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Detection of human papillomavirus DNA in an ameloblastoma using the in situ hybridization technique

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Van Heerden WFP, van Rensburg EJ, Raubenheimer EJ, Venter EH: Detection of human papillomavirus DNA in an ameloblastoma using the in situ hybridization technique.

HPV type 18 DNA was identified in an intrabony ameloblastoma using radiolabelled in situ hybridization. The viral DNA was found in a verrucous lesion in a cystic area of the tumor. The absence of HPV DNA in other epithelial areas of the ameloblastoma is suggestive of a secondary infection. HPV is not considered to be an etiological factor in the pathogenesis of this ameloblastoma.

Key words: ameloblastoma; DNA; human papillomavirus type 18; in situ hybridization

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Human papillomaviruses (HPVs) are DNA viruses that infect only squamous epithelium at selected locations in the skin and mucosa. The virus usually infects the basal cell layers and viral DNA are observed in low copy numbers in these cells. An increase in copy numbers of replicating viral DNA are found in the more differentiated epithelial cells while production of viral particles is restricted to the fully differentiated superficial epithelial cells (1). Since this state of differentiation has not yet been achieved in culture, it has not been possible to reproduce HPV in the laboratory to study their natural life cycle (2). These viruses induce papillomatous, hyperplastic or verrucous lesions depending on the site of infection and the HPV type implicated. HPV involvement in upper respiratory and digestive tract lesions like focal epithelial hyperplasia, squamous cell papillomas, laryngeal papillomas, leukoplakia and squamous cell carcinoma has been demonstrated by means of immunohistochemical, DNA hybridization and polymerase chain reaction studies (2–6). More than 60 types of HPV have been isolated to date, of which types 1, 2, 4, 6, 7, 11, 13, 16, 18, 32 and 57 were found in the different oral lesions (7).

The association between HPV and odontogenic tumours and cysts has not been studied to a great extent. HPV 16 DNA has been demonstrated in an odontogenic keratocyst using Southern blot hybridization (8), while KHAN found HPV capsid antigen in 3 out of 10 ameloblastomas in young persons (9). The purpose of this study was to investigate an ameloblastoma with typical HPV histologic changes for HPV DNA using the in situ hybridization technique. This is a sensitive technique and has the advantage of localizing viral DNA in tissue sections to the extent of detecting them at the resolution of single cells.

Material and methods

A 25-yr-old man reported to the Maxillofacial and Oral Surgery clinic complaining of a painless, bony hard swelling in the anterior mandible. Examination showed a tumor extending from the right mandibular angle to the contralateral first molar region. Expansion of the lingual and buccal cortices was evident with thinning and erosion of the buccal cortex in the right premolar area. Radiographs showed a well-circumscribed, multilocular lesion with root resorption of the associated teeth. No signs of mucosal ulceration were present.

After an incisional biopsy, a diagnosis of a follicular aneloblastoma was made and the tumor was resected through an intraoral approach. The specimen was fixed in 10% buffered formalin for pathologic examination.

Macroscopic examination showed a gray-white solid tumor with cystic areas of varying size. A papillomatous lesion presenting as a luminal growth was present in one cyst. Microscopy revealed a follicular ameloblastoma with acanthomatous as well as granular cell differentiation (Fig. 1). The papillomatous lesion showed verrucous hyperplasia with hyperparakeratosis, elongation of the rete-ridges and groups of koilocytes lying in the upper part of the epithelium. These features resembled those of a verruca vulgaris (Fig. 2).

The biopsy material containing the papillomatous lesion, as well as blocks exhibiting the characteristic ameloblastomatous epithelium and including areas of granular cell and acanthomatous differentiation, were examined for the presence of HPV antigen using an ABC immunoperoxidase kit for the HPV group specific antigen (Lipshaw Corporation, Detroit) as well as in situ hybridization.

For HPV typing, the specific DNA probes of HPV 2, 6, 7, 11, 13, 16, 18 and 30 cloned in either pBR322 or pUC19 were used (kindly provided by Dr E-M de Villiers, Human Papillomav-

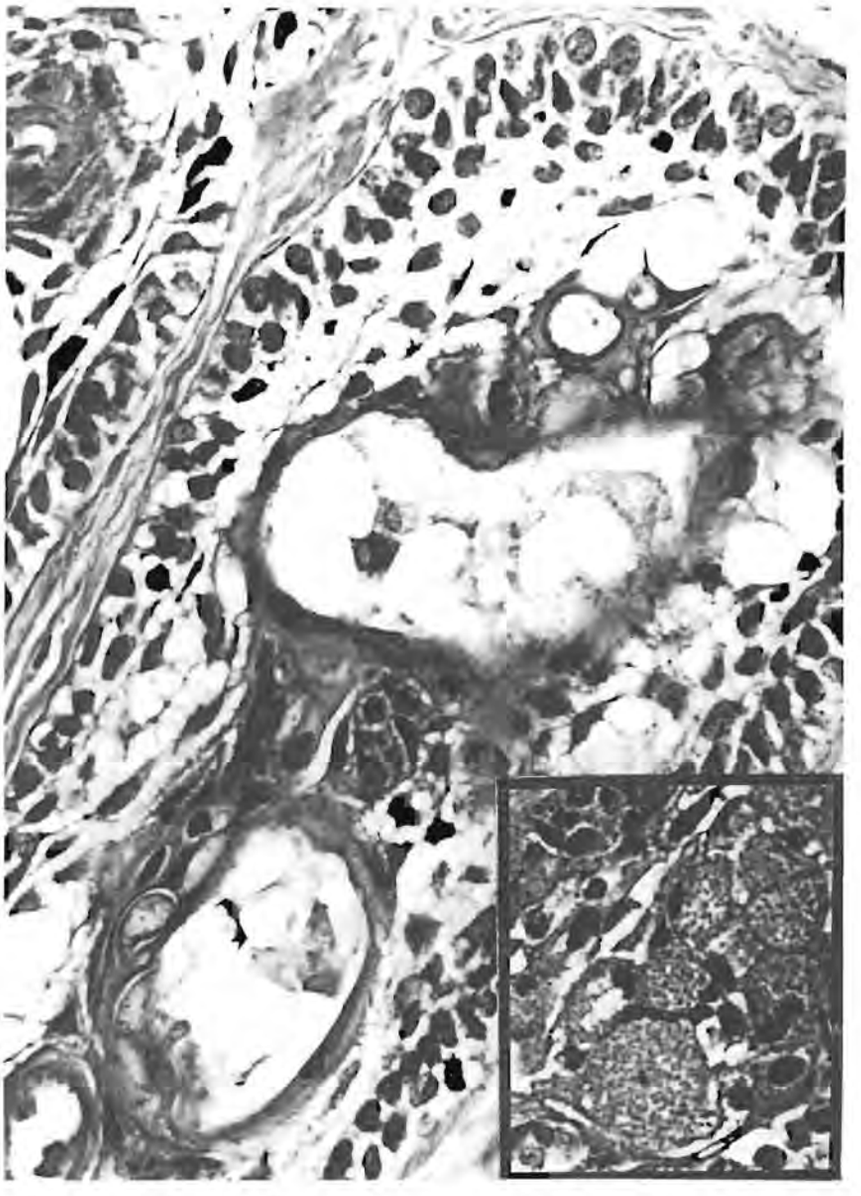

Fig. 1. Follicular ameloblastoma with central acanthomatous differentiation. HE × 200. *Inset.* Areas of granular cell differentiation. HE × 200.

irus Reference Centre, DKFZ, Heidelberg, Germany). The probes were labelled with ^{32}p dCTP using the multiprime DNA labelling system (Amersham, U.K.). The labelled probes had a specific activity of $2–5 \times 10^8$ counts/min/μg of DNA. For in situ hybridization 5–10 ng of each probe was used on individual sections.

pBR322 and pUC19 vectors served as negative control probes. Two paraffin embedded tissue sections (a cervical intraepithelial neoplasia and vulvar carcinoma positive for HPV 6 and 16 respectively) were used as positive control slides.

The tissue sections were incubated at 60°C overnight using 3-aminopropyltriethoxysilane coated slides (10). Slides were deparaffinised and rehydrated by sequential immersion into xylene (3 × 10 min) and ethanol. They were then incubated in 0.2 N HCl for 20 min at room temperature and transferred to 2 × SSC at 70°C for 10 min. The tissue sections were digested with a 2 × SSC, 0.1% SDS buffer solution containing Proteinase K (Boehringer, Mannheim, Germany) at a concentration of 0.01 μg/ml at 37°C for 30 min. Sections were post-fixed for 5 min in 4% paraformaldehyde, 2 × SSC and 5 mM $MgCl_2$; 5 min in 50% formamide, 2 × SSPE and acetylated (2 × 5 min). The slides were prehydridised (50% formamide, 10% dextran sulfate, 2 × SSPE, 100 mM glycin, 0.1% SDS, 2% 50 × Denhardts, 10 mM Tris pH 7.4 and 200 μg/ml salmon sperm DNA) for 30 min at 52°C prior to the application of the probe solution. Heat-denatured probe solution (either HPV 2, 6, 7, 11, 13, 16, 18 or 30) was added to each section and slides were incubated for 16 hours at 52°C in a humidified chamber.

Following hybridisation, the slides were washed twice in a 2 × SSPE/50% formamide solution and once in 50% fomamide, 0.1% SDS, 2 × SSC, each wash for one hour at 37°C. Slides were dehydrated through graded ethanols containing 0.3 M NH_4 acetate. Slides were dipped in LM-1 emulsion (Amersham, UK), following instructions of the manufacturer. After exposure, slides were developed, fixed and counterstained with hematoxylin-eosin. The presence of HPV DNA sequences in the lesions was indicated by the condensations of black silver grains superimposed on the nuclei of cells.

