Habits	Smokers	42	(84.0%)	22	(73.3%)	9	(64.3%)	1	(100%)	**74**	(77.9%)
	Non-smokers	8	(16.0%)	8	(26.7%)	5	(35.7%)	0	(0.0%)	**21**	(22.1%)
Leukoplakia	Homogenous	42	(84.0%)	26	(86.7%)	12	(85.7%)	1	(100%)	**81**	(85.3%)
	Non-homogenous	8	(16.0%)	4	(13.3%)	2	(14.3%)	0	(0.0%)	**14**	(14.7%)
Clinical types	Homogenous	42	(84.0%)	26	(86.7%)	12	(85.7%)	1	(100%)	**81**	(85.3%)
	Erythroleukoplakia	3	(6.0%)	3	(10.0%)	1	(7.1%)	0	(0.0%)	**7**	(7.4%)
	Nodular leukoplakia	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	**0**	(0.0%)
	Verrucous leukoplakia	4	(8.0%)	1	(3.3%)	0	(0.0%)	0	(0.0%)	**5**	(5.3%)
	Proliferative verrucous	1	(2.0%)	0	(0.0%)	1	(7.1%)	0	(0.0%)	**2**	(2.1%)

The relation of the various grades of epithelial dysplasia in relation to ethnicity is given in table XXII.

Table XXII: Cross tabulation of histopathological diagnosis and ethnicity

	White (n=76)	Black (n=13)	Asian (n=5)	Coloured (n=1)	**Total** (n=95)
No dysplasia	37 (48.7%)	10 (76.9%)	2 (40.0%)	1 (100%)	**50** (52.6%)
Mild dysplasia	27 (35.5%)	2 (15.4%)	1 (20.0%)	0 (0.0%)	**30** (31.6%)
Moderate dysplasia	11 (14.5%)	1 (7.7%)	2 (40.0%)	0 (0.0%)	**14** (14.7%)
Severe dysplasia	1 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**1** (1.1%)

CHAPTER 6

6. DISCUSSION

The data from this study on oral leukoplakia has been obtained from a biased sample of patients who had been consulted by dental practitioners for assessment of oral white lesions and had their biopsies microscopically examined. Therefore comparison of the data in this study with results of other studies of oral leukoplakia obtained by other selection criteria (house to house survey, hospital survey etc.) may not be applicable; and the results of this study with regard to the ethnic predilection of oral leukoplakia should be interpreted with caution since they may reflect a referral bias.

In this study we excluded white lesions caused by snuff use since these lesions have a well-known etiological agent (smokeless tobacco), and run a different course to that of oral leukoplakia, and therefore should be classified separately (12, 48).

The epidemiology of oral leukoplakia in black persons is not well documented, but it appears that the frequency of oral leukoplakia in blacks is lower than in whites (9, 46). When evaluating biopsy reports of oral lesions with a histopathological diagnosis of epithelial dysplasia, Kaugars, Burns (49) reported that black persons accounted for about 8% of cases while white persons for 91%; and Silverman, Gorsky (46) reported that of the 257 patients with oral leukoplakia, 97% of them were whites. In a South African study of archived histopathological material, it was found that 86% of oral leukoplakia were from whites and 9% from blacks (9).

The methodology of the present study is similar to the South African study mentioned above, and in the present study 80% of oral leukoplakias were found in white patients, 14% in black patients, 5% in asian patients and 1% in coloured patients (Table 3). The results of this study add further weight to the notion that in South Africa the frequency of oral leukoplakia in black persons is lower than in white persons (9), as it appears to be the case in the United States (46, 49).

This low prevalence of oral leukoplakia in black South Africans is difficult to explain. While one can argue that during apartheid time (pre-1994) black people may have had limited access to government/provincial health facilities and therefore less patients were diagnosed with oral leukoplakia, in the post-1994 era, all South Africans regardless of their ethnic background have equal opportunities to receive medical care in government/provincial hospitals.

The low frequency of oral leukoplakia in black persons may be attributed to the fact that in general, black persons seek medical treatment late in the course of their disease, and by the time of diagnosis, the oral leukoplakia has already undergone malignant transformation and progressed to oral squamous cell carcinoma (9, 50); and to the fact that in South Africa, black persons smoke tobacco less than do white persons (10, 11). Indeed, in this study, 62% of black patients with oral leukoplakia smoked tobacco compared to 82% of white patients with oral leukoplakia (Table VII).

This retrospective study of 95 patients with oral leukoplakia confirms other previous reports that oral leukoplakias usually occur between the fourth and seventh decades in life (2, 9, 33, 46); that oral leukoplakia may affect equally males and females (33, 51, 52); and that any site of the oral mucosa may be affected (2, 37, 46). While the buccal mucosa is the most frequently reported site affected by oral leukoplakia (2, 12, 46), in this study the floor of the mouth was the site most commonly affected. However data in the literature with regard to the sites affected by oral leukoplakia are highly variable owing to differences in factors such as selection criteria, methods of data collection and geographic location of the investigated population (8, 37, 52, 53).

In this study, 78% of the oral leukoplakias were associated with tobacco smoking, conforming to reports in the literature that between 70% and 90% of oral leukoplakias are associated with tobacco smoking (9, 46, 54); and 85% of the cases were of the homogenous type, in line with other studies reporting that more than 60% of oral leukoplakias are of this type (9, 52).

