



P53 AND Ki-67 (MIB-1) EXPRESSION
IN
ODONTOGENIC KERATOCYSTS.

Thesis submitted in partial fulfillment of the requirements for the
degree of Master of Dental Surgery

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This thesis is dedicated to

Ayah & Ibu

Hanim, Sasha and Della

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available for loan and photocopying.

Adi Rahmadsyah

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CHAPTER 1
INTRODUCTION

Odontogenic keratocysts (OKs) are developmental cysts of the jaws and are known to express growth and behavioural characteristics more analogous to benign neoplasms than to simple jaw cysts. A number of studies on odontogenic keratocysts have emphasized that the aggressive behavior of these cysts might be associated with epithelial cyst lining activities.

The unusual behavior and the characteristic histological features of OKs have been the subject of many studies. More recently the results of a number of immunohistochemical studies have been reported in the literature. For example a small number of studies have indicated that p53 (p53 protein is a product of the p53 gene and acts as a checkpoint which monitors the efficacy and completion of the obligatory events in the cell cycle and blocks uncontrolled cell division) expression in OKs is higher than in other jaw cysts and that the location of p53 positive cells was more likely in the suprabasal epithelial cells. However, in these studies it is apparent that there is considerable variability when the results of different investigations are compared.

In addition to studies concerning p53 distribution in OKs, there have been a very small number of studies investigating the presence and distribution of another cell proliferation marker Ki-67 (MIB-1). To date the results of these few studies have indicated that OKs express Ki-67 (MIB-1) at a level greater than for p53.

Apart from recent studies by Slootweg (1995) who studied the distribution of p53 and Ki-67 in a sample of 13 OKs and Li et al (1996) who undertook a similar study on a sample of 22 OKs there are few data available describing the correlated distribution of p53 and Ki-67 (MIB-1) in OKs.

The **aims** of the present study were to:

- Analyse p53 and MIB-1 distribution in a large sample of OKs.
- Determine whether or not staining patterns correspond for each marker.

A small sample (9) of ameloblastomas were also analysed.

Fifty cases of OKs were examined for p53 and MIB-1 (Ki-67) expression using immunohistochemical techniques following microwave pretreatment of histological sections. The primary antibody used for immunohistochemical staining of OKs and ameloblastoma were monoclonal p53 (Dako-DO-7) and MIB-1 (Zymed).

CHAPTER 2
LITERATURE REVIEW



2.1. THE ODONTOGENIC KERATOCYST

Odontogenic keratocysts (OKs) are developmental cysts of the jaws. The cysts are thought to originate from odontogenic epithelium, such as dental lamina or its remnants within bone or oral mucosa, and from the proliferation of basal cells of the oral epithelium (Wilson and Shear, 1992; Browne, 1970b). Histologically, these cysts are characterised by keratin formation.

The term odontogenic keratocyst was introduced by Philipsen (1956) and its essential clinical and radiological features were described by Pindborg and Hansen (1963). At first, the term was applied to any odontogenic cyst which had undergone keratinization. However, Hansen (1967) concluded that odontogenic keratocysts were not of inflammatory origin.

Prior to classification of their terminology in the nineteen sixties OKs had long been known by different terms. In his book *Cysts of the Oral Region*, Shear (1992) stated that this cyst was described as a dermoid cyst by Mikulicz (1876) and then as a cholesteatoma by Hauer (1926) and Kosticka (1929). Although some authors had suggested the term "primordial cyst" as a synonymous term for odontogenic keratocyst, the former term is still not accepted worldwide because of the lack of evidence to support it.

The term primordial cyst had been used to describe cysts developing in place of a tooth through degeneration of the enamel organ. The term was first used by Robinson (1945) and, subsequently, used by the World Health

Organization in 1971. In 1985, Main and Shear suggested the term “odontogenic keratocyst” be used to designate this cyst of the jaws.

Histologically, the OK has a characteristic appearance. It can occur as a single cyst or multiple cysts. It can also be associated with the Basal Cell Naevus Syndrome (BCNS).

2.1.1. Clinical features

The clinical features of OKs, such as age, incidence, sex, the site of occurrence and the number and size of lesions are relatively variable.

The incidence of OKs reported in studies conducted between the 1960s and 1990s also varies. Compared to other jaw cysts, the incidence of OKs is relatively low. A study by Toller (1967) reported that 33 (11.0%) of 300 jaw cysts of all types were OKs. In contrast, a study by Hoffmeister and Harle (1985) showed that only 51 (1.5 %) of 3353 cases of jaw cysts recorded were OKs.

The incidence of OKs varies between white and black populations, with the white population more likely to suffer OKs than the black population. According to The World Standard Population, the incidence of OKs is higher in white males than in white females, black males and black females (Rachanis and Shear 1978).

OKs have been found in patients ranging in age between the first and ninth decades. However, the highest incidence of OKs (40-60 %) occurs between the second and third decades. Some authors have reported a bimodal age distribution with a frequency peak occurring in younger patients (second and third decades) and a second peak in the fifth decade or later (Toller 1967; Forsell 1980; Ahlfors et al 1984; Woolgar et al 1987). Although the number of cases encountered in a younger age group studied by Rachanis and Shear (1978) was higher than that of an older group, the latter group was actually at greater risk of diagnosis of OKs. It is unlikely that these cases represent different varieties of OKs. Rather it is likely that the cysts in the older age group have been present, but undiagnosed, for many years (Brown 1975). This accords with a characteristic of OKs, namely, that they may significantly extend in a proximal - distal direction, with little or no bone expansion.

On the other hand, the average age of patients with multiple keratocysts, whether accompanied or not by Basal Cell Naevus Syndrome (BCNS), as pointed out by Woolgar et al (1987), is considerably lower than the mean age of patients with single non-recurrent keratocysts. In this study, the average age of patients at the time of the removal of the cysts was 26.2 years, with a frequency peak at the age range of 10 - 19 years. By contrast, in the study conducted by Kakarantza-Angelopoulou and Nicolatou (1990), most patients, of whom only 7 were BCNS related, were in their fifth and sixth decades.

OKs occur more frequently in males than in females, and this sex predominance is more prominent in blacks than in whites, the ratios being

1.5:1 and 5.0:1, respectively (Shear 1992). Overall, data from different studies show a varying male to female ratio ranging from 1.35:1 (Brannon 1976) to 1:2 (Shear 1992). In the Kakarantza–Angelopoulou and Nicolatou (1990) sample, however, more than 73% of patients were males. In contrast, in patients with multiple keratocysts, with or without BCNS, females seemed to be more affected than males. Brannon (1976) reported that the incidence of OKs associated with BCNS was lower in males than female patients, with a ratio 1:1.71. Browne (1977) found the female to male ratio was 2:1, whereas, according to Woolgar et al (1987), it was 1:1.22 compared with 1:0.62 in patients with single OKs. The reasons for the differences in these findings have not been elucidated.

Basal Cell Naevus Syndrome, which is better known as Gorlin-Goltz Syndrome, has as its major features multiple basal cell carcinomas, multiple jaw cysts, skeletal anomalies, parietal and temporal bossing, calcification of falx cerebri, palmar and plantar pits and hypogonadism. There is a report describing squamous cell carcinoma arising from an odontogenic keratocyst in a patient with this syndrome (Moos and Rennie 1986).

Although OKs can occur anywhere in the jaw, the mandible is affected more frequently than the maxilla. The frequency of mandibular involvement in studies from the late 1960's to the late 1980's is such that more than two thirds of the OKs were reported from the lower jaws (75 %, Shear 1991; 77 %, Hansen 1967; 83 %, Browne 1970a; 65 %, Brannon 1976; 72 %, Vedtofte and Praetorius 1979; 78 %, Forsell 1980; 75 %, Ahlfors et al 1984; and 69 %,

Voorsmit 1981). These findings are supported by recent studies, such as that of Kakarantza-Angelopoulou and Nicolatou (1990), in which 65% of OK cases were found in the mandible, and the study of Brondum and Jensen (1991) which reported that 44 (82%) of 54 cases of OKs were in the mandible.

Most of the cases found in the mandible occurred within the posterior portion of the body, with or without extension to the ascending ramus. This finding was also noted by Kakarantza-Angelopoulou and Nicolatou (1990) and by Brondum and Jensen (1991), who reported that 50 % of cases involved the angle of the mandible and ascending ramus. Only 10 (18 %) of cases were found by Brondum and Jensen (1991) in the maxilla, of which 70% were located in the anterior and canine region, and 30% were in the molar area. However, Woolgar et al (1987), as cited by Shear (1992), reported that after the age of 50 years, there is a greater possibility of OKs being found in the maxilla.

Forsell et al (1980) observed that OKs found in the maxilla, were generally smaller and seemed to be clinically detected much earlier than those in the mandible. It was suggested that cysts in the maxilla are likely to become infected even when they are small in size.

OKs vary considerably in size. In Forsell's (1980) study, approximately 50 % of cases were 40 mm or more in size, cysts in the angle of the mandible and ascending ramus in particular being in this category, while cysts in the maxilla, and those involving the body of the mandible, were more likely to be smaller in

size. In addition, the study of Brondum and Jensen (1991) showed that 4 of 44 cases had cysts of more than 50 mm in diameter, 3 cases had cysts of 20 to 49 mm and only one case had a cyst of 15 mm in diameter.

There are no characteristic features by which a clinician might be guided to the diagnosis of OKs, and patients vary widely in their clinical presentation. On many occasions, patients are free of symptoms until the cyst has reached a large size. This is because, as previously mentioned, OKs tend to extend along the cancellous bone without causing significant bone expansion on the cortical plate until late in the clinical course of the lesion (Browne 1970a; Shear 1992). On presentation some patients will complain of pain, while those with large lesions will show evidence of bone expansion in the involved area. About one third of maxillary keratocysts will show buccal expansion, whereas palatal expansion is rarely seen. In the mandible, about half of all cases, particularly those involving third molars and/or an ascending ramus region, will exhibit buccal expansion, but lingual expansion is also not infrequently encountered. Like many other intrabony lesions, a large keratocyst may cause displacement of teeth, proptosis of eyeballs, bone perforation, destruction of the floor of the orbit and neurological symptoms (Voorsmit 1981; Lund 1985; Browne 1970a; Brannon 1976). If the cyst is infected or inflamed it may give rise to clinical presentations including cellulitis, abscess, trismus and fistula formation (Browne 1970a; Brannon 1976).

Most patients with OKs present with a single lesion, although it is sometimes a very extensive one. Some patients, however, may present with multiple OKs

at the time of diagnosis, or may develop multiple cysts throughout their lives. These patients may either have multiple cystic lesions of the jaws alone or the cysts may be related to the Basal Cell Naevus Syndrome (Gorlin-Goltz syndrome). This autosomal dominant condition is an inherited abnormality which expresses skin lesions, jaw and other skeletal lesions, central nervous system and eye lesions, as well as some fairly typical facial features, of which enlargement of the size of the skull, due to bossing of the frontal and parietal bones, and over-development of the supra-orbital ridges, are particularly common and noticeable features. The presence of OKs is one of the most common features of Gorlin-Goltz syndrome, affecting 65-75 % of all cases, and this tendency increases with age (Browne 1991; Shear 1992). No clear relationship between the occurrence of BCNS and OKs has been established, but the cysts are thought to be derived directly from dental lamina (Shear 1992).

2.1.2. Radiological features

The radiographic appearance of OKs is variable. Generally it is described as a well-demarcated, round to ovoid, radiolucent area with a distinct radiopaque border. The margin can sometimes be scalloped, with a smooth periphery, or can be partly diffuse (Browne 1970a; Brannon 1976; Shear 1992).

OKs can also present unilocular and multilocular radiographic features. The majority of lesions are unilocular radiolucencies, as shown in the study conducted by Browne (1970a). In 83 cases of OKs from both jaws, 47 (57 %)

of the cases in the study were unilocular, 17 cases (20 %) were loculated and 19 (22.0 %) were multilocular lesions. This distribution is similar to the work reported by Brannon (1976) in which 32 (61.5 %) of 52 cases were unilocular, 5 cases (9.6 %) were bilocular, 12 (23.0 %) were multilocular, and 3 had an ill-defined border.

2.1.3. Histopathological features

The histological features of OKs are characteristically distinguishable from other types of odontogenic cysts. The histological features of an OK can be divided into three parts. These are as follows: the cyst cavity which consists of protein fluid or a lumen full of keratin or blood; the lining epithelium, with keratinised stratified squamous epithelium; and the capsule, which comprises fibrous connective tissue.

OKs, as Browne (1970b), Brannon (1977), Forsell (1980) and Shear (1992) have described, are cysts with the characteristic microscopical feature of a thin-walled, collapsed and folded-lining epithelium.

Pindborg et al (1960) (as referred to by Brannon (1977)) designated the following histological criteria for OKs:

1. the cysts are usually lined by a very thin epithelium and uniform in thickness, with little or no evidence of rete ridges;
2. the basal layer is well defined and is cuboidal or columnar in shape with a palisaded arrangement;

3. the spinous cell layer is thin and often shows a direct transition from the basal cell layer, and the cells commonly show intracellular edema;
4. the keratin layer can be parakeratinised and orthokeratinised and the surface is often corrugated.

The terms orthokeratinised and parakeratinised describe the stage of keratinisation of the epithelial layers. Parakeratinisation is described as incomplete keratinisation that demonstrates the appearance of nuclei in the keratinised layer of the epithelium, whereas orthokeratinisation describes a complete keratinisation of the epithelial layers without nuclei in the keratinised layers (Lucas 1984; Domenici-Lombardo et al 1996).

The majority of the reported cases of OKs (80-90%) are parakeratotic, and only a few (10-20%) are orthokeratotic (Brannon 1977; Cohen and Shear 1980; Voorsmit 1984). Sometimes, different parts of the same cyst can be both parakeratotic and orthokeratotic. The different types of keratinisation present could possibly be due to the type of underlying basal cell layer.

A common feature of the lining epithelium of OKs is a keratinised stratified squamous epithelium 5-8 cell layers thick without rete ridges. However, there may be acanthotic irregularities and rete ridge proliferation which can occur in both parakeratinised and orthokeratinised cysts. The form of the rete ridges can be irregular in shape and formation, forming, for example, a broad, blunt type or a budding type (Brannon 1977).

Cysts with orthokeratinised lining often present cuboidal basal cells. On the other hand, a corrugated surface is often seen in parakeratinised cysts with a palisaded basal layer containing columnar or cuboidal cells or both.

Most of the cysts described in the study by Kakarantza-Angelopoulou and Nicolatou (1990) were lined by a thin, stratified squamous, parakeratinised epithelium with a corrugated surface without rete pegs. The epithelia had varying thicknesses, ranging between 5 to 15 cell layers thick. Only 24.14 % of cases had orthokeratinised epithelium and a granular cell layer. Columnar and cuboidal basal cells were found separately in some cysts or formed a mixed population in other lesions. Thirty-nine cases contained a flattened basal cell layer, which was thought to be related to the presence of orthokeratinised lining epithelium.