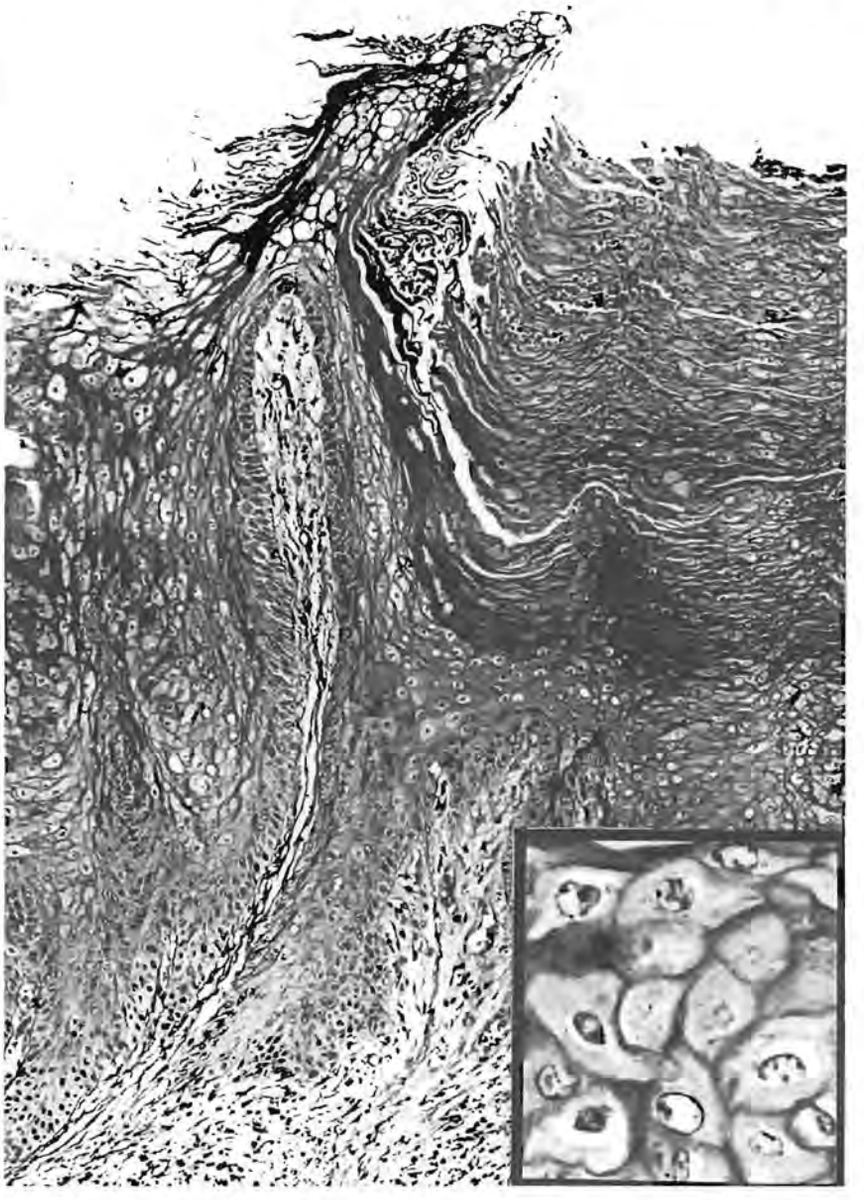

Fig. 2. Micrograph of the papillomatous lesion showing parakeratosis, epithelial papillary processes and elongation of rete-ridges. HE × 100. *Inset.* High power detail of koilocytes showing enlarged cells with slightly irregular nuclei surrounded by a halo. HE × 250.

Results

Immunohistochemical examination of both the papillomatous lesion and typical ameloblastoma areas was negative. The in situ hybridization technique revealed HPV DNA type 18 in the papillomatous lesion (Fig. 3). The blocks containing the typical ameloblastoma features, including foci of granular cell and acanthomatous differentiation, were negative for the HPV DNA types examined.

Discussion

Radiolabelled HPV DNA in situ hybridization was used instead of the more commonly used biotinylated DNA in situ hybridization because it is a more sensitive method to detect HPV DNA (11). The sensitivity of the radiolabelled HPV DNA probe is 20–100 genome copies per cell compared to the 100–800 genome copies per cell of the biotin-labelled HPV DNA probe (1). The negative immunohistochemical staining in our study may be due to the fact that this technique identifies only the productive phase of the viral infection. Furthermore, as this method is based on an antigen-antibody reaction, the target antigenic determinants may be distorted by heating in paraffin, fixation in formalin or digestion by trypsin (12).

The presence of HPV type 18 DNA in a primary intrabony tumor of odontogenic epithelial origin is difficult to explain. Contact between the tumor epithelium and the oral mucosa may have facilitated cross-infection between oral epithelium and the ameloblastoma. Although no ulceration of the oral mucosa or skin was noted in this patient, expansion of the buccal and lingual cortices with erosion of the buccal cortex in the right premolar area were present. This area corresponded with the location of the papillomatous lesion in the ameloblastoma. The specimen was thoroughly examined for the presence of similar lesions without success, supporting the link between the HPV-associated lesion and the eroded bone cortex. Direct contact between tumor epithelium and surface epithelium could not be demonstrated.

Cox *et al.* demonstrated HPV 16 DNA in an odontogenic keratocyst

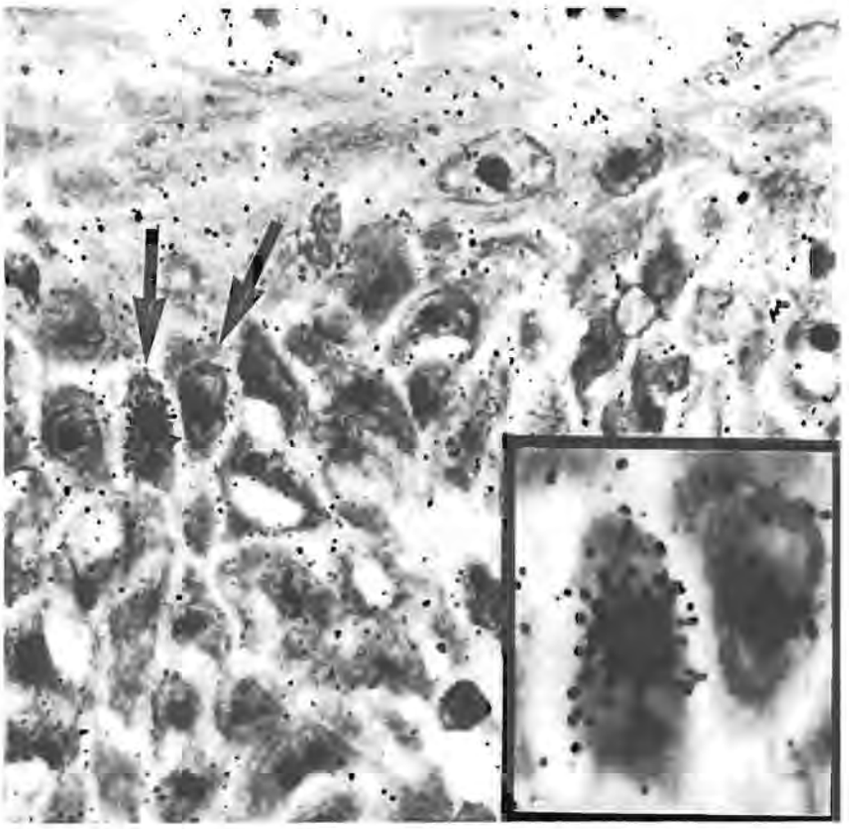

Fig. 3. Micrograph of the verrucous lesion subjected to in situ hybridization shows the presence of HPV 18 DNA as condensations of black silver grains superimposed on the nuclei (arrows). ×200. *Inset.* High power detail of positive cells. ×400.

lacking the typical histologic features of an HPV infection (8). This HPV was implicated in the pathogenesis of the odontogenic keratocyst because the keratin-producing lining of the cyst provided squamous epithelium for viral persistence as well as completion of the virus life cycle. HPV 18 has an even higher oncogenic potential than HPV 16 (13), and has also been detected in oral epithelial dysplasias and oral squamous cell carcinomas (14). In our case HPV DNA was detected only in the solitary papillomatous lesion and not in the other epithelial regions permissive for viral infections, i.e. the acanthomatous and granular cell areas. We feel that the restriction of HPV DNA positivity to the verrucous lesion represents a secondary infection and is therefore not an etiological factor in the pathogenesis of this ameloblastoma.

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Clinico-pathological study of 30 unicystic ameloblastomas

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Keywords: ameloblastoma; odontogenic tumour.

SUMMARY

The clinico-pathological records of 30 unicystic ameloblastomas collected over a period of 10 years were studied. The mean age at diagnosis was 18,0 years (SD ±8,1), most lesions were located in the mandible and were frequently associated with impacted teeth, root resorption and tooth displacement. The unicystic ameloblastomas in 11 patients (4 females and 7 males) exhibited invasion of the fibrous wall, 4 cases (1 female and 3 males) showed intra-luminal proliferation and the remaining 15 specimens (9 females and 6 males) were lined by non-proliferating ameloblastic epithelium. Two cases recurred 3 and 7 years after initial surgical removal. This study reveals the potential aggressive behaviour of unicystic ameloblastomas and underlines the importance of a thorough microscopic examination for sub-classification.

OPSOMMING

Die klinies-patologiese rekords van 30 unisistiese ameloblastome, wat oor 'n tydperk van 10 jaar versamel is, is bestudeer. Die gemiddelde ouderdom by diagnose was 18,0 jaar (SD ±8,1). Die meeste letsels was in die mandibula en was met geïmpakteerde tande, wortelresorbsie en tandverplasing geassosieer. In 11 pasiënte (4 vroulik, 7 manlik) het die unisistiese ameloblastome die fibreuse wand binnegedring, in 4 gevalle (1 vroulik, 3 manlik) was intraluminale proliferasie teenwoordig en in die oorblywende 15 gevalle (9 vroulik, 6 manlik) was die sist deur 'n nie-prolifererende ameloblastiese epiteel. In twee gevalle het herhaling onderskeidelik 3 en 7 jaar na die aanvanklike chirurgiese verwydering voorgekom. Hierdie studie bevestig die potensiële aggressiewe gedrag van unisistiese ameloblastome en beklemtoon die noodsaaklikheid van deeglike mikroskopiese ondersoek vir subklassifikasie.