In this study, as it was also reported previously (9), there was no significant differences in site, clinical presentation (homogenous, non-homogenous) or in histological features between tobacco smoking associated and idiopathic leukoplakia (Table IX, XII, XIV).

There are several clinical, histopathological and cytogenetic factors associated with a greater risk of malignant transformation of oral leukoplakia: older age, larger lesions, specific sites (floor of the mouth, ventrolateral surface of the tongue, maxillary retromolar

and adjoining soft palate), idiopathic lesions, non-homogenous lesions, high grade epithelial dysplasia and lesions in which the keratinocytes harbour cytogenetic alterations associated with malignant transformation (8, 12, 35, 37).

The presence of epithelial dysplasia, particularly of the high-grade type, is an important indicator of possible future progression of the oral leukoplakia to squamous cell carcinoma (8, 14, 35, 37, 55). On reviewing the histopathological features of these study cases, 54% had no epithelial dysplasia (Table XIV). Of all the leukoplakic lesions with epithelial dysplasia, 67% had mild epithelial dysplasia, 31% had moderate epithelial dysplasia and 2% had severe epithelial dysplasia. The degree of dysplasia was not significantly associated with tobacco associated leukoplakia or the clinical type, but it was significantly associated with the oral site involved (floor of the mouth, ventrolateral surface of the tongue, p-value< 0.05).

Interpretation of the results of different studies with regard to the relation between epithelial dysplasia and different parameters of oral leukoplakia needs to be done with caution since the exercise of grading epithelial dysplasia is highly subjective with low interpersonal reproducibility (7, 50).

This study has two main limitations. Firstly, it is a retrospective study; and secondly it is based on archived histopathological reports. Most importantly the number of black persons was small, not giving optimal statistical strength to the results. However, despite

these limitations this study provides some important differences in the clinicopathological features of oral leukoplakia between black persons and white persons.

CHAPTER 7

7. CONCLUSION

In keeping with the prime objective of this study, the clinicopathological features of oral leukoplakia were defined in the different racial groups in the greater Johannesburg area of South Africa, and especially in the black population.

As mentioned previously, only 14% of oral leukoplakia occurred in black persons compared to 80% in white persons. In contrast to white persons, black persons were diagnosed with oral leukoplakia at a younger age; there were more males affected than females; and the proportion of idiopathic leukoplakias was greater. There were significantly more black people (23%) with non-homogenous leukoplakia oral leukoplakia than white people with non-homogenous leukoplakia (13%), but there were significantly more white people (51%) than black people (23%) with dysplastic oral leukoplakia; and while in white people the floor of the mouth was the most frequently affected site, in black people it was the buccal mucosa.

APPENDIX

1: Data collection form

Oral Leukoplakia in a South African sample - A clinicopathological study

Form ID

Research project DP _____

File no _____ **Date** D D M M Y Y

Age ____ **Gender** Male | Female **Race** Black | White | Asian | Coloured

Alcohol Yes | No How many/ day _____ How long _____

Smoking Yes | No How many/ day _____ How long _____

Snuff Yes | No How many/ day _____ How long _____

Arecanut Yes | No How many/ day _____ How long _____

Family history of cancer Yes | No **Type** _____

Oral lesions Single | Multiple

Clinical classification Homogenous | Verrucous | Erythroleukoplakia | Nodular

Oral site	Single	Multiple	Midline	Right	Left
Commissure					
Floor of the mouth					
Upper lip					
Lower lip					
Upper labial mucosa					
Lower labial mucosa					
Buccal mucosa					
Upper alveolar mucosa					
Lower alveolar mucosa					
Upper gingival					
Lower gingival					
Dorsal aspect: tongue					
Ventral aspect: tongue					
Lateral aspect: tongue					
Base: tongue					
Oropharynx					
Soft palate					
Hard palate					
Upper retromolar plexus					
Lower retromolar plexus					

Histology

Hyperkeratosis without dysplasia	Hyperkeratosis with dysplasia	Mild dysplasia
Moderate dysplasia	Severe dysplasia	Carcinoma in situ
Carcinoma		

Histopathological report _____

2: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Rakesh Chandran

CLEARANCE CERTIFICATE	**M110614**
PROJECT	Oral Leukoplakia in a South African Sample-A Clinicopahtological Study
INVESTIGATORS	Dr Rakesh Chandran.
DEPARTMENT	Department of Oral Pathology
DATE CONSIDERED	24/-6/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 24/06/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Shabnum Meer

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the [illegible] **completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

3: Permission letter

health and social development
Department: Health and Social Development
GAUTENG PROVINCE

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Office of the Chief Executive Officer
Charlotte Maxeke Johannesburg Academic Hospital
Tell: 011 488 3792
Fax: 011 488 3753
Email: lindiwe.mngomezulu@gauteng.gov.za
Date: 20th May 2011

Dr. Rakesh Chandran
MSc. Dental (Oral Pathology)
University of the Witwatersrand

Dear Dr. Chandran

RE: "Permission to view clinical records of patients treated at the Charlotte Maxeke Johannesburg Academic Hospital"

Please note that your above request is provisionally approved. Your study can only commence once ethics approval is obtained.

Yours sincerely

Dr. T. Selebano
Chief Executive Officer

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