Some of the keratocysts described by Kakarantza-Angeloupuolo and Nicolatou (1990) and by Brondum and Jensen (1991) had a loose connective tissue capsule with subepithelial hyalinisation, with the epithelium separated from underlying connective tissue.

According to Forsell (from Brondum and Jensen 1991) OKs can be classified into five groups as follows;

- 1a thin, band-like parakeratotic cyst epithelium; basal cells cuboidal or columnar, accentuated, and palisaded;
- 1b epithelium composed mainly of basal cell layer only; basal cells accentuated;

- II orthokeratotic cyst epithelium with distinct stratum granulosum; accentuation of basal cells not pronounced;
- III cyst epithelium exhibiting areas of orthokeratinisation and nonkeratinisation;
- IV parakeratotic cyst epithelium resembling the oral mucous membrane;
- V cyst epithelium thin and parakeratotic; basal cells not accentuated.

The study by Brondum and Jensen (1991) demonstrated that 27 of 44 patients in their study fell into group Ia of the Forsell classification; no patients were in group Ib; 5 were in group II; only 1 female patient was in group III; 3 patients were in group IV and group V contained 8 patients. In their investigation, all patients had a variety of risks of recurrence according to the group into which they fell.

Toller (1967) described that cyst fluids from keratinising cysts had less than 3.5g per 100ml soluble protein, whereas non-keratinising cysts contain between 5.0 - 11.0g per 100ml, with a mean of 7.0g per 100ml. Toller (1967) concluded that OKs could be diagnosed if the cyst fluid contained less than 4.0 g per 100 ml of soluble protein.

There are some rare variations in the epithelial lining of OKS, which may be found. Brannon (1977) and Browne (1970b) reported that the epithelial cells may undergo metaplastic changes, as evidenced by the presence of mucous cells (reported in 2 - 4% of cases) and ciliated cells (occurring in 1 - 4% of cases). Hyaline bodies can occur, along with foci of inflammation where the

characteristic cyst structure is destroyed and the epithelium is non-keratinising (Browne 1970b; Yamaguchi 1980).

Although mitotic figures are a normal feature of OKs, it is interesting that most of the mitotic activity (90%) appears in the suprabasal layers of the epithelium (Browne 1970b; Brannon, 1977) whereas the occurrence of this activity within the suprabasal layer of the epithelium is unusual for normal stratified epithelia. Moreover, the mitotic value of OKs reported by Browne (1975) is relatively high (3.9 to 8.0), compared to the mitotic value from other types of cysts.

2.1.4. Behaviour

OKs have clinical growth behavioural features, which are more aggressive than those of other odontogenic cysts. Kakarantza-Angelopoulou and Nicolatou (1990) reported that the growth potential of epithelial cells from OKs is higher than that of the epithelium from non-keratinised dentigerous cysts. It has also been suggested that the type of keratin present in OKs can cause differences in the behaviour of OK. In the conclusion to their study, Brondum and Jensen (1991) pointed out that parakeratotic OKs with a thin, band-like epithelium and palisade-like basal cells have a higher recurrence rate than orthokeratotic OKs.

Partridge and Towers (1987) described OK cases showing paradoxical clinical behaviour in that they featured behavioural features of both benign neoplasms

and simple cysts. The authors suggested that surgeons should be aware of this because of the high rate of recurrence of the cysts.

A study conducted by Kakarantza-Angelopoulou and Nicolatou (1990) demonstrated that nine (10.4%) of 87 OK cases recurred. The authors suggested that the cause of the recurrences were not just due to the intrinsic nature of the lesion, but was also due to the differences in surgical technique and the experience of the surgeon. A study by Crowley et al (1992) demonstrated that the recurrence rate for OKs with parakeratinised epithelium was 42.6% (of 449 cases), and 2.2% for orthokeratinised OKs. There was 1 case (14.3%) in which both parakeratinised and orthokeratinised features recurred. The mean recurrence time was 6.7 years, with a range from 6 months to 41 years.

The reported average time for recurrence of OKs varies between studies. According to a study conducted by Forsell et al (1987), the recurrence rate of keratocysts in a 5-year follow-up study range from 35% to about 50%. In this study, the first recurrent cyst was seen 3 years after operation. The authors suggested that an active annual follow-up, over a 3-year period, should be sufficient for practical purposes in the context of monitoring for cyst recurrence.

2.1.5. Histogenesis and pathogenesis of odontogenic keratocysts

Although it has been suggested that OKs originate from remnants of odontogenic epithelium, there is still a lack of evidence concerning the origin of OKs.

The origin of OKs has been speculated upon by Soskolne and Shear (1967). They suggested that the cyst originates directly from dental lamina tissue, in addition to deriving from preappositional enamel organ epithelium. This suggestion was based on their examination of OKs from patients with BCNS. They also suggested that satellite microcysts in the wall of the main cysts also originate from dental lamina related to the main cysts. Even though many studies have been carried out to search for the link between keratocysts and BCNS, an answer has not yet been found. Some researchers have suggested, however, that there exists in some individuals a predisposition to keratocysts (Woolgar et al 1987; Lombardi et al 1995).

Given that some researchers consider the remnants of the dental lamina to be the source of cysts, such as OKs, one would expect such cysts to be located in the gingiva or in the adjacent tissue. However, some keratocysts are located in the ascending ramus, which does not have a relationship to a tooth follicle or dental lamina (Shear 1992). Another suggestion concerning the source of OKs is that they originate from basal cell offshoots or basal cell hamartias from the overlying oral mucosa (Shear 1992).

There are a number of reported cases of OKs associated with BCNS. Seven (8.8%) of the 87 OK cases reported by Kakarantza-Angelopoulou and Nicolatou (1990) were part of the BCNS, and Farndon et al (1992) concluded that recurrent OKs are one of the characteristics of BCNS. A study conducted by Wicking et al (1994) suggested that there may be a gene alteration that is responsible for BCNS. The gene responsible for BCNS has been mapped as 9q22.3-q31 in a 12-cM interval between the micro-satellite marker loci D9S12.1 and D9s109 (Wicking et al 1994) or between DNA markers D9S12 and D9S53 (Farndon et al 1992). However, no study has yet been conducted to locate the gene that is responsible for OKs.

Although the histologic and behavioural features of the OKs were delineated approximately 3 decades ago, additional studies by a number of investigators have not shed any new light on the biology of these lesions. However, the relatively recent development of a variety of immunohistochemical “probes” which allow investigation of both normal and pathological tissue relationship to such things as “growth promoters” and “inhibitors” affords new opportunities for investigation of OKs.

Two such “probes” are p53 and Ki-67 and both of these can provide data relative to the histologically expressed growth activity of tissue such as the epithelium of OKs.

In recent years there have been reports deriving from a small number of studies, which describe p53 distribution in OKs. More recently data describing

Ki-67 distribution in OKs have been reported. Analysis of these data shows that relatively small sample sizes have been employed by researchers and that results are variable. There is a need for further investigation of p53 and Ki-67 expression in OKs.

In the subsequent sections of this literature review the topics of p53 and Ki-67 are reviewed with reference to OKs where relevant.

2.2. p53

p53 is recognised as the most frequently mutated gene in a wide range of human cancers. Mutant p53 can be involved in programmed cell death, apoptosis and cell transformation. p53 is also a protein that can regulate transcription (Levine 1992a&b).

2.2.1. Initial identification of p53

p53 was first identified by Lane and Crawford (1979), in studies investigating the effects of the simian virus (SV40) on cells in culture. SV40 was found to have the ability to transform cells in culture and to produce tumours if injected into isogenic animals. Furthermore injected animals were found to produce antibodies against SV40. Subsequent analysis of animals injected with SV40-transformed cells resulted in the discovery and partial characterisation of p53 (Levine 1992; Linzer and Levine 1979).

2.2.2. p53 Structural characteristics.

p53 consists of three components. The first part is the amino terminus, which lies within residues 1-43. The amino acids have 5 spaced cluster domains, I to V, which are mainly involved in p53 activity. The acid rich residues of the N-terminus are responsible for its transcriptional activation.

The second portion is the central part of the molecule where there are highly conserved blocks, which consist of sequence-specific DNA binding-sites, which lie approximately within residues 100-300. Although this portion of the p53 molecule is recognised as having the highest sequence-specific DNA binding activity, it appears to have far less nonspecific DNA-binding activity compared to the full-length protein. This central portion of the p53 protein is also reported to be the location at which most mutations occur (Bargonetti et al 1993).

The third component is the carboxyl terminus. This portion lies within residues 300-393 where the sequences for oligomerisation and nuclear localisation occur. This highly basic C terminus has the ability to negatively regulate p53 binding to DNA, specifically in bacteria (Ko and Prives 1996). This terminus is subdivided into three regions according to function. The first region, known as a flexible linker, lies within residues 300-320 and connects the DNA binding domain to the tetramerisation domain. The second region is the real tetramerisation domain, which lies within residues 320-360. The third region is the extreme carboxyl terminus known as the basic rich region

(Bargonetti et al 1993; Prives 1993; Gottlieb and Oren 1996; Ko and Prives 1996).

Although the ability of p53 to regulate growth requires the full length of the protein, it appears that the carboxyl domain is the most complex and busiest portion of p53 (Prives 1993).

2.2.3. Molecular weight and location of p53 gene.

The human p53 gene is a single gene located in the short arm of chromosome 17, (which corresponds to chromosome 11 in mouse chromosomes) (Benchimol et al 1985). The location is specified as position p13.1.

The p53 protein is a product of the p53 gene. Human p53 has a molecular weight of 53,000 daltons (53 kDa).

2.2.4. p53 protein levels in cells

The level of p53 is high in a wide variety of transformed cells and tumour cells, regardless of the transforming agent (Benchimol et al 1982; Thomas et al 1983). Modulation of the half-life or stability of the p53 protein, is the major mechanism for controlling the p53 level in normal and transformed cells (Oren et al 1981; Reich et al 1983). Low levels of p53 with a half-life of about 20 minutes have been detected in normal cells, whereas much higher levels of p53 with a half-life of hours can occur in transformed cells (Reich et al 1983).

Researchers hold many different opinions about what induces the level of p53 in cells. One of the commonly accepted views is that p53 levels are at least partly controlled by a post-transcriptional mechanism associated with a decline in replicative DNA synthesis. (Kastan et al 1991). Other p53 inducing agents include ionising radiation, ultraviolet light irradiation (Greenblatt et al 1994; Midgley et al 1995), or the presence of DNA strand breaks (Ko and Prives 1996). Hypoxia, heat and starvation have also been found to induce a high level of p53 (Ko and Prives 1996).

2.2.5. Types of p53

The different behaviours of p53 are believed to be due to the different types of p53 found in various cell types. Wild type p53 and mutant p53 are the two main types of p53 involved in progression and control of the cell cycle.

2.2.5.1. Wild-type p53

Wild type p53 as found in normal cells is believed to be a negative regulator of cell growth.

Over-expression of wild type p53 in cells will block cell division in the G₁ phase of the cell cycle, which will prevent progression into the S phase (DNA synthesis) (Levine 1992a&b). The role of p53 during the late G₁ phase of the cell cycle, acting as a transcription factor or a protein regulating transcription factors in the cell, can be explained in two ways. First, p53 may regulate

several genes or products essential for entry into the S phase of the cell cycle. Secondly, p53 could play a role in DNA replication or the initiation of DNA replication (Lane and Benchimol 1990).

The relationship between expression of p53 and G1 arrest in cell cycles is due to the role of p53 in modulating damaged DNA before entering the S phase. p53 will initiate the arrest that allows cells time to repair and maintain genetic stability (Kastan et al 1992). This checkpoint activity of p53 does not just occur in the G1 phase, but also in the G2 phase. Transient G2 delay in human ovarian cancer cells is basically caused by an over-expression of p53.

2.2.5.2. Mutant p53

p53 mutations are the single most common genetic alteration observed in human cancers. Mutation in tumour suppressor genes is a complex biochemical process which appears to be due to a combination of both endogenous biological processes and exogenous carcinogens.

Loss of function in alleles is thought to be one of two indicators of p53 mutation. Loss of allele function could be induced by deletions, insertions or re-arrangements in one or both p53 alleles. Missense mutation in one allele will produce an altered protein, while the other allele may be inactivated, be lost or not produce gene products. The other indication for missense mutation is increasing oncogenic activity (Levine et al 1991; Vogelstein et al 1992).

Mutation in tumor suppressor genes is quite distinct from that in oncogenes. Mutation in oncogenes commonly occurs in 1 of 2 alleles of the gene, which act in a transdominant fashion out-weighing the growth suppressor effects of endogenous wild type p53 (Finlay et al 1989). Consequently, the alleles endorse mutations that fundamentally or permanently signal the cell to divide. Mutation in oncogenes appear to arise spontaneously in somatic tissues over the lifetime of the organism and naturally occurring inherited forms of these mutations are not known.

Mutation in tumor suppressor genes occurs in several different codons, but is not randomly distributed in the gene. In several murine and human tumours, mutation occurs in both alleles of the tumour suppressor gene (Mowat et al 1985; Baker et al 1989). Mostly however, mutation occurs in one allele of a tumor suppressor gene that will inactivate the function of its protein, and the second allele is lost by lessening the tumor cell or by gene conversion to homozygosity (Levine et al 1991).

Mutation of p53 has now been recognised in a wide variety of human tumours. The origins of mutation of p53 protein are distinct in different types of tumours. The cell type, capacity for DNA repair and metabolic differences together with exogenous factors are among the factors that could account for differences in the origin of p53 mutations (Hollstein et al 1991).

Temperature is one of the factors that can regulate the mutation of p53 protein. Most of this protein in cells is in a mutant conformation at 39.5 °C, and

will be localised in the cytoplasm, whereas at 32.5 °C, the majority of this protein is in the wild-type (normal) conformation and is located in the nucleus. Transformed cells will multiply rapidly during the G1 phase of the cell cycle at 39.5 °C, whereas at 32.5 °C, the growth of these cells is stopped and the cells are terminated at the G₁/S border of the cell cycle. Wild-type proteins enter the nucleus at the beginning of the S phase (Hinds et al 1987; Michalovitz et al 1990 and Martinez et al 1991). At 37 °C, both mutant and wild type p53 proteins are present in the transformed cells and are located in the cytoplasm during the G1 phase of the cell cycle. Mutant p53 protein is not temperature-sensitive and remains in the cell cytoplasm throughout the cell cycle (Hinds et al 1987).

The description that led to the idea of p53 as an oncogene was based on studies that showed some DNA and p53 clones in culture could immortalise cells (Jenkins et al 1984; Finlay 1989; Levine 1992a&b). The clones contributed to transformation of the cells if transfected into rat embryo fibroblasts along with the activated *ras* oncogene (Eliyahu et al 1984).