INTRODUCTION

The unicystic ameloblastoma is a unilocular, cystic epithelial odontogenic tumour initially described by Robinson and Martinez in 1977. Males and females are affected approximately equally. The lesions usually occur in the mandible and especially in the molar-ramus area, while the maxilla is only occasionally affected (Ackerman, Altini and Shear, 1988). They usually occur in the second to fourth decades and the mean age at the time of diagnosis is reported to be 23,8 years. The lesions appear radiologically as a well defined unilocular radiolucency of varying size (Ackermann *et al.*, 1988). When associated with an unerupted tooth, the appearance closely resembles a dentigerous cyst. Involvement of the roots of teeth may give it a radicular cyst-like appearance (Lucas, 1984) and in many cases can only be distinguished from odontogenic keratocysts by microscopic examination. Unicystic ameloblastomas are divided into three groups. Group 1 include the simple cystic lesions lined by an epithelium that does not infiltrate into the fibrous cyst wall. Lesions in Group 2 show intra-luminal epithelial proliferation and the epithelium in Group 3 lesions invade the fibrous cyst wall. Group 1 and 2 lesions may be treated by enucleation, whereas Group 3 lesions should be treated more radically to prevent recurrences (Ackermann *et al.*, 1988).

As a rule unicystic ameloblastomas behave more aggressively than other odontogenic cysts. It is important therefore to recognise the clinical features which may facilitate an accurate diagnosis of the condition. This study was undertaken to determine the clinico-pathological features of unicystic ameloblastomas in a rural black population.

MATERIALS AND METHODS

Microscopic sections of all unicystic lesions that were biopsied between 1982 and 1992 at Medunsa were retrieved and re-evaluated. The unicystic ameloblastomas were subdivided into three groups using the criteria of Ackermann *et al.*, 1988. Clinical data and radiographs were obtained from patient files. The site of occurrence was designated as molar-ramus, premolar or incisor according to the centre of the radiolucent lesion on a panoramic radiograph.

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RESULTS

Thirty cases were diagnosed as unicystic ameloblastoma, 16 were males and 14 females. The mean age of the patients was 18,0 years (SD =±8,1) (range 8-43 years) and 63,3 per cent occurred in the second decade (Fig. 1). Twenty seven of the lesions were present in the mandible and only 3 in the maxilla (Fig. 2). The lesions varied in size from 2,5-12 cm mesio-distally and 22 of the lesions were more than 5 cm in diameter on the panoramic radiographs. One mandibular tumour was associated with a pathological fracture.

Radiologically, 11 of the lesions were associated with impacted teeth (Fig. 3), 13 with root resorption, 15 with tooth displacement and 8 with tooth displacement and root resorption. The impacted teeth associated with the lesions were mainly the mandibular third molars (n=7), followed by mandibular second molars (n=3) and mandibular canines (n=2). Two of the 3 maxillary lesions presented in the "globulo-maxillary" area. Of the 14 lesions in females, 9 were classified as Group I (Fig. 4), 1 as Group II (Fig. 5) and 4 as Group III. There were 6 Group I lesions and 7 Group III lesions amongst the 16 males, the remaining 3 were Group II lesions (Fig. 6).

In 7 unicystic ameloblastomas, 50 per cent or more of the lining was a nondescript type of epithelium (Fig. 7). Three out of 4 Group III lesions had a plexiform intra-luminal proliferation, the other had multiple mural nodules projecting into the lumen (Fig. 8). Inflammation in 3 lesions was associated with extensive epithelial arcading and 4 showed sub-epithelial hyalinization. Two cases recurred as polycystic ameloblastomas 3 and 7 years after surgical treatment respectively. The first was originally classified as Group III (Fig. 9), whereas the other case was a Group I unicystic ameloblastoma.

DISCUSSION

Since the original description of unicystic ameloblastoma (Robinson and Matinez, 1977) various reports on the aggressive behaviour of this cystic lesion have appeared (Ackermann *et al.*, 1988; Gardner, Morton and Worsham, 1987; Kahn, 1989; Keszler and Dominques, 1986; Punnia-Moorthy, 1989). Two patients in our study, which extends over a period of 10 years, presented 3 and 7 years after initial surgery with recurrences. Both recurrences exhibited the growth pattern of a polycystic ameloblastoma. Although Ackermann *et al.*, (1988) propose a more radical form of treatment for Group III lesions, a Group I lesion recurred in our study and this emphasizes the potentially aggressive behaviour of all unicystic ameloblastomas and

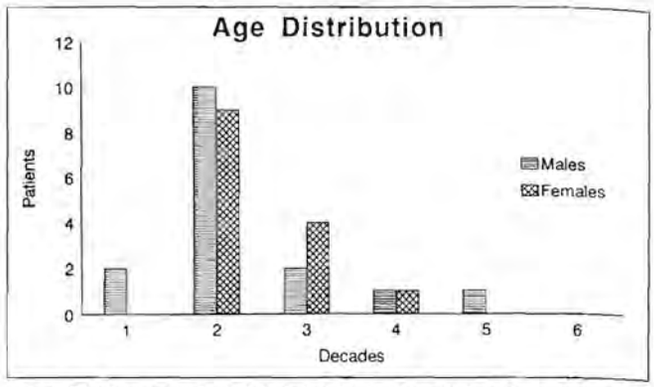

Fig. 1: The histogram of the age distribution of males and females.

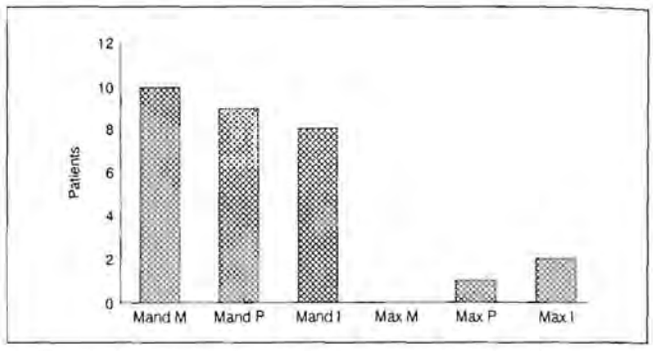

Fig. 2: The site distribution of 30 unicystic ameloblastomas. Mand=mandibular, Max=maxilla, M=molar ramus area, P=premolar area, I=incisor area.

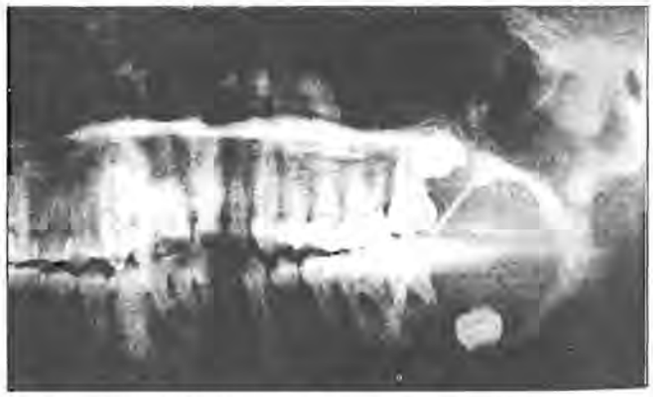

Fig. 3: Panoramic radiograph of an unicystic ameloblastoma associated with an impacted mandibular third molar.

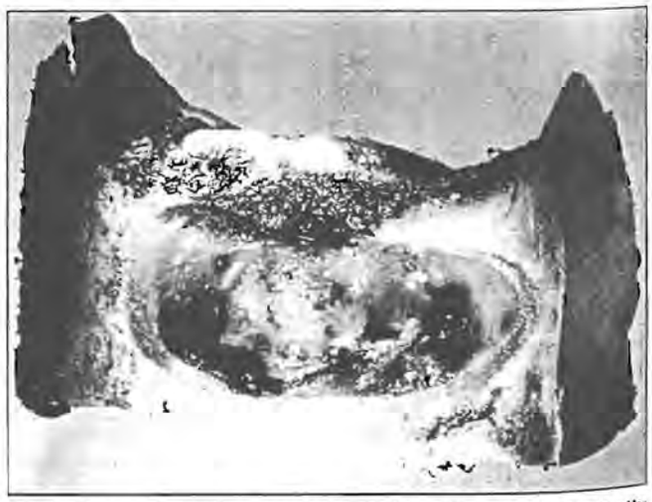

Fig. 4: Section through a resected mandible with a microscopically confirmed Group I lesion. Note the simple unicystic cavity.

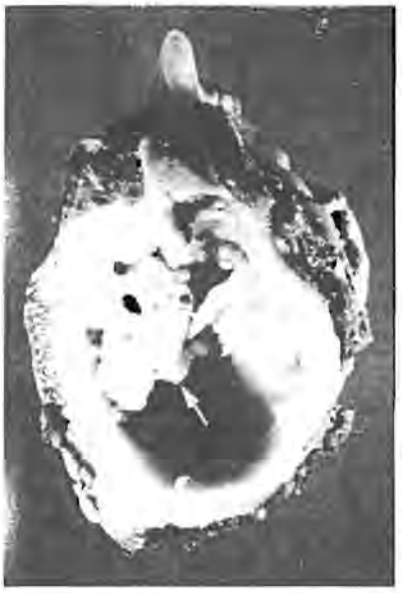

Fig. 5: Cross section through a microscopically confirmed mandibular Group II lesion. Note the intra-luminal proliferation (arrow).