Transformation and immortalisation activities are thought to be two separate activities (Eliyahu et al 1985). According to Eliyahu et al (1988) and Finlay et al (1989) the wild type p53 gene does not transform cells. In fact, as previously described it could inhibit the transformation of cells and block the growth of the cell in the late G1 stage of the cell cycle. On the other hand, mutant p53 behaves like an oncogene and although over-expression of

mutant p53 protein will lead to a high potential for tumourigenic activity in cells, it will not cause gross morphological changes (Eliyahu et al 1985).

2.2.6. Biological functions of p53

Although there are differing opinions among researchers concerning the functions of p53 (and this has led to some confusion), the functions of p53 can be categorised as falling into several main areas. For example it has been suggested that p53 could act as a checkpoint control in the cell cycle and in genetic stability (Prives and Manfredi 1993). It has also been suggested that p53 could also have a role in programmed cell death (apoptosis) if the repair process is halted (Gottlieb and Oren 1996). A role for p53 in cellular differentiation has also been suggested at least in relation to hematopoietic lineages (Gottlieb and Oren 1996; Prives and Manfredi 1993).

To fulfill these functions, it has been suggested that p53 could act as transcription factor in gene expression in cells. Together with other proteins (by binding to specific binding-sites), or by itself in an oligomeric protein complex, p53 could recognise DNA sequences in enhancer and/or promoter elements of a gene such that it effectively controls that gene (Levine 1992a&b).

2.2.6.1. Checkpoint of cell cycle.

Checkpoint of cell cycle is a mechanism that controls and imposes dependency on each of the phases in the cell cycle. Every phase in the cell cycle should depend upon the completion of earlier events. A cell cycle with lack of checkpoint such as occurs in mutations would decrease the dependency of the events. For example mitosis could occur without completion of DNA synthesis (Hartwell and Weinert 1989).

p53 is classified as one of the tumor suppressor genes. This type of gene product negatively regulates cell growth or division. p53 has DNA binding properties and is commonly thought of as a negative regulator of cell growth. In the cell cycle, p53 acts as a checkpoint which monitors the efficacy and completion of the obligatory events in the cycle and blocks uncontrolled cell division (Fields and Yang 1990; Hartwell and Weinert 1989; Levine 1992a&b; Levine et al 1994).

It has also been suggested that in response to cell DNA damage, p53 activate one of two pathways in damaged cells. In damaged cells, division will stop until the DNA has been repaired. This pathway is known as the growth-arrest pathway. This ability of p53 to act as a 'guardian of the genome' has been suggested as an important role for p53 in response to DNA damage and in the developmental processes (Clarke et al 1993; Lane 1994; Gottlieb and Oren 1996).

2.2.6.2. Apoptosis

Wild type p53 is now believed to be also involved in programmed cell death (apoptosis). Evidence for this role has accumulated from a number of studies. For example, Yonish-Rouach et al (1991) showed that cells with a lack of endogenous p53 expression, such as the murine myeloid leukaemic cell line, will continuously lose viability and will die if the production of wild type p53 is stimulated. Involvement of p53 in physiological apoptosis has been observed in p53 deficient thymocytes. Lymphoid stem cells of the small and large intestine in p53 (-) mice were resistant to apoptosis when the mice were irradiated, but p53 (+) mice did not respond to the treatment (Clarke et al 1993; Lowe et al 1993).

Accumulation of p53 protein contributes to the extent of apoptosis. A clear inverse relationship has been noted between p53 positivity and apoptosis. Wild-type p53, which has a short half-life, is an effective apoptosis inducer, whereas mutant p53, due to its longer half-life and its inability to transactivate the *bax* gene (which encodes a protein that binds to bcl-2, a well-known apoptosis suppressor) is not efficient at inducing apoptosis (Eerola et al 1997).

2.2.6.3. Cellular differentiation

The involvement of p53 in cell and tissue differentiation and development is not clear. However, there is some accumulating evidence that p53 is involved

in such activities. For example, during normal tissue regeneration after injury, over-expression of p53 inhibits cell proliferation which will downregulate cellular growth in the healing stage. The highest expression of p53 has been recorded during the fifth day after injury, at the same time as epithelialisation of injured tissue. (Antoniades et al 1994). Expression of wild-type p53 has also been found to inhibit angiogenesis, one of the factors needed for tumour growth (Dameron et al 1994).

2.2.7. Detection of p53 in cells

p53 protein can be detected using antibodies by the standard avidin-biotin peroxidase (ABC) immunohistochemistry technique. This bridge technique is one of the most common indirect techniques used in immunohistochemistry. The technique is carried out using a complex of primary antibodies against the antigen and avidin is bound to biotinylated peroxidase (the secondary antibody). The ABC complex is a lattice-like, three-dimensional formation and will capture several molecules of horseradish peroxidase at the site of the antigen in the section.

2.2.8. Application of p53 in tumour diagnosis.

Expression of p53 protein has been extensively used as a marker of malignancy and pre-malignancy in humans for almost two decades. More than 50% of human cancers have been found to express p53 protein. Detection of p53 mutation in cancers can contribute to increased understanding of the

aetiology and molecular pathogenesis of neoplasms (Hollstein et al 1991; Levine et al 1991). Further, p53 has an important role in studies aimed at better defining the diagnosis, prognosis and treatment of a variety of cancers.

The literature relating to p53 and tumours is vast. Review of this field is outside the reach of this study. However, brief reference is made to a small number of recent studies to illustrate examples of work which has been carried out.

There have been reports suggesting that colorectal cancers express genetically altered p53 tumour suppressor genes in the range of 44.8% (Freedman et. al 1996) to 76% (Ilyas et al 1996) of cases. These investigators also suggested that there is a correlation between colorectal cancers, familial history and diet.

p53 gene defects have been found to influence the behaviour and the prognosis of colorectal adenocarcinomas (Benhattar et al 1996; Goh et al 1996). Benhattar et al (1996) indicated that patients whose tumours expressed mutant p53 would have a shorter life expectancy compared to the patients with wild type p53. These findings demonstrated that expression of p53 protein in colorectal carcinomas could be a good indicator for diagnostic and prognostic purposes as well as for epidemiologic assessment.

Overexpression p53 protein has also been used as an indicator for diagnostic, prognostic and treatment purposes for tumours in the upper aerodigestive tract. (Bennet et al 1992; Tan et al 1996; Tan and Ogden 1997.)

Expression of p53 protein has also been used as an indicator for prognostic outcome assessment in prostate cancer. Over-expression of p53 protein was reported within the range of 5% - 62% of stained cells in prostate cancers (Apakama et al 1996; Brook et al 1996). Brook et al (1996) observed that the number of positive cells and the density of staining in metastatic cases was higher compared to the stainability of p53 in primary cases (Brook et al 1996).

Kato et al (1996) found that over-expression of p53 protein in breast cancer patients only had significant correlation with oestrogen and progesteron receptors but not with tumor size, nodal status, obesity, and histopathological tumour grade. The investigators concluded that over-expression of p53 protein was not a significant prognostic indicator for recurrence and survival in human breast cancer cases. Kristen et al (1997) suggested that p53 over-expression did not effect overall survival of patients with node negative breast cancer but that the expression of p53 had significantly correlated with tumour size.

Expression of p53 protein has also been described as a useful indicator for prognostic purposes in relation to neoplasms such as lung cancers and ovarian cancers. For example expression of p53 protein was found to be

similar in primary (62%) and secondary (66%) ovarian cancer sites. (Daidone et al 1996).

Evidence demonstrating the presence of p53 in benign neoplasms is equivocal. p53 expression was used by Yaziji et al (1996) to differentiate reactive from neoplastic lesions such as in gliosis and malignant astrocytic lesions. In a study conducted by Lee and Teh (1994) these investigators demonstrated that p53 expressed in all (100%) cases of pseudoepitheliomatous hyperplasia, 78.8% of cases keratoacanthoma and 75% of squamous cell carcinomas. These investigators also found that the stainability for p53 in pseudoepitheliomatous hyperplasias and keratoacanthomas was less dense compared to that of squamous cell carcinoma.

Ueda et al (1993) found that p53 (Pb240 which stains only mutant p53) was completely negative in all benign and reactive lesion.

2.2.9. p53 protein expression in oral lesions

Recent studies show that most invasive squamous cell carcinomas, some carcinomas *in situ*, and dysplasia from various sites in the human body, including the oral mucosa, have higher rates of p53 expression than normal epithelium (Ogden et al 1992b; Warnakulasurya and Johnson 1992; Regezi et al 1995, Piffko et al 1995). Regezi et al (1995) have suggested that the appearance of p53 in dysplastic epithelium and *in situ* carcinoma, and in the

normal appearing keratinocytes at the margin of carcinomas, may designate an early stage in the development of malignancy.

The study conducted by Regezi et al (1995) showed that 6 of 13 cases with oral epithelial dysplasia stained positively for p53 protein. The oral dysplasia of 3 of these patients progressively developed into p53 positive invasive carcinoma, 1 led into more advanced grade p53-positive dysplasia, 1 developed into p53-negative verrucous carcinoma, and 1 represented a p53-positive dysplasia developing 5 years after treatment of a p53-positive carcinoma (Regezi et al 1995). A high level of p53 has also been reported in pre-invasive and invasive oesophageal carcinomas (Bennet et al 1992).

Ravi et al (1996) suggested that p53 protein in oral malignancies would be of a mutant p53 type. This opinion concurs with that of Kusama et al (1996) who stated that p53 gene mutation may be involved in any stage of carcinogenesis of the oral epithelium.

There is some evidence that p53 can be found in normal mucosa. In a recent study conducted by Ogden et al (1997a&b) it was found that p53 protein was expressed on tissue taken from clinically and histopathologically normal mucosa of cancer patients and non-cancer patients. The authors suggested that expression of p53 protein in normal mucosa did not necessarily indicate the evidence of a further tumour.

The efficacy of p53 protein as a diagnostic and prognostic indicator for odontogenic cysts has yet to be confirmed. The presence of p53 has been identified in a variety of jaw cysts (Ogden et al 1992a; Slootweg 1995; Li et al 1996). Specifically, expression of p53 positive cells has been found in the epithelial lining of keratocysts (ranging from 40 - 80% of the cysts investigated), dentigerous cysts (40%) and radicular cysts (Ogden et al 1992a; Slootweg 1995; Lombardi et al 1995; Li et al 1996).

2.3. Ki-67 and its functions

2.3.1 Ki-67

Ki-67 is a monoclonal antibody that reacts with a human nuclear antigen associated with cell proliferation. This antibody was first generated against antigens in proliferating cells in a Hodgkin's disease cell line (Gerdes et al 1984; Gown and Leong 1993).

Ki-67 was first identified in studies on the production of monoclonal antibodies against nuclear antigens specific to Hodgkin's and Steinberg-Reed cells. It was found that most monoclonal antibodies generated against the nuclei of the Hodgkin's cells were reactive with the nuclear structure of all the cells tested, whereas only Ki-67 reacted with a nuclear antigen restricted to cells that are known to proliferate (Gerdes et al 1983). Furthermore, Gerdes et al (1984) and Gown and Leong (1993) reported that the antigen disappeared when proliferating cells were induced to differentiate into resting cells.

Experiments have shown that the Ki-67 nuclear antigen is present in the S, G2 and M phases, but is absent in the G0 phase (Gerdes et al 1983). Ki-67 is absent in the early events of mitosis, but occurs positively in G1 after mitosis.

Despite the fact that Ki-67 has been employed in experiments to indicate proliferative activity since 1984, it was not until 1991 that Gerdes et al (1991) described the characteristics of the Ki-67 antigen through immunobiochemical and molecular biology techniques. Gerdes et al (1991) found that the Ki-67 antigen was highly susceptible to protease treatment and could not be extracted by 0.1 normal HCl, which meant that it was a non-histone protein. Furthermore, it was found that the Ki-67 antibody recognised a huge protein doublet assembled by polypeptide chains with a molecular weight of 345 kd and 395 kd. through immunoblot analysis. The bands were detectable in lysates extracted from proliferating cells but were absent in quiescent cells (Gerdes et al 1991).

The Ki-67 gene was described by Gerdes et al (1991) by screening a lambda gt11 human cDNA expression library and by identifying a 1095 base pair (bp) partial clone which encoded the Ki-67 epitope. Analysis showed that the clone consisted of three highly repetitive and very basic DNA sequences with a high level of homology, each about 366 nucleotides in length. A very high conserved region (62 bp long) and cysteine residue were found in the repeats (Gerdes et al 1991; Sawney and Hall 1992).

Fonatsch et al (1991) encoded the Ki-67 gene by using a large mRNA transcript, which was expressed in cycling cells. By using cDNA, the location of Ki-67 was then confirmed as having chromosome location 10q25 (Fonatsch et al 1991).

2.3.2. MIB-1 as an alternative to Ki-67

Although Ki-67 has been widely used as a cell proliferation-associated marker since its discovery in 1984 by Gerdes et al, the use of this monoclonal antibody is restricted to frozen tissue sections only. It is believed that the epitope of the antigen is damaged during tissue processing by such agents as fixatives. This restriction has been an impediment for retrospective studies, which mostly employ formalin-fixed and paraffin-embedded tissue. Not until 1991 was a Ki-67 equivalent murine monoclonal raised. The new monoclonal antibody, called MIB-1, was generated from part of the Ki-67 molecule as reported by Gerdes and colleagues (1991). This antibody can be used with both formalin-fixed and routinely processed tissue.

MIB-1, MIB-2 and MIB-3 were confirmed as true Ki-67 equivalents and have shown a similar immunostaining pattern to that of Ki-67 in frozen sections, with the epitope reacting with MIB-1 staining more strongly than it does when labelled with Ki-67. However, the MIB-2 antibody, is negative in paraffin sections (Cattoretto et al 1992; Cuevas et al 1993).

The distribution of cells stained with Ki-67 and MIB-1 was reported by Hendricks and Wilkinson (1994) to be the same after microwave treatment of section prior to the immunostaining procedure. However, the stainability produced by the Ki-67 antibody was weaker than that of MIB-1.

Barbareshi et al (1994) found that the number of cells stained with Ki67 and MIB-1 was slightly different in frozen and fixed sections of breast carcinoma. They found that the mean and median values of MIB-1 Labeling Index (LI) were almost double the values of Ki67. They concluded that the difference was possibly due to the ability of the MIB-1 epitope to survive in frozen and acetone fixed circumstances. These investigations also suggested that MIB-1 would be a better substitute for the Ki67 monoclonal antibody, because of its potential for use in paraffin sections in retrospective studies, and because it produces clearer staining allowing optimal use with a computer-assisted image analysis system (Barbareshi et al 1994).

The qualities of immunostaining by cell proliferation-associated markers may be influenced by the effect of differences in fixation time and microwave heating time. Hendricks and Wilkinson (1994) reported 4 hours to be a suitable time for fixing tonsil tissue, prior to MIB-1 immunostaining, regardless of the duration of microwave pretreatment of sections. In contrast the stainability decreased if the tissue was fixed for 48 hours unless the heating time was increased to at least 14 minutes. They also suggested that prolonged heating time up to 49 minutes did not have any effect on stainability. These investigators suggested that tissue should be fixed as

soon as possible and concluded that the effects of a postponing of fixation may vary between tissue type.