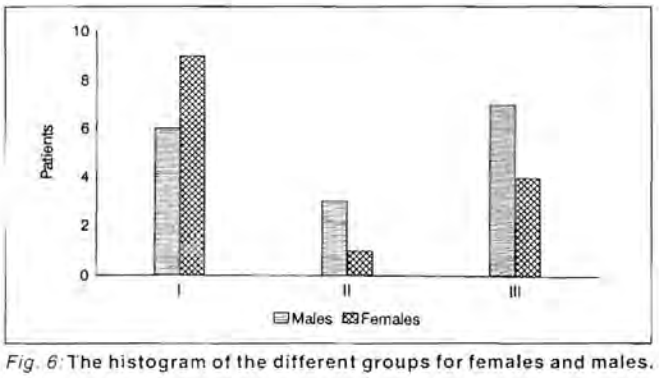

Fig. 6: The histogram of the different groups for females and males.

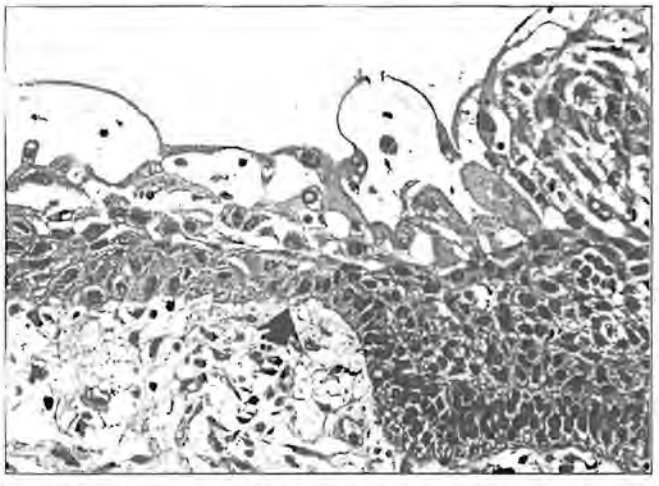

Fig. 7: Photomicrograph of a Group I lesion. Note the nondescript epithelium (left) and the sharp transition (arrow) to typical ameloblastic epithelium. (HE, X300).

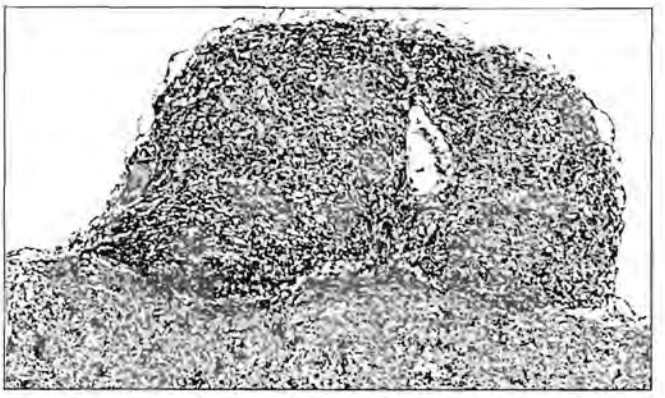

Fig. 8: The lining of a Group II lesion showing an intra-luminal nodular proliferation (HE, X100).

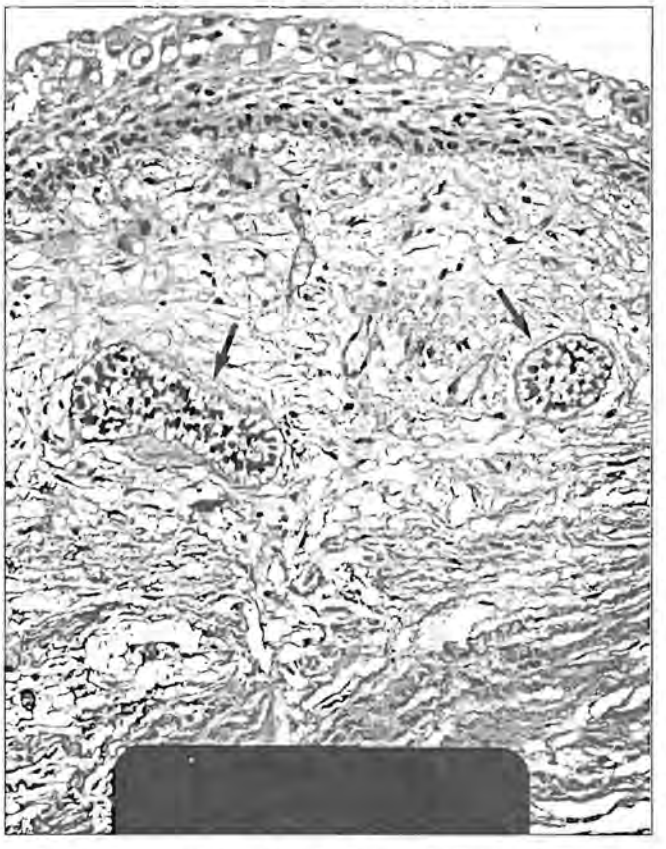

Fig. 9: The lining of the Group III lesion that recurred. Note the islands of ameloblastic epithelium in the connective tissue wall (arrows). (HE, X180).

highlights the importance of complete surgical removal. This recurrence may, on the other hand, reflect an inherent weakness in the proposed sub-grouping of unicystic ameloblastomas. If the whole cyst wall is not examined microscopically, an exercise which is highly impractical if not impossible in larger examples, mural invasion cannot be excluded categorically. The diagnosis of an unicystic ameloblastoma on a small biopsy specimen is not recommended. Moreover, the frequent occurrence of nondescript epithelium and inflammation may mask the typical characteristics of the ameloblastic epithelial lining. This microscopic sub-classification of unicystic ameloblastomas should therefore not be attempted on anything less than a thorough microscopic examination of the whole cyst wall. After such an examination the number of lesions placed in Group III would probably increase.

Our study supports the finding that there is an equal sex distribution for the unicystic ameloblastoma as well as a tendency for it to occur in young patients. Our cases however, presented on average 6 years earlier than those of Ackermann *et al.*,

(1989), probably because patients were seeking treatment sooner and had easier access to the hospital in the years 1981-1991. Most tumours occurred in the mandible and maxillary involvement was less common. A large number of mandibular lesions could be easily mistaken for dentigerous cysts, because of their association with impacted molars and canines. This is related further to the frequent occurrence of root resorption, a feature often found in dentigerous cysts (Shear, 1992). In order to establish a correct diagnosis, microscopic examination of all cystic jaw lesions is mandatory.

Group I lesions predominated in our study and then followed Group III and lastly Group II unicystic ameloblastomas. This is in contrast to Ackermann's 1988 study in which Group III lesions were most frequent. The ratio between female and male in Group I lesions was 1,5:1 and in Group III lesions 1:1,75. The significance of this finding is not known.

It is important to note that all unicystic ameloblastomas, irrespective of grouping, are neoplastic in nature and will recur if incompletely removed.

Although limited evidence is available on recurrences of unicystic ameloblastomas, it appears as if the latter may present either as a regrowth of the original unicystic lesion or as a multicystic ameloblastoma.

ACKNOWLEDGEMENT

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Serving Dentistry — ISO TC106

The 30th meeting of the International Standards Organisation's Technical Committee took place in Ottawa, Canada from 10-15 October 1994.

The countries represented were Australia, Brazil, Canada, China, France, Germany, Hong Kong, Italy, Japan, Netherlands, Norway, South Africa, Switzerland, Sweden, Thailand, United Kingdom and USA. The new South African flag was given a place of honour at the centre of the display seen in our photograph, which was taken at the conclusion of the opening ceremony. **Dr John Stanford,** Chairman of the Committee since 1991 is seen in the front of the picture with his predecessor, **Prof Pierre Laplaud** (1982-1990). the South African Bureau of Standards is a Participating Member of the Committee and Dr Heydt was their representative at the meeting. A detailed report on the work of the Committee will appear in a forthcoming issue of the ***JOURNAL***.

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JOURNAL OF CRANIO-MAXILLO-FACIAL SURGERY

A retrospective analysis of 367 cystic lesions of the jaw—the Ulm experience

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SUMMARY. **Out of 846 cyst-like lesions of the jaws, 367 cases were retrieved from the files of the Department of Oral and Maxillofacial Surgery at the University of Ulm and classified according to the new World Health Organization's classification for odontogenic tumours and cysts. Radicular and residual cysts comprised 56.9%, dentigerous cysts 21.3%, odontogenic keratocysts 10.6%, unicystic ameloblastomas 4.1%, nasopalatine duct cysts 2.7%, glandular odontogenic cysts 1.6% and paradental cysts, traumatic bone cyst, calcifying odontogenic cyst and lateral periodontal cyst each less than 1% of the sample. Nearly one third of the specimens were obtained from military patients; despite an expected bias towards young males, unicystic ameloblastomas presented one and a half decades later than is generally reported.**

INTRODUCTION

Before the recent adoption of the revised World Health Organization's classification of odonotogenic tumours and cysts (*Kramer* et al., 1992), epidemiological studies on cystic jaw lesions were difficult to interpret due to the omission of recently described entities, which had not been taken up in any classification system. Examples of these are the paradental cyst which arises from odontogenic epithelial residues stimulated into activity by inflammation (*Craig*, 1976) and the aggressive glandular odontogenic cyst, the exact origin of which is less clear (*Shear*, 1992). The calcifying odontogenic cyst, which is now classified as an odontogenic tumour, occurs both in neoplastic and cystic subtypes (*Hong* et al., 1991). Unicystic ameloblastomas are divided into three subtypes, a division which is based on the histological nature of its epithelial lining. Type I unicystic ameloblastomas exhibit a simple ameloblastic epithelial lining whereas Type II shows intraluminal proliferation and Type III mural invasion. The latter type is reported to be associated with a higher recurrence rate (*Ackermann* et al., 1988).

The purpose of this study was to revise and reclassify cystic lesions of the jaws diagnosed and treated in the Department of Oral and Maxillofacial Surgery, University of Ulm, over the last 5 years.