2.3.3. Ki-67/MIB-1 as indicators of cellular proliferation.

Since Ki-67 is present during the phases of the cell cycle designated as late G₁, S, M and G₂ (except for the G₀ phase), it has been employed for more than a decade as a marker for cell proliferation rate as an alternative to earlier methods of tumour proliferation, such as mitotic indices, which are restricted to one phase of the cell cycle.

An early report on the Ki-67 antigen (Gerdes et al 1984) showed that Ki-67 was absent between the G₀ and G₁ phases of the cell cycle, but present in the G₁ phase. The expression of the Ki-67 antigen increases as the cell cycle progresses, rising during the latter half of the S phase and reaching a peak in the G₂ and M phases. Ki-67 rapidly degrades after mitosis, with the half-life of the detectable antigen being an hour or less.

2.3.4. Expression of Ki-67 / MIB-1 in tumour cells

Cell proliferation rate is a useful indicator for determining prognosis and choice of treatment for patients with malignancies. Levine et al (1997) suggested that a high level of Ki-67 expression is one of few indicators for poor prognostic and poor survival rate in soft tissue sarcoma. Meanwhile, Valente et al (1994) found for oral squamous cell carcinomas that there is no

significant correlation between the number of Ki-67 positive cells at diagnosis and after treatment with radiotherapy. However, these investigators found that significant differences emerged after the first course of 10 Gy radiotherapy.

In recent years there have been a number of studies reporting the use of Ki67 or MIB-1 as cell proliferation markers in relation to a variety of malignant neoplasms. The growth fraction of breast cancers can be determined by using monoclonal antibody Ki-67. Gerdes et al (1986) demonstrated that the number of positive cells against Ki-67 in mammary lesions varied between benign and malignant lesions but showed no correlation between histological grades of malignancy and size of the growth fraction in breast cancers of their samples.

The measurement of proliferative activity of tumour cells, as defined by MIB1 immunohistochemical analysis, has a potential prognostic value in-patient with hepatocellular carcinomas. Ng et al (1995) found that the number of cells stained by MIB1 was significantly higher in less well differentiated, compared to well differentiated, hepatocellular carcinomas ($p=0.017$). Although statistically insignificant, the MIB-1 score was also higher in non-encapsulated, compared to encapsulated, hepatocellular carcinomas (Ng et al 1995).

Zidar et al (1996) found that the pattern of Ki-67 positive cells in the epithelium of laryngeal lesions correspond to their histological grade. In

normal laryngeal epithelium and reactive lesions such as simple hyperplasia, the location of most of Ki-67 positive cells is the basal layer, whereas in abnormal and atypical hyperplasia, the number of positive cells increases and the location of positive cells tends to be in the suprabasal layer (Hall et al 1990; Zidar et al 1996). Similar findings were also reported by Ichikawa et al (1997) where the number of Ki-67 positive cells increased according to the severity of dysplastic changes in hyperplastic, precancerous and cancerous oral lesions.

Zidar et al (1996) suggested that Ki-67 was a better proliferation marker compared to proliferative cell nuclear antigen (PCNA). In their study of epithelial lesion of larynx, Ki-67 staining demonstrated a clear border of positive stained cells of basaloid cells spinous cell layer, whereas PC-10 (an antibody for PCNA) expressed weak positive staining cells scattering in the spinous cell layer of laryngeal epithelium (Zidar et al 1996).

Expression of Ki67 has been used as an indicator of the malignancy of neoplasms. A clear relationship between MIB-1 staining and histological grade, tumour size, tumour type and survival rates of patients with breast carcinomas was reported by Pinder et al (1995). The investigators also suggested that MIB-1 labelling index can be used as a predictor for patients survival.

Li et al (1995) found that expression of Ki-67 was significantly higher in solid ameloblastoma of follicular type compared to cystic tumour lining, intra luminal

nodules and invading islands of unicystic ameloblastoma. They suggested that the results of their study indicated that epithelial proliferative potential was different in different areas of unicystic ameloblastoma and between solid and unicystic ameloblastomas. These findings concurred with the latest results of Ong'uti et al (1997) where the follicular type of ameloblastoma had significantly higher LI than plexiform ameloblastoma. Ong'uti et al (1997) found that there was no significant correlation between labeling index (LI) and clinical features such as age, sex and tumour size, but noted there was a correlation with histological pattern of the epithelium in ameloblastomas. Kato et al (1997) reported that Ki-67 Labeling index (LI) of 4.7% on sections from leiomyoma of the mandible did not seem to correlate with clinical features such as rapidity of growth.

Only a very small number of studies have been carried out using Ki-67 expression to emphasize the proliferative activity of odontogenic cyst epithelium (Woolgar et al 1995; Slootweg 1996; Li et al 1996). However, those studies involving analysis of expression of Ki-67 in odontogenic cysts had concluded that OK's have a greater number of Ki-67 positive cells compared to other odontogenic cysts and inflammatory cysts (Slootweg 1995; Li et al 1996).

CHAPTER 3
MATERIALS AND METHODS

3.1. Materials

3.1.1. Case selection

The odontogenic keratocysts used in this study were selected from the files dated from 1st January, 1970 until 31 December, 1990, in the Department of Oral Pathology and Oral Surgery, University of Adelaide. The specimens had been received by the Department for histological assessment from dental practitioners and medical practitioners in private practice, from various clinics of the Royal Adelaide Hospital and Queen Elizabeth Hospital in Adelaide, and from the School Dental Service of South Australia.

The cases were diagnosed as OKs by an authorised pathologist of the Department at that particular time with reference to the standard criteria characterised by Shear (1960) Brannon (1977) Browne (1970b) and the World Health Organization (1992). Clinical data recorded in the files included the location of the cyst, the age at which patients first noticed the appearance of the cyst, gender, relevant medical data and provisional diagnosis. The cases selected for this study were re-examined retrospectively by the author and one other observer relative to the criteria characterised by Shear (1960), Browne (1970b), Brondum (1970), and the World Health Organization (1992).

Also documented in the files was such information as behavior and history of the cyst (single, multiple, recurrent) and whether the patient had Gorlin-Goltz

Syndrome (BCNS). Histologically, the lining epithelium of the cyst was categorised with respect to the type of keratinisation.

Most of the OKs selected for this study were re-cut and restained. Sections five microns thick were cut and stained with hematoxylin and eosin (H&E) for routine observation and for selection of cases for immunohistochemical study.

From 124 OK cases recorded from 1970 to 1990, 50 cases were finally selected for immunohistochemical analysis. The selection was based on criteria such that:

1. the specimen should demonstrate the characteristic epithelial features of OKs (WHO, 1992);
2. the OK should not be a dentigerous keratocyst;
3. the specimen should not be so severely affected by inflammation that the characteristic features of OKs could not be clearly identified;
4. the specimen should not have undergone decalcification.

Clinical data including age and sex of the patients and site of the lesion were recorded for these 50 cases.

Nine specimens of ameloblastoma from the Oral Pathology and Oral Surgery Department files between 1970 and 1990 were also selected for a pilot analysis regarding expression of p53 and Ki-67.

Two primary antibodies were used for immunohistochemical analysis as follows:

1. DO-7 - a monoclonal antibody that reacts with wild and mutant types of the intercellular p53 protein. DO-7 is a mouse anti-human antibody, which will show the accumulation of mutant p53 protein in tumors from a broad spectrum of cell lineages. DO-7 is produced by DAKO (Australia) Pty Ltd.
2. MIB-1 (Zymed) - a murine monoclonal antibody that reacts with the Ki-67 nuclear antigen. MIB-1 reacts against a nuclear antigen expressed in proliferating cells. It recognizes the antigen within cells in the G₁, S, M and G₂ phases, but not within cells in the G₀ phase.

3.2. Methods

3.2.1. Preparation of routine histologic material

Where sufficient paraffin-embedded file tissue remained, fresh 5 micron sections were cut from blocks pertaining to the 124 cases selected for analysis. In a number of cases insufficient file material remained and in these cases file copies of H&E stained sections were reassessed.

In the case of freshly cut sections, 1 of 4 sections from each specimen selected was restained using hematoxylin and eosin (H&E) and mounted on a glass slide, previously coated with APT (3-aminopropyl-triethoxy-silane). The

slides were dried overnight in a 37° C oven. All fresh and previously H & E stained sections were examined to confirm the diagnosis of OK.

3.2.2.2 Assessment of clinical data and histologic material

3.2.2.1 Clinical data

Clinical data relating to each case was recorded as follows:

3.2.2.1.1. Age

The age of subjects was taken from the recorded clinical examination data available in the files.

3.2.2.1.2. Sex

The sex of patients was recorded according to the clinical examination available in the files.

3.2.2.1.3. Site

The site of occurrence of the cyst was determined on the basis of the clinical information in the files and was recorded according to the classification described by Raden and Reade (1973a).

3.2.2.2. Histological assessment

Histological examination of the 124 original cases was carried out using an Olympus CH optical microscope. Photomicrographs were taken using a Leitz Ortholux unit and Pan X film.

3.2.2.2.1. Epithelial lining

The epithelial linings of OKs in this study were classified according to the type of keratinization present. Keratinization of the epithelial lining was characterised as parakeratinised, orthokeratinised, or a combination of both. Irregularities, such as abnormality in thickness, rete-ridge budding and metaplasia in the epithelial lining were also recorded.

3.2.2.2.2. Number of cysts and behavior

The number of cysts occurring at one time, and the appearance of microcysts, were recorded from the history and clinical data in the files. Information on recurrence was also obtained from the file or from surgery.

3.2.2.2.3. Inflammation

Inflammation was recorded according to its presence type and severity. The presence and severity of inflammation was classified such as mild, moderate or severe. The presence of and the severity of inflammation in the cyst

capsule and lining epithelium of the cyst were criteria that were considered in selecting cases for immunohistochemical study. Only cases showing an absence of inflammation or one or two focal areas of inflammatory cells in the cyst capsule were considered for the immunohistochemical study phase.

3.2.4. Immunohistochemical phase

For the immunohistochemical phase of this study, the sections were dewaxed, rehydrated and placed in phosphate-buffered saline (PBS). Immunohistochemical staining for p53 and Ki-67 (MIB-1) was performed after digestion with trypsin II at 37°C using standard techniques.

After immunostaining, sections were examined using an optical microscope. Stained sections were scored for the presence or absence of positive staining and according to the level of positivity of the staining for each antibody used. Levels of positivity of staining were recorded as follows:

- (-) no positive staining cells in the epithelial lining;
- (+) scattered positive cells in the epithelial lining;
- (++) few or moderate foci, discontinued positive cells along the epithelial lining;
- (+++) continuously positive cells in the epithelial lining.

Data deriving from this phase of study were collated and subjected to Chi-square analysis.

Photomicrographs were taken of representative sections using a Olympus camera and kodak print film.

CHAPTER 4

RESULTS

4.1. Clinical data

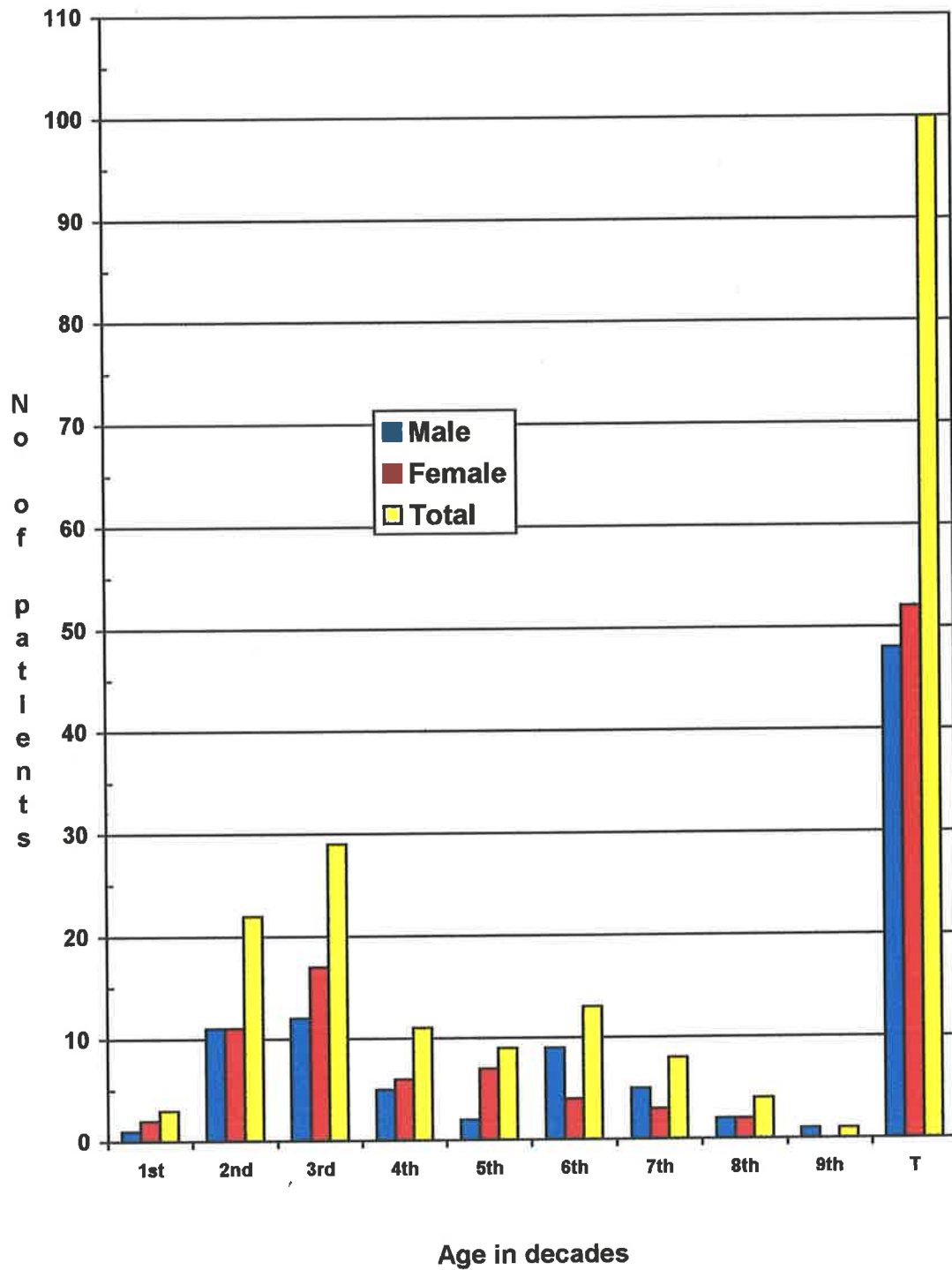
One hundred and twenty four cases of odontogenic keratocyst (OK) from 112 patients were recorded in the histopathology files Oral Pathology, Department of Dentistry for the years 1970 to 1990.

4.1.1. Age distribution of total OK pool

The OK cases recorded in the Oral Pathology files between 1970 and 1990 occurred in patients between the ages of 7 and 81 years (mean 34.9 ± 19) (Figure 1). The age of 12 individuals was unknown. Although the mean age of patients involved in this study was 34.9 ± 19 years, there tended to be 3 peaks of age distribution in the second, third and sixth decades (Figure 1).

4.1.2. Age distribution of selected OK cases

Fifty cases of OK were randomly selected from the original 124. The cases selected for immunohistochemical staining were from 46 patients with an age range from 7 to 79 years and a mean age of 31.6 ± 17.4 years. The peak age distribution of the sample was in the second decade (Figure 2). All four cases associated with Gorlin-Goltz Syndrome occurred in patients with an age range of age between 9 to 14 years.



**Figure 1: Age, sex distribution of all 100 patients recorded in files as having odontogenic keratocyst and for whom age/sex distribution data were available.
T = total male and female and total male plus female.**

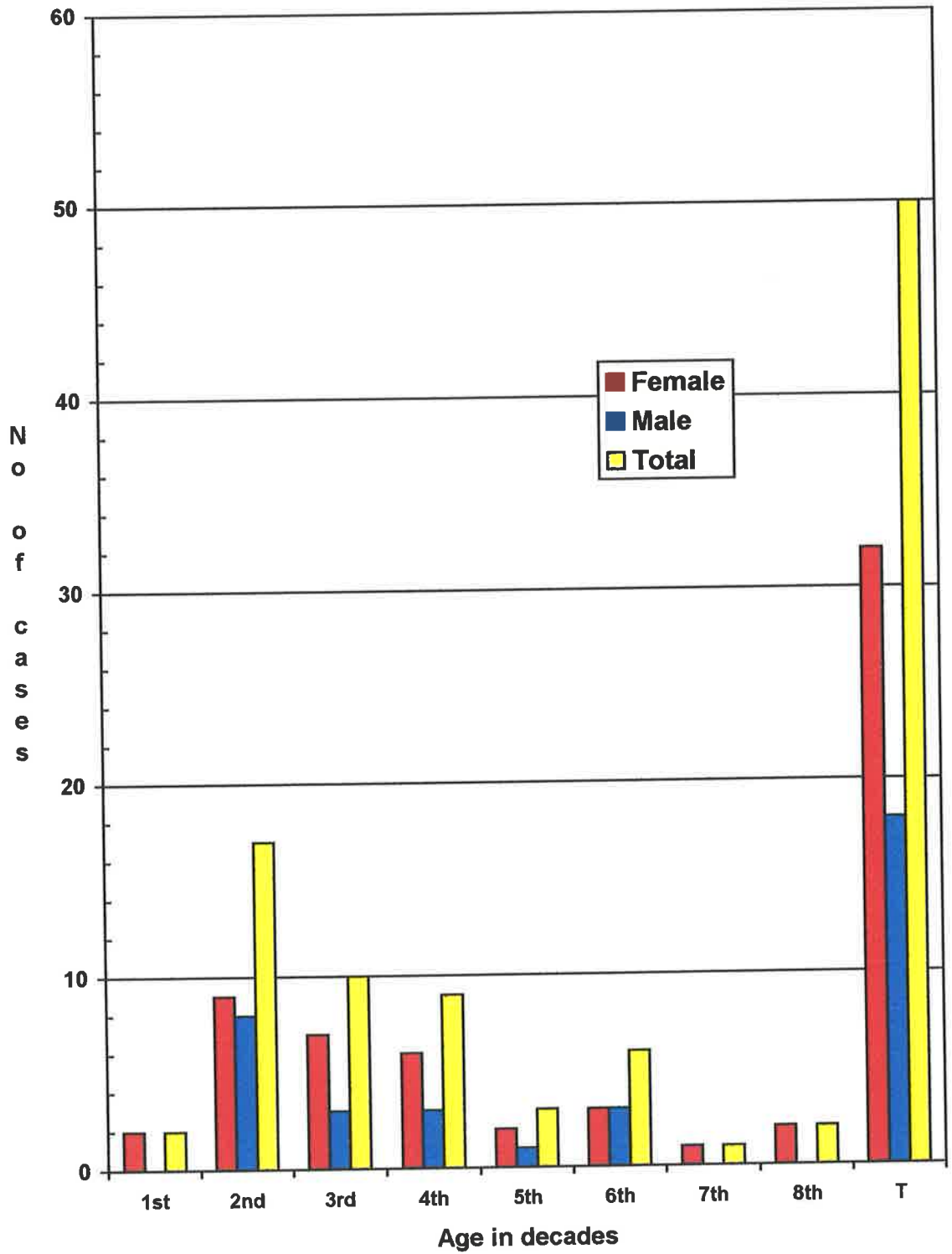


Figure 2: Age and sex distribution of the fifty OK cases selected for immunohistochemical study.
 T = total male and female and total male plus female.

4.1.3. Sex distribution of total OK pool subjects and immunohistochemistry pool subjects

Fifty-nine (52.67%) of the 112 patients were male and 53 (47.32%) were female. One female and 3 males, were recorded as having the features of Gorlin-Goltz Syndrome. Thirty-two (64%) of the 50 cysts selected for immunohistochemical study were from female subjects and 18 (36%) were from males (Figure 2).

4.1.4. Site distribution of total OK pool

The OKs analysed were distributed in the mandible and maxilla. Eighty-seven (70.02 %) of the 123 cases occurred in the mandible and 36 (29.08%) of the cases occurred in the maxilla (Figure 3). Three patients had cysts in both the maxilla and the mandible, while for 1 patient there was no record of the site of the occurrence of the cyst.

Most of the OKs occurred in the posterior area of the jaws. Sixty-four (75.56%) of the mandibular cases were in the posterior mandible and 9 cases were in the anterior mandible. The specific site of occurrence of 14 mandibular cases were not recorded.

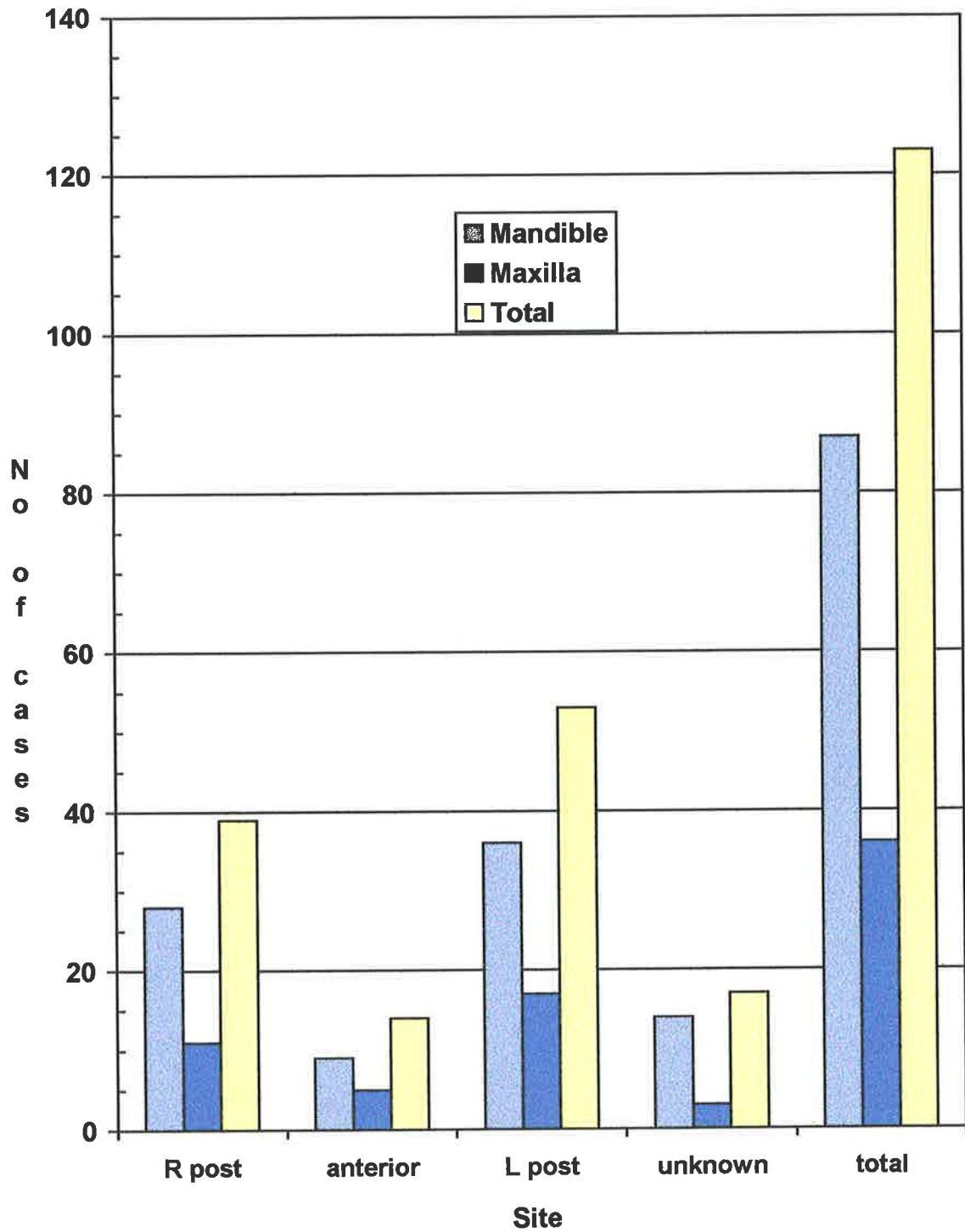


Figure 3: Site distribution of 123 OKs recorded in files

R post - right posterior

L post - left posterior

Unknown - site not recorded

Twenty-eight (77.7%) of the 36 cases occurring in the maxilla were in the posterior region, and 5 were in the anterior maxilla. The specific site of 3 cases in the maxilla was not recorded.

Of the 50 cases selected for immunohistochemical staining, 14 were in the maxilla and 36 were in the mandible. The pattern of distribution of these cases was similar to all cases archived. Eleven (78.5%) of the 14 cases appearing in maxilla were in the posterior region of the maxilla and 27 (75%) of the 36 cases occurring in the mandible were in the posterior region of the mandible. The specific site of occurrence of 8 cases from the mandible and one from the maxilla was not recorded (Figure 4).

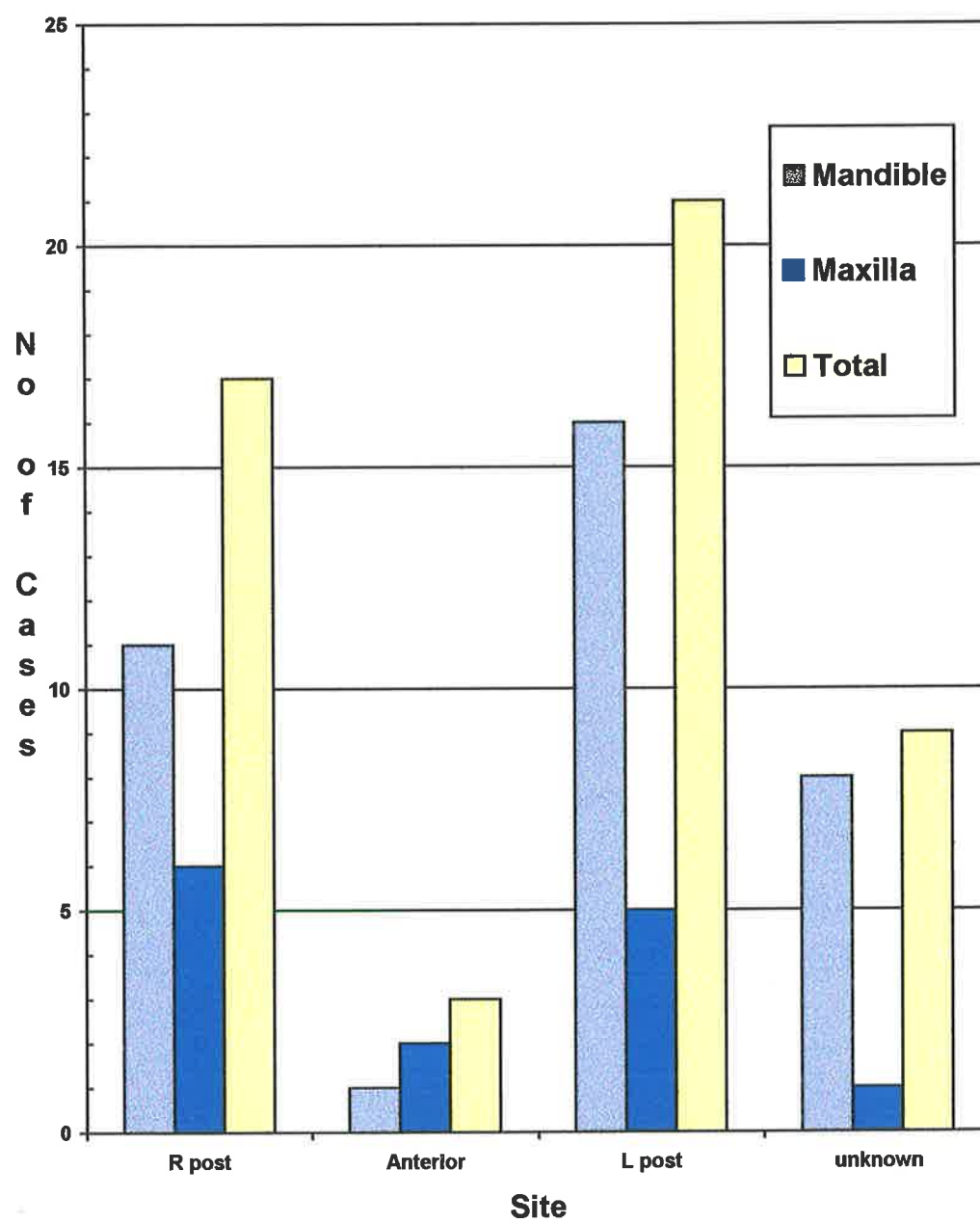


Figure 4: Site distribution of the fifty cases of OK selected for immunohistochemical study
 R post - right posterior
 L post - left posterior
 Unknown - site not recorded

4.2. Histologic observations

4.2.1. Epithelial lining of cases selected for immunohistochemical analysis.

Although most most of the cysts (more than 75%) were affected by inflammation, the cases selected for immunohistochemical staining demonstrated the characteristic epithelial features of OKs with minimal effects of inflammation.

The majority (92%) of 50 OKs were lined by a parakeratinised stratified squamous epithelium (Figure 5). Three (6%) cases were lined by an orthokeratinised stratified squamous epithelium (Figure 6). One (2%) case had both an orthokeratinised and parakeratinised stratified squamous epithelium as the cyst lining (Figure 7).