MATERIAL AND METHODS

The clinical examination forms and radiographs of all cystic lesions affecting the jaws were retrieved from the files of the Department of Oral and Maxillofacial Surgery at the University of Ulm, Germany. 367 out of 846 microscopic sections were supplied for re-examination by the Department of Pathology at the Bundeswehrkrankenhaus Ulm as well as from the University Department. Only cases on which clinical information, a radiograph and representative microscopic sections where available, were included in the study. Each case was re-evaluated and classified independently according to the criteria set in the second edition of the World Health Organisations classification of jaw cysts and tumours (*Kramer* et al., 1992) by two oral pathologists.

RESULTS

367 cases were included in the study and 22 excluded due to a lack of radiographs and/or unrepresentative microscopic sections. Nearly one third of the cases

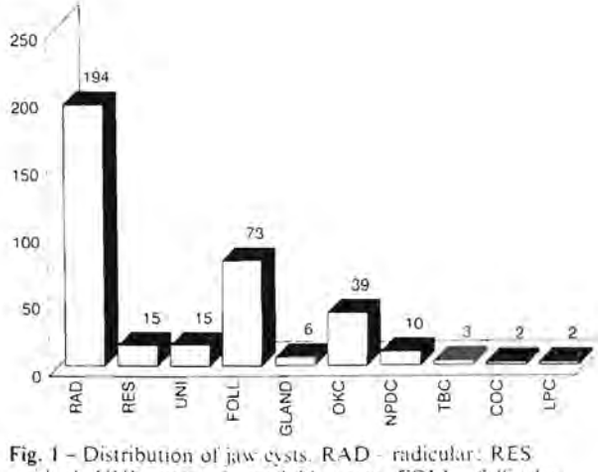

Fig. 1 – Distribution of jaw cysts. RAD – radicular; RES – residual; UNI – unicystic ameloblastomas; FOLL – follicular; GLAND – glandular odontogenic; OKC – odontogenic keratocyst; NPDC – nasopalatine duct cyst; TBC – traumatic bone cyst; COC – calcifying odontogenic cyst; LPC – lateral periodontal cyst.

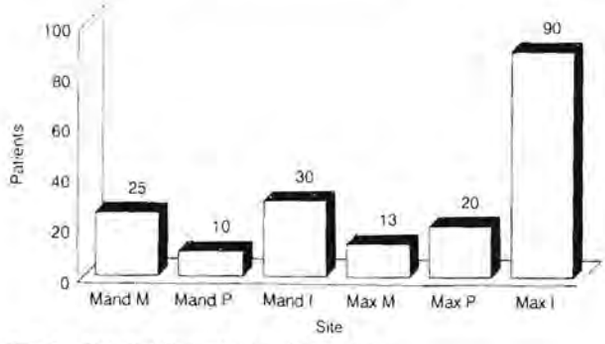

Fig. 2 – Site distribution of radicular cysts.

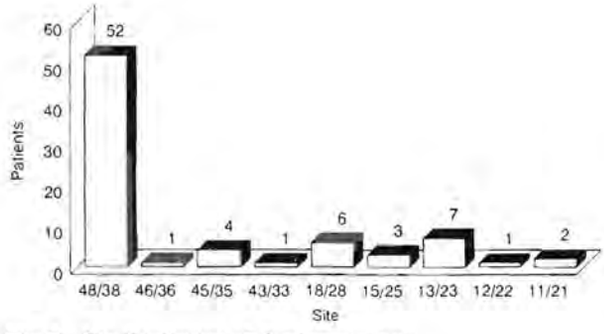

Fig. 3 – Site distribution of dentigerous cysts.

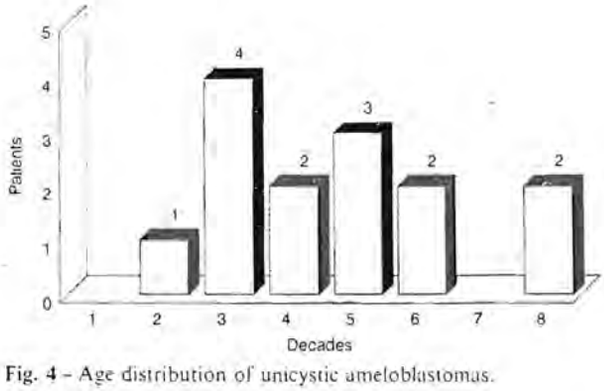

Fig. 4 – Age distribution of unicystic ameloblastomas.

recorded were military patients treated in the Bundeswehrkrankenhaus. The distribution of cystic jaw lesions in this study is reflected in Figure 1.

Radicular (n = 194) and residual cysts (n = 15) comprised 56.9% of lesions diagnosed. The mean age at presentation of radicular and residual cysts was 34.4 years (SD = 14.2) and 52.7 years (SD = 13.2) respectively. Radicular cysts occurred most commonly in the maxillary incisor region (Fig. 2).

Dentigerous cysts (n = 78) comprised 21.3% of the sample and presented at a mean age of 37.1 years (SD = 15.3 years). The mandibular third molars were most frequently involved (Fig. 3).

Three patients out of a total of 39 with odontogenic keratocysts, the latter comprising 10.6% of the sample, suffered from the basal cell naevus syndrome. The mean age at presentation of odontogenic keratocysts was 40.3 years (SD = 19.5 years) and the majority of cases (n = 21) involved the mandibular molar area. In 10 of those cysts, X-ray examination showed teeth or rudiments inside the cavity which led to a primary misdiagnosis of dentigerous cyst.

Unicystic ameloblastomas (n = 15) comprised 4.1% of the sample and presented at a mean age of 40.7 years (SD = 18.8 years). The youngest patient was in the second decade of life whereas 2 cases presented in the eighth decade (Fig. 4). 8 unicystic ameloblastomas occurred in the mandibular molar area, 6 of which appeared radiographically as dentigerous-like cysts, and 4 lesions affected the maxilla. 13 unicystic ameloblastomas were lined by non-invasive odontogenic epithelium (Type I) and 2 cases exhibited foci of mural invasion (Type III).

The mean ages at presentation of nasopalatine duct cysts (n = 10, 2.7% of the sample) and glandular odontogenic cysts (n = 6, 1.6% of the sample) were 44.9 years (SD = 13.5 years) and 46 years (SD = 14.3 years) respectively. The paradental cyst (n = 3), traumatic bone cyst (n = 3), calcifying odontogenic cyst (n = 2) and lateral periodontal cyst (n = 2) each contributed to less than 1% of the sample. No examples of gingival cysts of infants and adults, eruption cysts and nasolabial cysts were found in this study.

DISCUSSION

Accurate diagnosis of cystic lesions of the jaw is crucial as various types are aggressive and may lead to local recurrence if incorrectly diagnosed and inappropriately treated. Many cystic lesions of the jaw share clinical and radiographic features and microscopic examination forms an important part of the diagnostic process. For this purpose, an in-depth knowledge of an internationally accepted classification system, such as that proposed by the World Health Organization (*Kramer* et al., 1992) is essential.

The description of new cyst entities in combination with the new WHO-classification on the one side and improbable lack of diagnosed ameloblastomas on the other prompted this retrospective investigation. It shows the incapability of a general pathologist to make a correct and specific diagnose of jaw cysts and necessitates cooperation with an experienced oral pathologist.

Due to their association with the ghost cell odontogenic tumour, the calcifying odontogenic cyst is no longer grouped amongst cysts in this classification but is classified as a benign tumour originating from the odontogenic apparatus. This cystic tumour, as well as the odontogenic keratocyst (*Brown*, 1970; *Niemeyer* et al., 1985), unicystic ameloblastoma (*Ackermann* et al., 1988) and glandular odontogenic cyst (*Patron* et al., 1991), are notorious for their aggressive behaviour and high recurrence rates (*Machtens* et al., 1972). This implies that in the present study, 17% of the total sample of cystic jaw lesions, required more than simple enucleation as a curative surgical procedure. Type III unicystic ameloblastomas, of which 2 cases were diagnosed in this study, exhibit infiltrative features and should be treated

similarly to the polycystic types, with wide excision or even resection of the involved jaw segment (*Ackermann* et al., 1988). These results have induced a recall of those patients with diagnosed aggressive cysts or ameloblastomas in order to prove the necessity for further treatment.

A large percentage of patients in this study were military personnel and our data is probably biased towards young males. The high mean age of 40.7 years for unicystic ameloblastomas was therefore surprising as these cystic tumours are reported to occur most frequently in the first half of the third decade (*Robinson* and *Martinez*, 1977; *Gardner*, 1981; *Ackermann* et al., 1988). As no literature is available on unicystic ameloblastomas in the German population, this finding may point towards an older age incidence for unicystic ameloblastomas in Germany. Unicystic ameloblastomas frequently involved the mandibular molar area where impaction of a mandibular third molar in the cyst wall was common. Unless these lesions are examined microscopically, they will be misdiagnosed as dentigerous cysts.

The frequency of the different cyst types encountered in this study, as well as the sites of involvement of radicular, residual, dentigerous and odontogenic keratocysts and unicystic ameloblastomas, are in agreement with the recent literature (*Shear*, 1992). The lack of examples of gingival cysts of infants and adults and eruption cysts, as well as the infrequent occurrence of paradental cysts is the result of exclusion of all cases without a microscopic diagnosis. Most of these lesions either go unnoticed or are not submitted for microscopic examination after removal and are probably more common than is reflected in a study of this nature.

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SPECIAL ARTICLE

Classification of Odontogenic Cysts of the Jaws

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Recent developments in the classification and diagnosis of cysts of the jaws necessitate a revision of the topic. This paper discusses the revised World Health Organisation classification of odontogenic cysts and illustrates short descriptions of cyst types with appropriate examples.