The thickness of the epithelial lining ranged between 3 to 8 cells. The thickness and the arrangement of the epithelial cell lining were sometimes disrupted by the presence of inflammation.



Figure 5 : Photomicrograph of an OK with a parakeratinised lining epithelium (E). Such an epithelium was present in 46 of the 50 cases of OKs examined in this study (L – cyst lumen, C – cyst capsule). Haematoxylin and Eosin. Original magnification X 200.

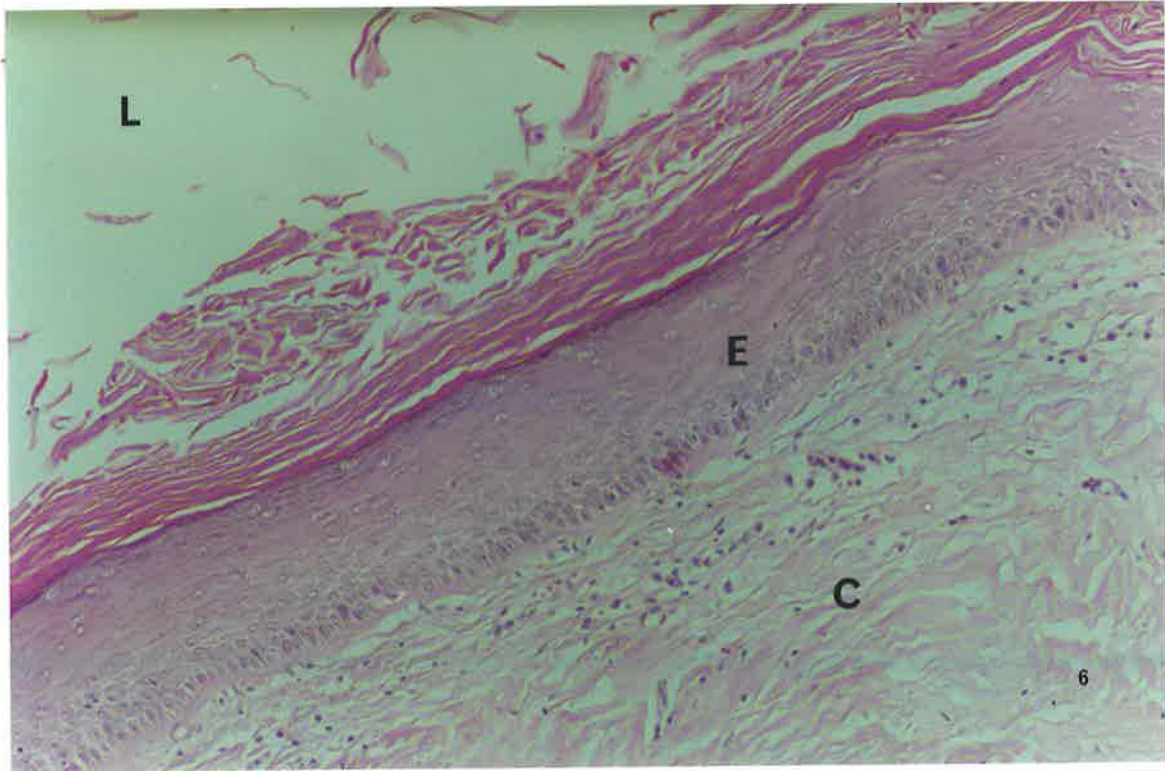


Figure 6: Photomicrograph of an OK with an orthokeratinised lining epithelium(E). Such an epithelium was present in 3 of 50 cases of OKs examined in this study (L – cyst lumen, C – cyst capsule). Haematoxylin and Eosin. Original magnification X 200.

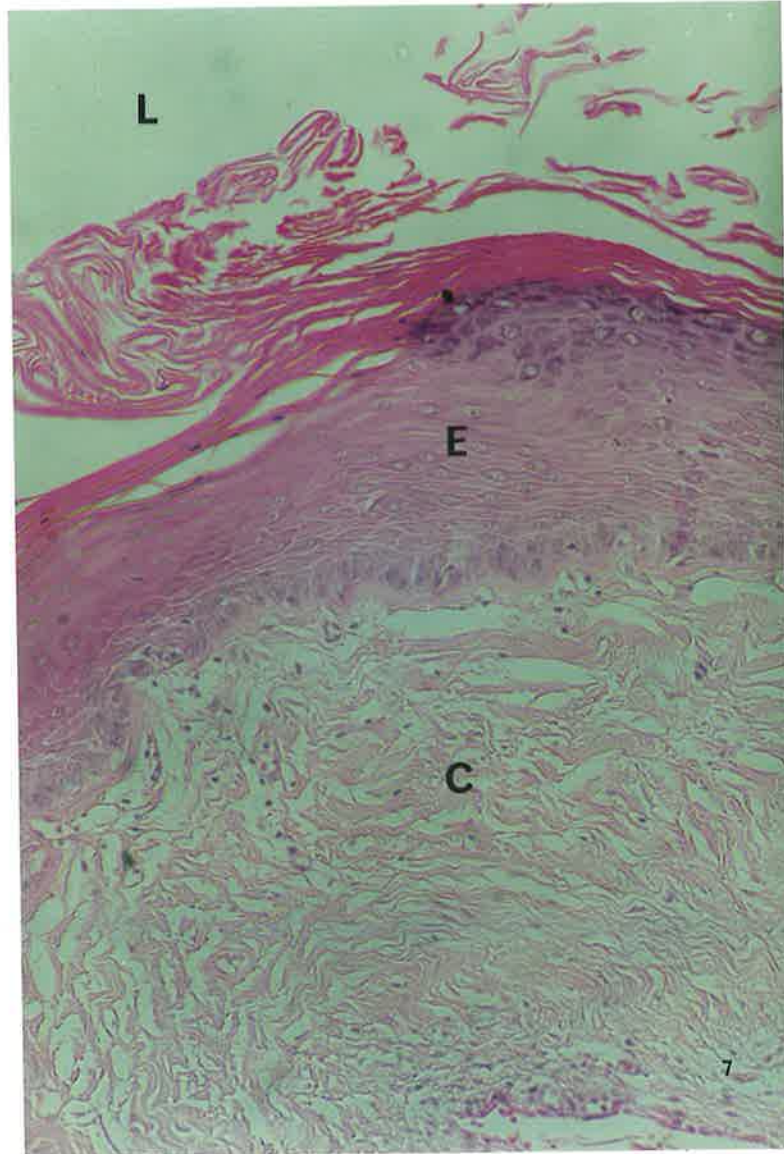


Figure 7: Photomicrograph of an OK with mixed orthokeratinised and para-keratinised lining epithelium (E). Such an epithelium was present in one out of fifty cases of OKs examined in this study (L – cyst lumen, C – cyst capsule). Haematoxylin and Eosin. Original magnification X200.

4.2.2. Immunohistochemical Staining

4.2.2.1. p53 staining

Sections were examined for the presence of brownish staining nuclei (Figure 8). The degree of positivity of p53 staining varied between densely positive and faintly positive (Figure 9). Only cells classified as having unequivocal positivity were counted as positive. Cells having faint positivity (Figure 10) were scored as negative.

Twenty-two (44%) of the 50 OKs stained positive for p53. Most of the p53 positive stained cells were located in the supra-basal cell layers. The number of p53 positive stained cells was higher in areas affected by inflammation.

Two of 4 cases associated with Gorlin-Goltz syndrome in this series were p53 positive whereas 2 p53 negative cases were recurrent cysts, which had been previously enucleated.

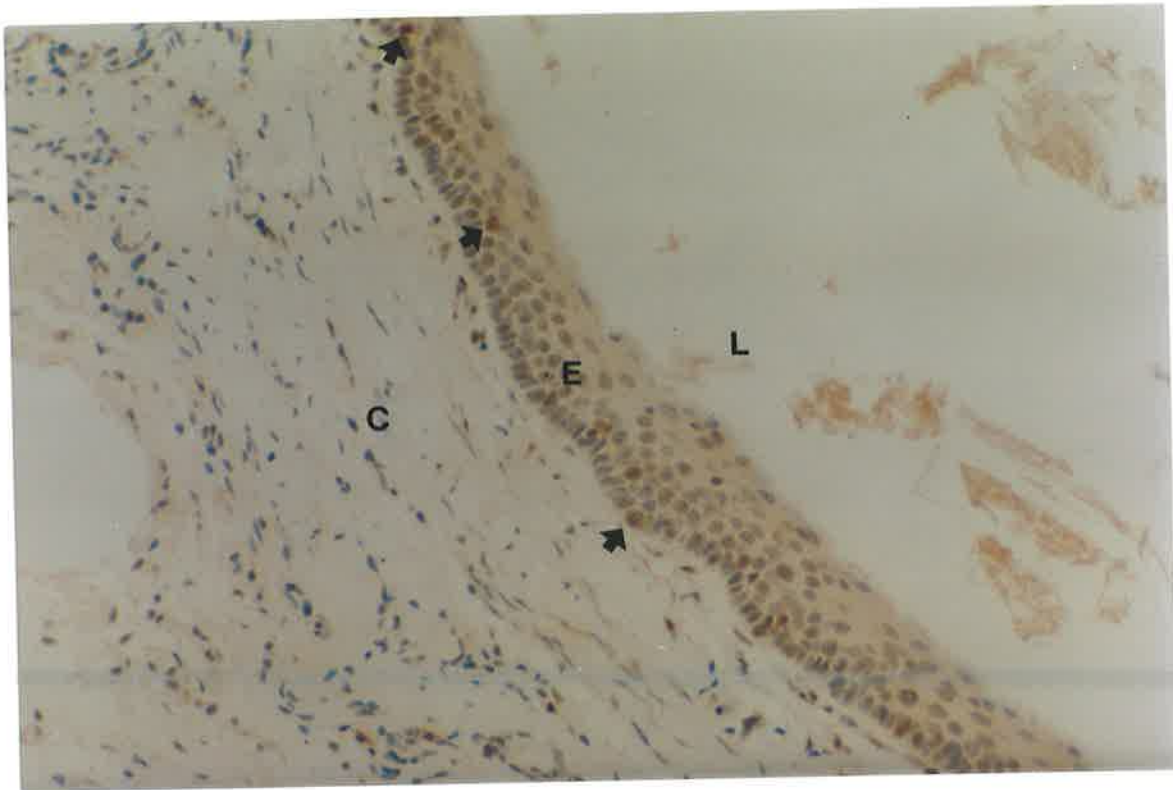


Figure 8: p53 labelled section on an OK. There is some general background staining of the epithelium as a whole and of cyst capsule. Within the epithelium (E) distinctly brown stained cell nuclei are evident (arrows). This intensity of nuclear staining was used as the standard for scoring p53 positivity. L – cyst lumen, C – cyst capsule. Original magnification X 100.

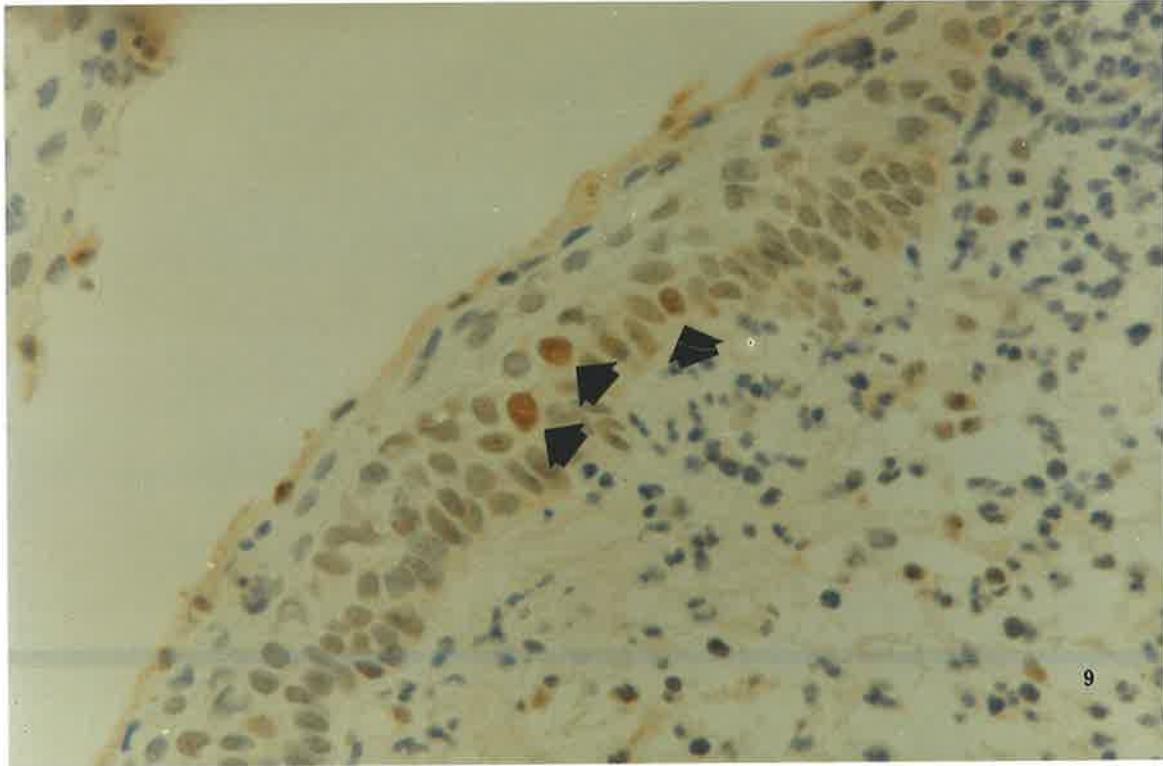


Figure 9: Photomicrograph of p53 staining in OK epithelium. Such positive stained nuclei (arrows) were present in 22 (44%) of 50 OK cases in this study. Original magnification X 400.

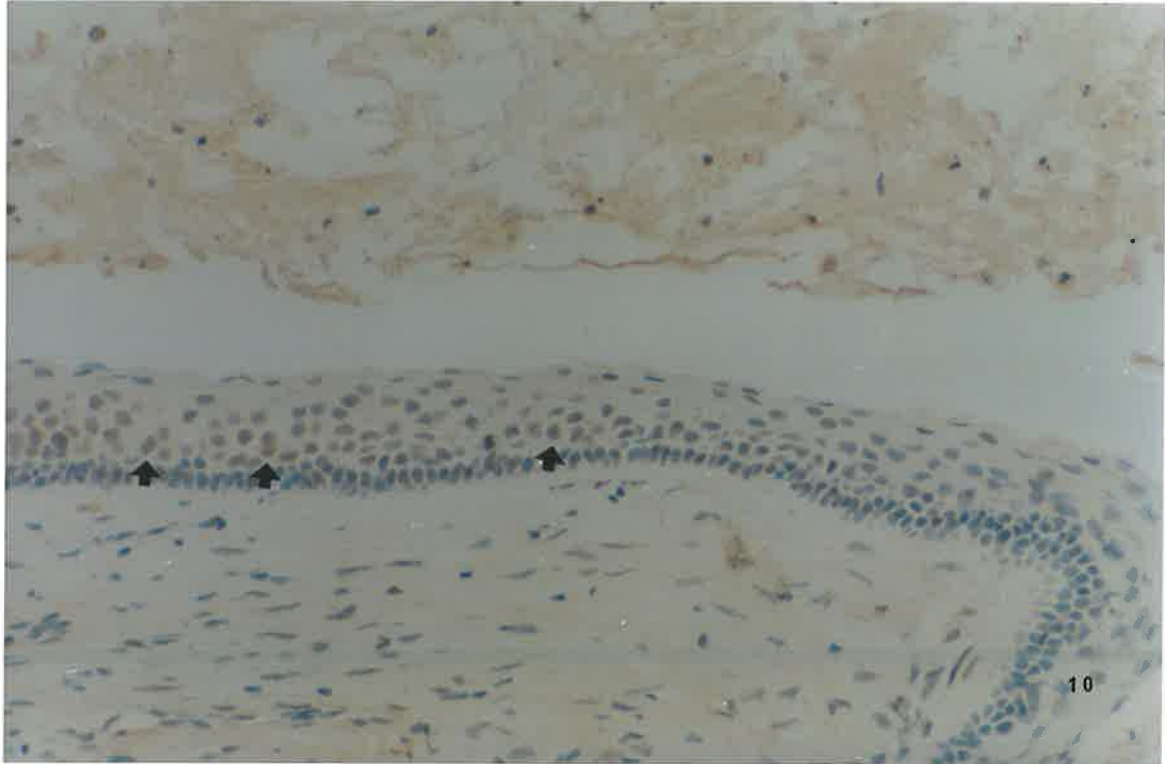


Figure 10: Photomicrograph of an OK in which the epithelium was scored as negative for p53. There is general background staining of cyst lumen contents, parts of the epithelium and capsule component. Some of epithelial cell nuclei (arrows) exhibit a faint brown staining. However, these were scored as negative. Original magnification X 100.

4.2.2.2. Relationship between p53 staining and inflammation

There was no relationship between p53 positivity and the presence of inflammation. Seventeen (77%) of p53 positive cysts were associated with varying degrees of inflammation, whereas 21 (75%) of p53 negative cysts were associated with inflammation ($X^2 = 0.034$; $p < 98$) (Figure11).

4.2.2.3. MIB-1 Staining.

Forty one (92%) of the 50 OKs stained positive for this marker (Figure 12). Most of the positive stained cells were located in supra basal cells. The intensity of positive staining varied, but was always denser and clearer than that for p53.



Figure 12: Photomicrograph of MIB-1 staining in an OK. Brownish stained nuclei indicated MIB-1 positivity. Such positive stained nuclei were present in 41 (84%) of 50 OKs in this study. Original magnification X 200.

4.2.2.4. Relationship between MIB-1 positivity and inflammation

The stainability of MIB-1 in epithelial cells of OKs was analysed according to the degree of inflammation (Figure 13). Thirty-three (80.5%) of 41 MIB-1 positive specimens were associated with capsular inflammation. Five (55.5%) of 9 MIB-1 negative stained specimens demonstrated inflammation. Chi-square analysis demonstrated a poor correlation between MIB-1 positivity and the presence or absence of inflammation ($\chi^2 = 2.515$, $P < 0.2$).

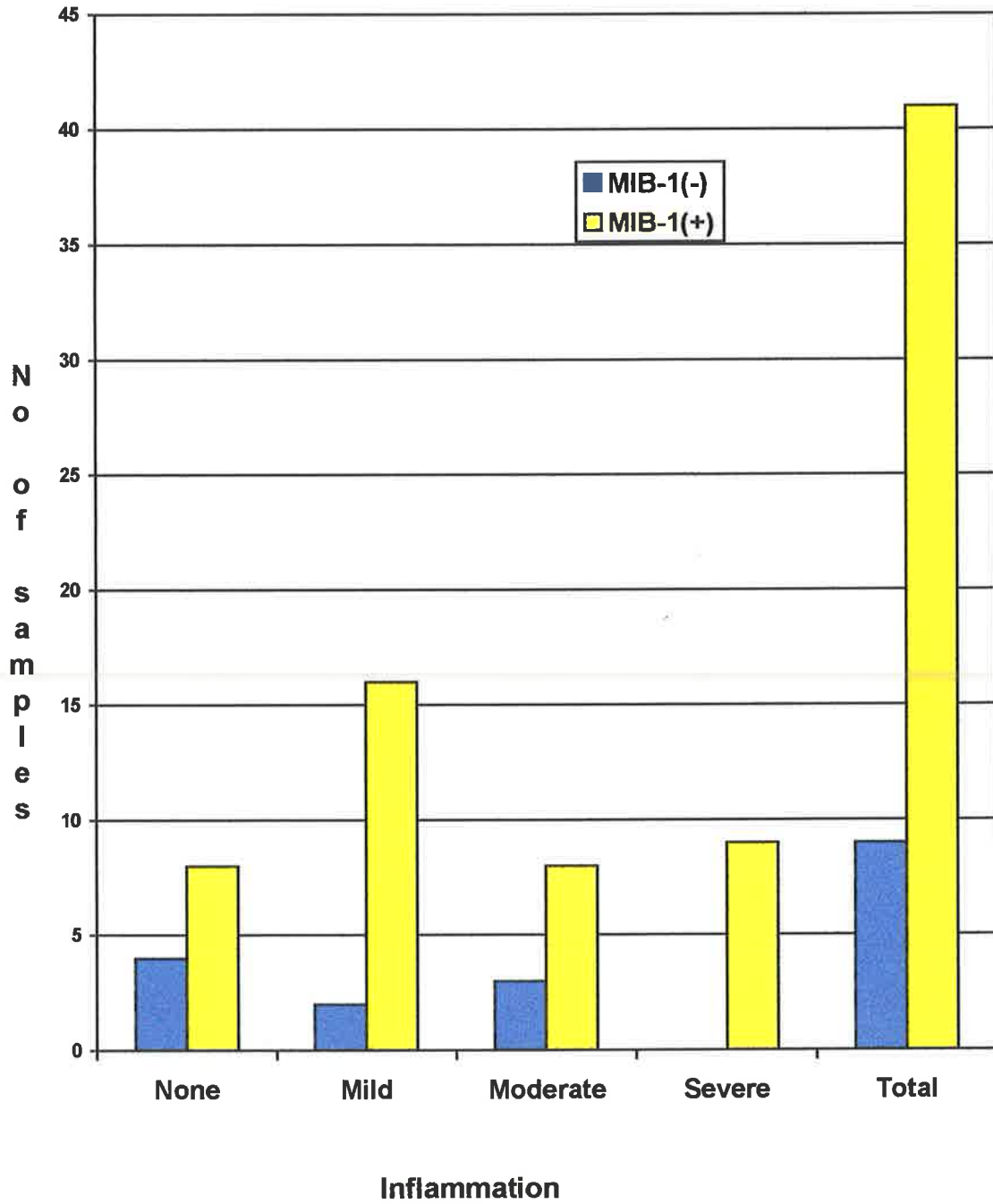


Figure 13: Relationship between MIB-1 positive staining and severity of inflammation.

4.2.2.5. Relationship between p53 staining and MIB-1 staining

All 22 p53 positive cysts were also positive for MIB-1. Of the 28 p53 negative samples 19 (67.8%) were MIB-1 positive, whereas 9 (32.1%) were MIB-1 negative ($\chi^2 = 8.62$; $P < 0.01$) (Figure 14). A majority of dense p53 positive nuclei appeared to correspond with the position of MIB-1 positive nuclei. All of the MIB-1 negative specimens were also negative for p53 protein. These phenomena demonstrate a possible relationship between p53 and MIB-1 stainability in the epithelium of OKs. Chi-square analysis confirmed an association between p53 and MIB-1 positivity in the epithelium of OKs ($\chi^2 = 8.62$, $P < 0.01$).

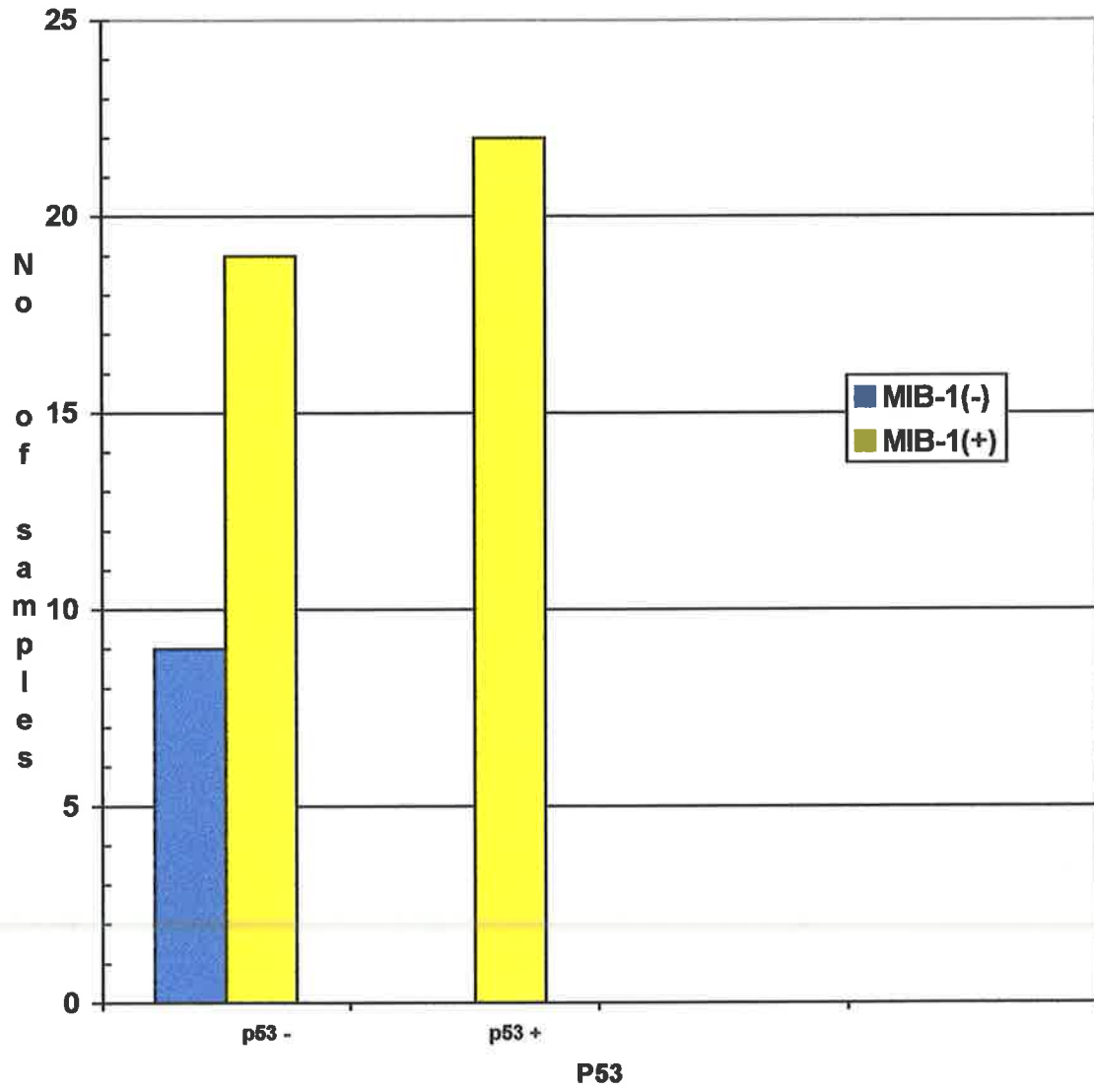


Figure 14: Relationship between p53 staining and MIB-1 staining.

4.2.2.6. Ameloblastoma

Nine cases of ameloblastoma were selected from the histopathology files of department of Oral Pathology and Oral Surgery, Department of Dentistry. The University of Adelaide. Table 1 shows the various subtypes of those 9 cases.

Table 1: p53 and MIB-1 stainability in ameloblastoma

Case No.	Subtype	P53	MIB-1
1. 266/77b	Follicular	+	+
2. 37/72	Predominant follicular	-	-
3. 536/83	Unicystic	+	+
4. 449/85b	Unicystic	-	+
5. 127/79A	Basal cell type	+	-
6. 276/75	Flexiform	+	+
7.233/79	Follicular	+	+
8.172/72	Plexiform	-	-
9. 795/85	Plexiform	+	+

Six (66.67%) of 9 cases of ameloblastoma in this series were positive for p53 (Figure 15) and 6 (66.67%) were positive for MIB-1 (Figure 16). However, there were more p53 positive cells compared to MIB-1 positive cells.

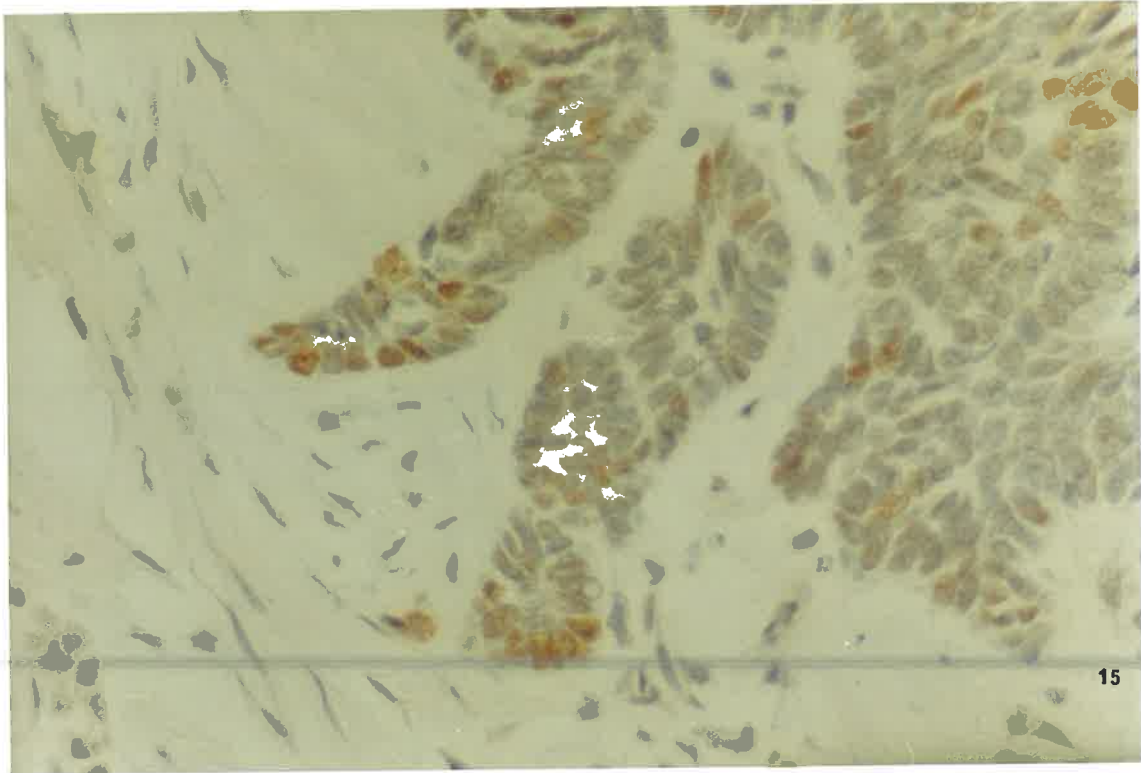


Figure 15: Photomicrograph of p53 staining in ameloblastoma. Such positive stained nuclei (arrows) were present in 6 (66.66%) of 9 ameloblastoma cases in this study. Original magnification X 400.