INTRODUCTION

Cysts are pathological, fluid filled cavities lined by epithelium. They are more common in the jaws than in any other bone because of the epithelial rests remaining in the tissue after dental development. Cysts of odontogenic origin are the most common cause of chronic swelling of the jaws and have been recognised as diagnostic problems for a long time. During the past few years, numerous articles have appeared regarding the pathogenesis, behaviour, diagnosis and treatment of the different types of jaw cysts and various new concepts have since emerged. In order to standardize the diagnoses of jaw cysts, utilization of uniform diagnostic criteria is essential. The purpose of this article is to present the revised World Health Organisation's classification of odontogenic cysts of the jaws and to illustrate the typical features with appropriate examples obtained from the files of the Department of Oral Pathology, Medical University of Southern Africa.

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CLASSIFICATION

The classification of the odontogenic cysts of the jaws is based on that recommended in the World Health Organization's (WHO) publication *Histological Typing of Odontogenic Tumours*[1] and a recently published textbook on oral cysts[2] (Table 1). The histogenetic division into 'Developmental' and 'Inflammatory' groups remain unchanged.

Table 1.

1. Developmental
- 1.1 Gingival cyst of infants
- 1.2 Odontogenic keratocysts
- 1.3 Dentigerous (follicular) cyst
- 1.4 Eruption cyst
- 1.5 Lateral periodontal cyst
- 1.6 Gingival cyst of adults
- 1.7 Botryoid odontogenic cyst
- 1.8 Glandular odontogenic cyst

2. Inflammatory
- 2.1 Radicular cyst (apical and lateral)
- 2.2 Residual cyst
- 2.3 Paradental cyst
- 2.4 Inflammatory collateral cyst

This classification excludes the calcifying odontogenic cyst (which is now categorized as an odontogenic tumour) as well as other cystic tumours like the unicystic ameloblastoma. It is furthermore noteworthy that the concept of cysts developing in the closure lines of embryologic processes (such as median palatine cyst, median mandibular cyst and globulo-maxillary cyst) which were previously classified as of non odontogenic origin, has been rejected after detailed clinical[3,4] and embryological[5,6] studies.

DEVELOPMENTAL

Gingival cysts of infants

Gingival cysts of infants, also referred to as Bohn's nodules, occur commonly on the alveolar processes of newborn infants (Figure 1). They soon disappear through involution and are seldom seen after three months of age. These cysts arise from the dental lamina and although rarely biopsied, are lined by keratinizing squamous epithelium.[2]

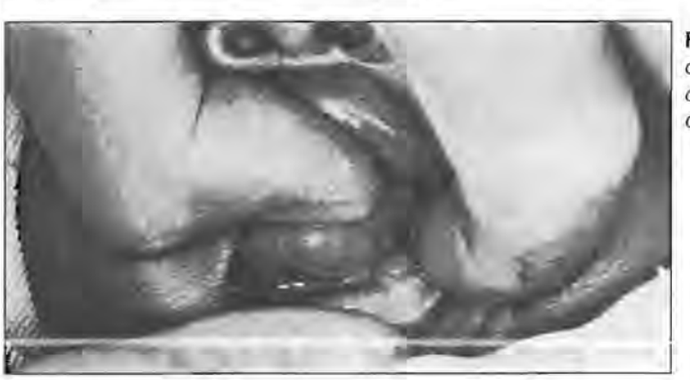


Figure 1. *Gingival cyst of the infant on the left mandibular alveolus.*

Odontogenic keratocysts

The term 'primordial cyst', which was often used synonomously with odontogenic keratocyst, has fallen in disuse because no convincing evidence for development from a tooth primordium has yet been forwarded. There is however, evidence supporting origin from primordial odontogenic epithelium, that is, dental lamina or its remnants[7,8]. Although other odontogenic cysts may exhibit foci of squamous metaplasia, odontogenic keratocysts are primarily recognised by their stretched and keratinized epithelial lining with a well defined, often palisaded basal cell layer. Basal cell budding, as well as daughter cyst formation are found in odontogenic keratocysts and are especially pronounced in patients with the naevoid basal cell carcinoma syndrome in which multiple keratocysts occur. These phenomena as well as the fragility of the cyst wall are the primary causes for incomplete surgical removal and the high recurrance rate. Odontogenic keratocysts .nay occur in the place of a tooth (replacement variety), around the crown of an impacted tooth (envelopmental variety) in the ramus of the mandible (extraneous variety) or between the roots of adjacent teeth (collateral variety)[9]. Although the majority present as unilocular radiolucencies (Figure 2), scalloped margins may be misinterpreted as multilocularity leading to an erroneous diagnosis of ameloblastoma[2]. The envelopmental variety is often indistinguishable radiologically from a dentigerous cyst and the collateral variety from a lateral periodontal or lateral placed radicular cyst.

Dentigerous (follicular) cysts

A dentigerous cyst is one which encloses the crown of an unerupted tooth by expansion of its follicle, and is attached to its neck[2] (Figure 3). It probably developes by accumulation of fluid between the reduced enamel epithelium and the enamel after formaton of the crown has been completed. The diagnosis of dentigerous cyst should not be made on radiographic evidence only, otherwise keratocysts of the envelopmental variety and unilocular ameloblastomas involving adjacent unerupted teeth, are liable to be misdiagnosed. The wall of a dentigerous cyst is lined by thin epithelium of two to three layers of undifferentiated cells derived from reduced enamel epithelium.

Figure 2. *Odontogenic keratocyst in the anterior mandible. Note the sclerotic margin surrounding the cyst.*

Figure 3. *Panoramic view of a dentigerous cyst surrounding the crown of an impacted maxillary central canine. Note the displacement of the permanent lateral incisor and canine.*

Eruption cyst

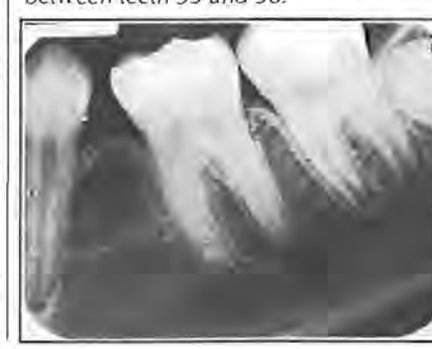
An eruption cyst is in effect a dentigerous cyst which occurs in the soft tissues. There is usually no radiographic evidence of bone involvement. The cyst is exposed to masticatory trauma and many eruption cysts burst spontaneously with only few requiring surgical exposure of the involved tooth.

Lateral periodontal cyst

The designation 'lateral periodontal cyst' is confined to those cysts which occur in the lateral periodontal position and in which an inflammatory aetiology and a diagnosis of collateral keratocyst have been excluded on clinical and histological grounds.[10] Radiographs show a round or oval, well circumscribed, radiolucent area somewhere between the apex and cervical margin of a vital tooth (Figure 4). Various theories on the histogenesis of this cyst type were forwarded, of which the proposal that it arises initially as a dentigerous cyst developing by expansion of the follicle along the lateral surface of the erupting tooth is an attractive one[11]. Most commonly, the lateral periodontal cyst is lined by a thin, non keratinized layer of squamous or cuboidal epithelium with small inconspicuous nucleii and convoluted epithelial plaques, which develop as a result of localized proliferation of cells[2].

The botryoid odontogenic cyst is a multilocular variant of the lateral periodontal cyst. This rare cyst has a

Figure 4. *Periapical radiograph showing a lateral periodontal cyst in the alveolus between teeth 35 and 36.*



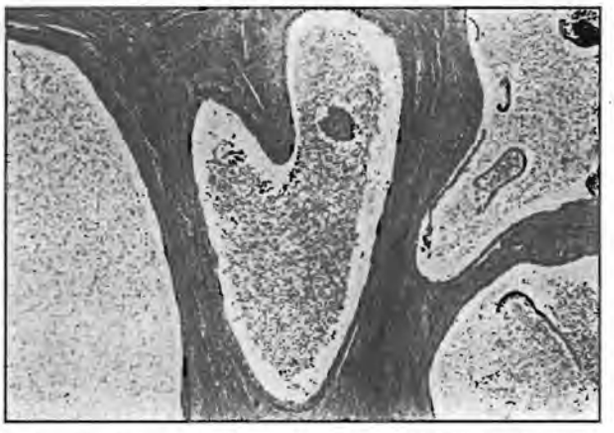


Figure 5. *Multicystic appearance of a botryoid odontogenic cyst (H&E stain X40).*

lining similar to the lateral periodontal cyst with thin connective tissue septae separating distinct cystic cavities (Figure 5).

Gingival cyst of adults

The gingival cysts of adults is located in the gingival soft tissue and presents as a gingival swelling without any radiographic signs of bone destruction. Although many theories have been proposed on its histogenesis, the most favoured is derivation from gingival odontogenic epithelial cell nests[2] or reduced enamel epithelium after the eruption of a tooth.[12,13] If the latter theory is accepted, gingival cysts in adults may represent the soft tissue counterpart of lateral periodontal cysts. This is supported by the numerous similarities both clinically and histologically between these two cyst types.

Glandular odontogenic cyst

A cyst with fairly typical histological features and which has some characteristics in common with lateral periodontal cyst has recently been reported[14,15]. Radiographically, some cases exhibit a unilocular radiolucency with either smooth or scalloped margins (Figure 6), while others are distinctly multilocular. The cyst may be lined in parts by a non-keratinized stratified squamous epithelium. The superficial layer of the epithelial lining consists of columnar or cubiodal cells with occasional cilia and the epithelium has a glandular or pseudo glandular structure, with intra-epithelial crypts lined by cells similar to those on the surface.