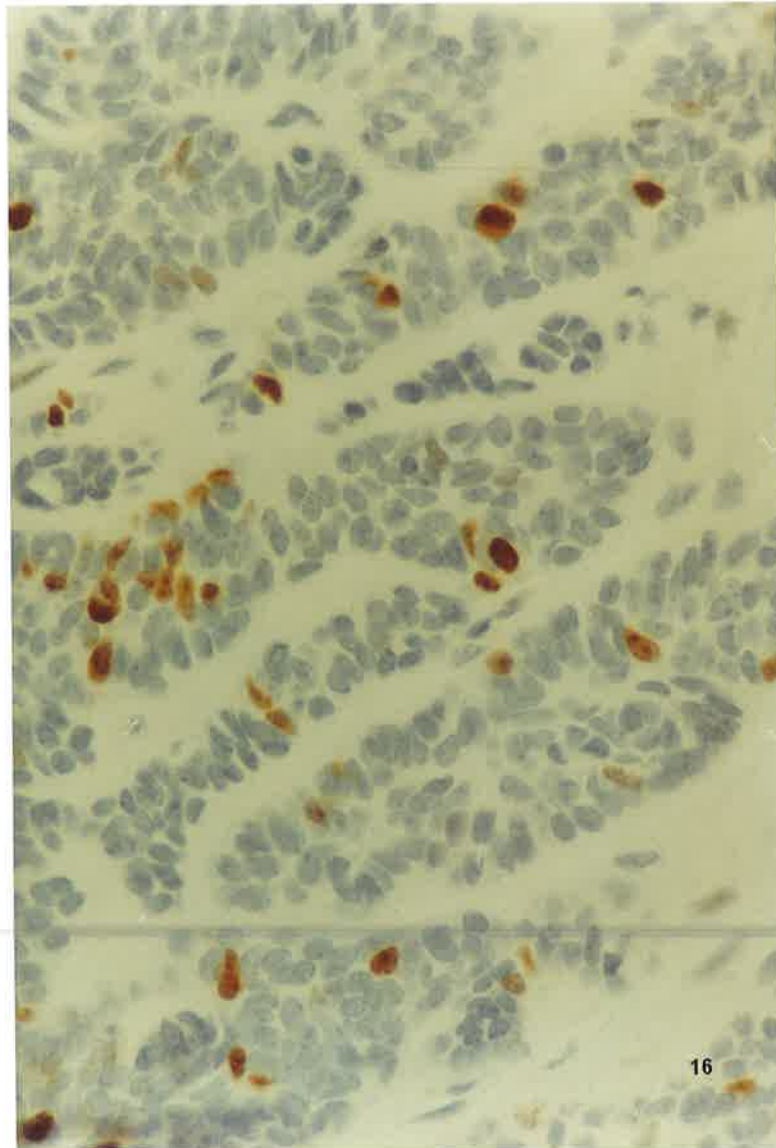


Figure 16: Photomicrograph of MIB-1 staining in ameloblastoma. Brownish stained nuclei indicated MIB-1 positivity. Such positive stained nuclei were present in 6(66.66%) of 9 ameloblastomas this study. Original magnification X 400

CHAPTER 5
DISCUSSION

5.1. Clinical and histological findings

The clinical findings of this present study regarding location of the cysts in the jaws and age and sex distribution of patients are in general accordance with previous studies (Shear 1960, Main et al 1970, Browne 1970a and 1971; Brannon 1976, Vedtofte and Pretorius 1979, Ahlfors 1984, Brondum and Jensen 1991). The age distribution of cases in this study is similar to that reported by Vedtofte and Praetorius (1979), Ahlfors et al (1984) and Forsell (1980). Similarly, the site distribution of cases in this study concurs with the findings of others. OKs in this study had a high predilection for location in posterior sites of the mandible.

The microscopic features of OKs observed in this study were similar to those described by others. Most (92%) of the OKs in this study were lined by parakeratinised stratified squamous epithelium. Three (6%) of OKs in this study were lined with orthokeratinised stratified squamous epithelium and only 1 (2%) case had both parakeratinised and orthokeratinised stratified squamous epithelium. This result is similar to the result of Kakarantza-Angelopoulou and Nicolatou (1990) and Crowley et al. (1992) where 98% and 86.2% (respectively) of their cases were lined by parakeratinised stratified squamous epithelium.

The presence of inflammation in the cyst capsule was recorded in most (76%) of the present cases. This finding is similar to the results of Kakarantza-Angelopoulou and Nicolatou (1990) where 72.4% of their cases were inflamed. Whether the presence of inflammation in the cyst capsules was a

result of previous biopsy, infection or was due to other factors could not be determined in the present study.

5.2. Technical aspects of immunostaining

Despite the fact that 22 (44%) of 50 OKs in this study were positive for the anti-mouse monoclonal antibody against p53 protein (DO-7, Dako Ltd. Australia), which stains both mutant and wild type of p53 protein, the density of nuclear staining varied between cases. Both faint and dense staining for p53 antibody was seen in the epithelial cells of the 22 positive stained cases. This observed variation in staining density could explain some of the differences between the results of the present study and those of other studies as far as p53 expression is concerned as interpretation of p53 positivity versus p53 negativity could vary between different observers.

The findings in this study demonstrated some similarity to and consistency with the staining patterns reported in other studies. Ogden et al (1992a) found that 5 (41.6%) of 12 cases of OKs were positive against CM-1 (a rabbit polyclonal antibody raised against both mutant and wild type p53), and Lombardi et al (1995) reported that 15 (50%) of 30 OKs were positive against CM-1. Slootweg (1995), on the other hand, found that 11 (84.6%) of 13 OKs were positive against the murine monoclonal antibody BP53-12-1 (Bio-genex, San Ramon, CA, USA) which reacts against both mutant and wild type p53. Li et al (1996) reported that BP53-12 gave a denser and greater number of positive-stained cells in their 12 OK samples, compared to clone 1801 and polyclonal CM-1, which stained 38% and 71% of cells, respectively. In fact,

BP53-12 positive cells were detected in the lining epithelium of all cyst types in their samples. Lombardi et al (1995) described the intensity of staining of p53 in OKs as weak and speckled. The differences in the results reported by these authors could also thus be an effect of the different antibodies used.

The relationship between p53 positive cells and proliferative activity in the epithelial lining of OKs has yet to be understood. Most of the p53 positive cells in the present study were distributed in the suprabasal layers of the lining epithelium. This concurs with the findings of Slootweg (1995), Li et al (1996) and Lombardi et al (1995) who also found that most of the p53 positive cells were located in the suprabasal layers of the lining epithelium. The location of p53 positive cells could be related to the mitotic activity in OK lining epithelium. Browne (1971) suggested that the majority of mitoses found in the lining epithelium of OKs are not located in the basal layer, only 10% of mitoses occurring there.

Ogden et al (1992a) found that p53 positive cells predominantly occurred in the basal layers. Although Ogden et al (1992a) and Lombardi et al (1995) used the same methods and the same type of antibody, the pattern and the intensity of staining in the study by Lombardi et al (1995) were distinctly different. These differences remained unexplained.

The density of p53 staining in the present study was not as dense as that observed for MIB-1. P53 stained light brown whereas MIB-1 staining was solid and dark brown showing a clear contrast, which was easily identified, as

positive. Slootweg (1995) also described the pattern of the staining of p53 protein and Ki-67 as being different.

5.3. Pre-treatment effects on staining

Another possible explanation for the different results among studies could be the different procedures used in immunostaining. A pretreatment procedure for antigen retrieval is one of the procedures in the avidin-biotin peroxidase method that can vary among laboratories. Some laboratories prefer to use a microwave-oven pretreatment technique for antigen retrieval, while others used boiled, freshly prepared citrate/HCl buffer (10 nmol) pH 6.0 for 15 min on an electric hotplate, together with protease digestion (Slootweg 1995, Shi et al 1991).

Pre-treatment for antigen retrieval in immunohistochemistry has been introduced to overcome the problem of the presumed effects of fixatives such as formalin, in tissues. This procedure is designed to expose antigenic sites hidden by cross-linked proteins (Gown and Leong 1993). Antigen retrieval has been recognised as having have some advantages including the following; better staining for long-term formalin-fixed tissue; reduced incubation times for primary antibodies and possible exclusion of procedures such as enzyme pre-digestion (Shi et al 1991). In the study, pretreatment for antigen retrieval was carried out by microwave heating sections prior to immunostaining.

Dowell and Ogden (1996) suggested that antigen retrieval could increase immuno-reactivity for p53 protein. Fifteen (68.18%) of 22 squamous cell

carcinomas (SCC) and 35 (97.2%) of 36 benign lesions from their study over-expressed p53 in samples in which antigen retrieval was applied. However, only 6 (27.27%) of 22 SCC and 1 (2.77%) of 36 benign lesions over-expressed p53 in samples in which antigen retrieval was excluded. On the other hand Li et al (1995) found that the stainability of Ki-67 in freshly cut tissue was similar to that for microwave-treated formalin-fixed tissues. No significant difference was found between the number of positive cells in sections from paraffin embedded and frozen tissue.

5.4. p53 staining

Positivity for p53 protein in any reactive lesion does not always connote mutation in the gene. Some commercially available antibodies stain both mutant and wild type p53 proteins. Accumulation of wild type p53 protein can be detected in normal tissue as a result of physiological processes, such as the maturation of haematopoietic cells (Kastan et al 1991, Dowell and Ogden 1996) and of lymphocytes with rapid proliferative activity (Mercer and Baserga 1985). In fact Li et al (1995) suggested that there are no mutations found in exons 5 – 10 of the p53 gene of odontogenic keratocysts. This finding leads to speculation that positive cells in OKs might be caused by an accumulation of wild type p53. Positive staining for p53 protein in OKs should therefore be cautiously interpreted.

Moreover, over-expression of p53 protein in reactive lesions such as keloids, fasciitis, myositis, pyogenic granuloma and benign neoplasms support the

opinion that p53 positivity is not just the result of over-expression of mutant p53 gene but could also be the expression of wild type p53 in physiological processes (Kastan et al 1991, Dei Tos et al 1993).

5.5. MIB-1 staining

The very few previous studies of MIB-1 expression in OK lining epithelium have reported positive cells located in the suprabasal layer of the lining epithelium. Forty-one (82%) of OKs in the present study had MIB-1 positive stained cells possibly indicating that most of the OKs in this study were in a stage of active proliferation. This result concurs with the results of other studies. In the present study, all p53 positive cases were also positive for MIB-1, and the pattern of the positive stained cells against both antibodies was for cells to be mainly located in the suprabasal layers. This similar pattern of site distribution of staining for both antibodies in OKs might indicate that p53 is acting as a checkpoint control in the cell cycle to either suppress and/or modulate cell proliferation.

5.6. p53 staining and inflammation

In this study p53 expression did not seem to correlate with the presence of inflammation. Seventeen (77%) of p53 positive cysts were associated with varying degrees of inflammation, whereas 21 (75%) of p53 negative cysts were associated with inflammation ($X^2 = 0.034$; $p < 98$). These results may indicate that inflammation in the capsule of OKs influences neither the lining epithelium nor the expression of p53 protein in the lining epithelium. These

results do not concur with those of the study by Antoniadou et al (1991), which demonstrated that p53 expression declined during active cellular proliferation in acutely-injured skin tissue and re-emerged during the healing process.

5.7. MIB-1 staining and inflammation

Even though statistically insignificant ($p < 0.2$), the expression of Ki-67 (MIB-1) in the epithelial lining could correlate with the occurrence of inflammation in the capsule of OKs. Thirty-three (80.5%) of 41 MIB-1 positive specimens in this study were associated with capsular inflammation. Five (55.5%) of 9 MIB-1 negative stained specimens demonstrated inflammation. To date there have not been any other reports regarding the occurrence of Ki-67 antigen in inflamed odontogenic cyst capsules. The finding of a lack of correlation between MIB-1 staining and inflammation thus stands as an isolated observation at present. However, it is interesting to speculate on the lack of correlation between inflammation and p53/MIB-1 positivity. This finding would suggest that the presence of inflammation in OK cyst walls does not influence lining epithelium proliferation rates.

5.8. Ameloblastoma

Six (66.67%) of 9 cases of ameloblastoma in this series were positive for p53. The results of this study were similar to those reported in other studies. Slootweg (1995) found that 6 (66.67%) of 9 cases of ameloblastoma were positive against p53 protein and all 9 cases of ameloblastoma were positive for MIB-1. In the present study, only 6 (66.67%) of 9 cases of ameloblastoma

were positive for MIB-1. The differences in the results of these and other studies for MIB-1 may be explained by differences in the procedures used in staining such as the type of antibody and the antigen retrieval method used.

Although the number of positive stained cells for both p53 and MIB-1 was exactly the same, a few cases showed different patterns and intensity of staining. In contrast to the OK portion of the study, there were more p53 positive cells in ameloblastomas compared to MIB-1 positive cells. Further work aimed at analysing p53 expression in odontogenic tumours such as ameloblastoma would be of value.

- **CONCLUSIONS**

CONCLUSIONS

The main purposes of this study were to;

- (a) assess the expression of two "growth indicator" markers (p53 and MIB-1) in a sample of OK's larger than that previously described in the literature
- (b) examine for any correlations in the expression of these markers
- (c) assess the expression of p53 and MIB-1 in a pilot sample of ameloblastomas.

On the basis of the results of this study it was concluded that:

1. Approximately half (44%) of the 50 OK's examined expressed p53 positivity. This figure is within the range of values reported by other investigators.
2. p53 and MIB-1 expression did not seem to correlate with capsular inflammation.
3. The majority (82%) of OK's in this study were positive for MIB-1. This observation is in general accord with the findings reported by three other groups of investigators who have examined MIB-1 expression in OK's. It is concluded that MIB-1 is a better marker of cellular proliferation activity in OK's than p53.
4. Although the number of cases of ameloblastomas studied was small p53 expression was more overt than MIB-1 expression.

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