INFLAMMATORY

Radicular cyst

A radicular cyst is one which arises from epithelial residues in the periodontal ligament as a result of inflammation[2]. The inflammation usually follows necrosis of the dental pulp and the identification of a non vital tooth associated with the cyst is an important diagnostic parameter. Although these cysts are usually located around the apex of a tooth (Figure 7), they may also be found on the lateral surfaces of a root in association with the opening of an accessory pulpal canal. Radiographically these cysts are characterized by round or ovoid radiolucencies surrounded by a narrow radio-opaque margin which extends from the lamina dura of the involved tooth. The size of the lesion is not reliable in distinguishing it from a periapical granuloma, unless it is larger than 2 cm in diameter in which case the lesion is most likely a radicular cyst.[16] Almost all radicular cysts are lined

Figure 6. *Glandular odontogenic cyst presenting as an unilocular cyst in the maxilla. Note the displacement of the adjacent teeth.*

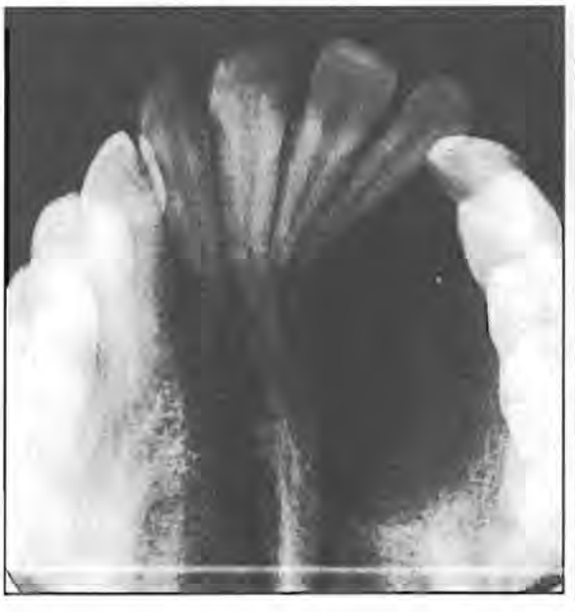
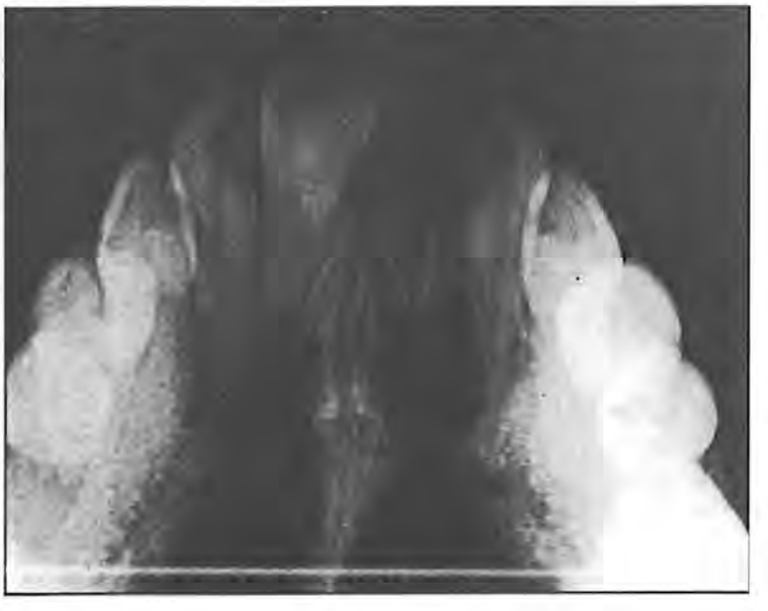


Figure 7. *Radiograph of a radicular cyst surrounding the apex of a maxillary incisor.*



wholly or in part by stratified squamous epithelium. The epithelial lining may proliferate and exhibits arcading and a considerable degree of spongiosis with an intense inflammatory infiltrate.

Residual cyst

A residual cyst can be described as a radicular cyst of which the associated tooth has been extracted. All the radiographic and histological features of radicular cysts except for the association with a non-vital tooth therefore apply to residual cysts.

Paradental cyst

Craig (1976) wrote the first detailed account of a cyst of inflammatory origin which occured on the lateral aspect of the roots of partially erupted mandibular third molars where there was an associated history of pericoronitis. In these cases the teeth are vital and radiographic examination shows a well demarcated radiolucency distally to a partially erupted tooth: Ackerman, Cohen and Altini[17] like Craig[18] favour origin from reduced enamel epithelium but suggested that cyst formation occurs as a result of unilateral expansion of the dental follicle secondary to inflammatory destruction of the periodontium and alveolar bone. This is different from the histogenesis of dentigerous cysts where expansion occurs primarily with consequent bone destruction.

Paradental cysts are microscopically indistinguishable from radicular cysts and a proper clinical history and radiograph must accompany the biopsy in order to facilitate a correct diagnosis.

Inflammatory collateral cyst

This rare cyst type occurs as a result of inflammatory process in the periodontal pocket[2]. The associated tooth is vital and the cyst is microscopically indistinguishable from radicular cysts. Microscopic diagnosis relies heavily on adequate clinical information. This cyst appears to favour developing buccally to the lower first or second molars.

CONCLUSION

Accurate diagnosis of cysts of odontogenic origin is important as various cyst types like odontogenic keratocysts and glandular odontogenic cysts are aggressive lesions and tend to recurr after incomplete removal. It is important that clinicians are aware of the unreliability of radiographic interpretations. On the other hand, a microscopic diagnosis of biopsies taken from densely inflamed cyst walls is often difficult, if not impossible, to interpret without clinical information and radiographs. A high degree of diagnostic accuracy, when dealing with jaw cysts, can only be achieved through communication between the clinician and resident pathologist.

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Differential diagnosis yst-like lesions: Clinico-pathologic features of 63 cases

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Keywords: odontogenic cyst; dentigerous cyst; unicystic ameloblastoma; keratocyst.

SUMMARY

A series of unilocular pathological conditions resembling dentigerous cysts was analyzed and the clinical and radiographic features correlated with the microscopic diagnosis. The most common lesions were found to be true dentigerous cysts followed by unicystic ameloblastomas and odontogenic keratocysts. Unicystic ameloblastomas with a dentigerous cyst-like appearance occurred most frequently in the mandibular third molar region and commonly caused expansion of the mandible. The adjacent teeth in these cases showed a high occurrence rate of root resorption. Unlike the site distribution of true dentigerous cysts reported in other series, 50 per cent of our cases occurred in the maxillary anterior and premolar regions. Our study emphasizes the importance of microscopic examination of all pericoronal cystic lesions.

OPSOMMING

Die kliniese- en radiologiese beeld van 63 gevalle van unilokulêre patologiese toestande wat tandhoudende siste naboots, is vergelyk met die mikroskopiese diagnose. Die mees algemene toestand wat voorgekom het, was tandhoudende siste, gevolg deur unisistiese ameloblastoom en odontogene keratosiste. Unisistiese ameloblastome met die radiologiese beeld van 'n tandhoudende sist, het mees algemeen in die mandibulêre derde molaar area presenteer en het dikwels beenekspansie veroorsaak. Die aangrensende tande het 'n hoë frekwensie van eksterne wortelresorpsie getoon. Anders as in ander reekse, het 50 persent van tandhoudende siste in die maksillêre anterior en premolaar areas voorgekom. Hierdie studie beklemtoon die belang van mikroskopiese ondersoek van alle perikoronale sisteuse letsels.

INTRODUCTION

A dentigerous cyst is defined as a unicystic cavity which encloses the crown of an unerupted tooth by expansion of its follicle and is attached to the neck of the tooth (Shear, 1992). In a radiographic context, a radiolucent area surrounding the crown of an unerupted tooth may be seen with odontogenic keratocysts of the envelopmental or follicular variety as well as unicystic ameloblastomas involving adjacent unerupted teeth, and these may be misinterpreted as dentigerous cysts. This could have prognostic consequences as the recurrence rates of the various pathologic lesions that envelop the crown of a tooth vary significantly. Simple enucleation is an adequate form of treatment for dentigerous cysts but more extensive surgery is required for unicystic ameloblastomas and odontogenic keratocysts. Even with adequate treatment, the recurrence rates of unicystic ameloblastomas and keratocysts are reported to be high (Vedtofte and Praetorius, 1979; Ueno *et al.*, 1986). Accurate diagnosis of jaw cysts is therefore essential for adequate treatment planning.

The purpose of this study was to appraise the clinico-pathologic features of pericoronal radiolucencies resembling dentigerous cysts.

MATERIAL AND METHODS

Sixty three lesions with a radiologic appearance of a dentigerous cyst were retrieved from the files of the department of Maxillo-Facial and Oral Surgery at the Medical University of Southern Africa. This hospital is a reference centre for the Northern Transvaal region and all patients in the study are Black and mostly of rural origin. The radiologic appearance with special reference to the size (longest axis measured on panoramic radiograph) and location of the lesion, the presence or absence of root resorption of adjacent teeth, expansion of cortical plates and displacement of the associated tooth, was compared with the age, sex and microscopic diagnosis of the lesion.

RESULTS

Sixty-three unilocular paracoronal cystic lesions resembled dentigerous cysts radiologically. The histological diagnosis of these lesions are listed in

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Fig. 1: Unicystic ameloblastoma with root resorption of the associated teeth.

Fig. 2: Adenomatoid odontogenic tumour causing tooth displacement and root resorption.

Fig. 3: Unicystic ameloblastoma of the left mandible showing enlargement in all dimensions.

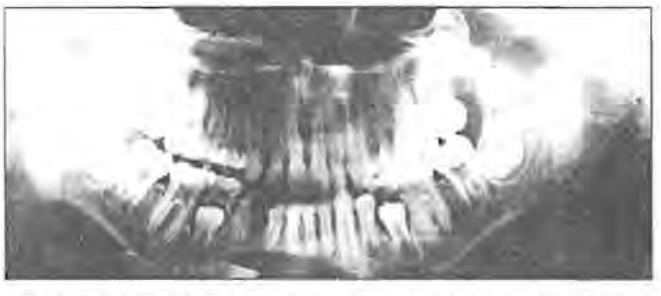

Fig. 4: Dentigerous cyst of the mandible showing enlargement along the medullary space.

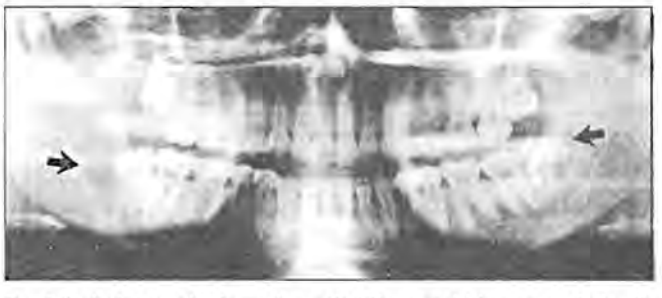

Fig. 5: Calcifying odontogenic cyst of the mandible associated with an impacted canine and exhibiting mural calcifications (arrows).

Table I. Three of the calcifying odontogenic cysts with a dentigerous cyst-like appearance were subclassified according to Praetorius *et al.*, (1981) as type IA, and one each as type IB and IC respectively. The sex distribution, mean age at presentation, average size of the cyst as measured on a panoramic radiograph and the presence of root resorption and tooth displacement are shown in Table I.

Fig. 6: Multiple keratocysts involving the left and right mandibular ramus and right globulomaxillary area in a patient with the naevoid basal cell carcinoma syndrome.

Unicystic ameloblastomas showed an equal sex distribution, while dentigerous cysts, odontogenic keratocysts and calcifying odontogenic cysts were more common in males. Adenomatoid odontogenic tumours were found in females only. The mean age at presentation of the six cyst types were not found to differ significantly. The mean size of the unicystic ameloblastomas were significantly larger than the odontogenic keratocysts and dentigerous cysts ($p<0,005$) while odontogenic keratocysts' mean size were significantly larger than that of dentigerous cysts ($p<0,05$). Root resorption was most frequently observed in unicystic ameloblastomas (64 per cent of cases) (Fig. 1) and calcifying odontogenic cysts (60 per cent of cases). Displacement of non-involved teeth was a constant finding in cystic adenomatoid odontogenic tumours (Fig. 2). The enlargement of unicystic ameloblastomas occurred in all dimensions and frequently caused bony expansion (Fig. 3). Enlargement of follicular and odontogenic keratocysts in the mandible appeared to follow the medullary space initially (Fig.4) with bony expansion seen only in the largest examples.

All paradental cysts were associated with partially erupted third molars. Six cystic adenomatoid odontogenic tumours occurred in the maxilla and one in the mandible. One very large lesion of the latter type extended across the maxillary midline. Three calcifying odontogenic cysts presented in the maxilla and two in the mandible. One cyst in each jaw showed radiographic evidence of calcifications (Fig. 5).

Thirteen dentigerous cysts were located in the maxilla, the majority of which were associated with impacted central incisors (4 cysts), canines (2 cysts) and premolars (4 cysts). In the mandible, only 3 dentigerous cysts involved third molars; one, a second molar, while two involved canines. One dentigerous cyst was associated with a primary maxillary canine. Eight odontogenic keratocysts were located in the mandibular (7 cases) or maxillary (one case) third molar areas and 5 presented in the canine region (3 maxillary and 2 mandibular). Two patients presenting with the basal cell nevus syndrome had multiple cysts

Table I: *Clinical data.*

	n	Sex		Mean Age (SD)	Mean Size in mm (SD)	Root Resorption	Tooth Displacement
		Male	Female				
Unicystic Ameloblastoma	14	7	7	15,5 ±6,3	80 ±22,7	9	5
Dentigerous Cyst	21	16	5	16 ±16,2	35 ±11,5	4	5
Odontogenic Keratocyst	13	10	3	15 ±12,5	45 ±20,1	1	3
Adenomatoid Odontogenic Tumour	7	0	7	12 ±3,9	45 ±15,7	1	7
Calcifying Odontogenic Cyst	5	4	1	23 ±7,0	40 ±6,5	2	3
Paradental Cyst	3	2	1	21 ±2,0	10 ±12,7	0	0

(Fig. 6). The unicystic ameloblastomas showed a predeliction for the mandibular third molar region (11 cysts) followed by the mandibular canine region (3 cysts). No unicystic ameloblastomas with a dentigerous cyst-like appearance occurred in the maxilla.

DISCUSSION

The importance of an accurate diagnosis of a lesion with a dentigerous cyst-like appearance, especially in a Black population sample in which dentigerous cysts are less common than in Whites (Shear, 1992), cannot be over emphasized. By the same token the presence of unicystic ameloblastomas must not be underestimated, being the second most common cystic lesion found in our patients. Outstanding characteristics of this potentially aggressive neoplasm is its large size when compared to the other cysts, its tendency to expand more symmetrically than other cystic lesion in the mandible as well as its common association with root resorption of adjacent teeth. Adenomatoid odontogenic tumours were found in females only but although the majority seem to affect the anterior maxilla, it also occurred in the mandible in one instance. Tooth displacement was more frequently observed in adenomatoid odontogenic tumours than in any of the other cystic lesions. Dentigerous cysts were more frequently encountered in the anterior maxilla and their most frequent association with impacted mandibular third molars (Shear, 1992) was not found in our study. The lower frequency of impacted third molars in Blacks (Brown *et al.*, 1982) may account for this observation in our exclusively Black sample. The attachment of the cyst wall to the impacted tooth is reported to extend more apically in ameloblastomas than dentigerous cysts (Ikeshima *et al.*, 1990). In large examples of dentigerous cysts the associated tooth is often rotated, making this measurement difficult to interpret on panoramic radiographs.

Our study does not support the report that there is a frequent occurrence of root resorption in association with dentigerous cysts (Struthers and Shear, 1976). The site distribution of odontogenic keratocysts in our study conform to that of another series (Shear, 1992). Forssell (1980) observed a relationship between the cyst and the crown of a tooth in 41 per cent of a series of 135 cases. McIvor (1972) however, demonstrated this relationship exclusively in the mandible. In our study, 4 maxillary odontogenic keratocysts presented in association with impacted teeth. The frequent association of odontogenic keratocysts with impacted teeth have led Altini and Cohen (1980) to introduce the term "follicular primordial cyst" for this group of lesions. They postulated that this association may arise following eruption of a tooth into a pre-existing cystic cavity in the same way as a tooth erupts into the oral cavity. Although we have no microscopic evidence, we believe that this hypothesis may be extended to all cysts in our series, except for the follicular and paradental cysts, in both of which types their association with an impacted tooth have been satisfactorily explained (Shear, 1992).

Although certain specific features seen on radiographs, such as the size of lesion, its location, the presence or absence of root resorption or tooth displacement and other factors such as age and sex of the patient may influence the clinical differential diagnosis, a thorough histological examination is essential in establishing an accurate diagnosis.

ACKNOWLEDGEMENT

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GLANDULAR ODONTOGENIC CYST

Willem F.P. van Heerden, MChD, Erich J. Raubenheimer, MChD, and Martin L. Turner, DipTech(Med)

Two cases of glandular odontogenic cysts are reported. The unique histological features, eg, the intraepithelial glandular structure, papillary processes, and eosinophilic cuboidal and larger granular superficial cells are sufficient to warrant glandular odontogenic cyst as a distinct entity. Electron microscopic examination of the superficial eosinophilic cuboidal cells are suggestive of a process similar to apoptosis. Eroded cortical plates suggest an aggressive behavior.
HEAD & NECK 14:316–320

The glandular odontogenic cyst (GOC) is a rare cystic lesion that is not incorporated in classifications of jaw cysts. Only a few examples of this lesion have been described in the literature. Gardner et al[1] collected eight cases of GOC. Padayachee and van Wyk[2] reported two cases, which they described as "sialo-odontogenic cysts."

The GOC has an equal sex distribution and occurs in both the mandible and maxilla of adults.[1,2] These lesions, which can attain a large size, appear on radiographs as uni- or multilocular lytic lesions. The histologic features described by Gardner et al[1] include a cyst lining consisting of stratified squamous epithelium of varying thickness that contains pools of mucicarmine-positive material. The superficial layer consists of eosinophilic cubiodal and occasionally mucous- and ciliated cells. Spherical structures produced by swirling epithelium and lack of cell polarization are focally present in the epithelium lining. Irregular-shaped calcifications are occasionally found in the subepithelial connective tissue.

This report describes the clinical, histopathologic, and ultrastructural features of two cysts.

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CASE 1

A 27-year-old woman reported to the clinic complaining of a painless swelling in the anterior mandible of three years' duration. Intraoral examination revealed a 6 × 3 cm sized swelling extending from the left first mandibular molar to the right second premolar with buccal as well as lingual bone expansion. The mucosa was intact, but the bone was eroded in areas causing the swelling to fluctuate on palpation (Figure 1). No sensory nerve fallout was found. Radiography revealed a well-defined unilocular radiolucent lesion with a scalloped border. Displacement of the anterior teeth was present (Figure 2). During biopsy, a unicystic cavity containing yellow serous fluid was found. Differential diagnoses included a unicystic ameloblastoma and odontogenic keratocyst.

Histologic examination of the incisional biopsy revealed a cyst lining consisting of a nonkeratinized epithelium. The epithelium varied in thickness from double-layer cuboidal to

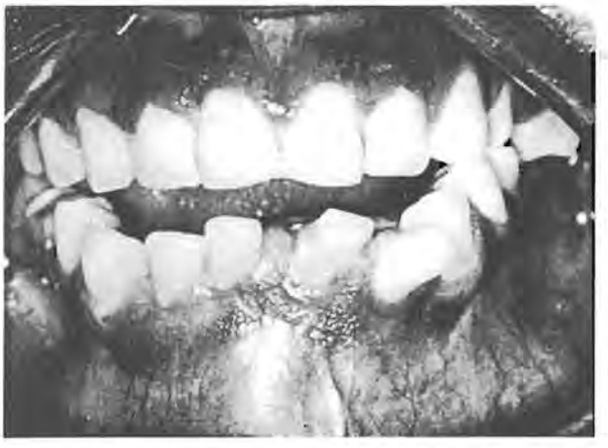

FIGURE 1. Mandibular lesion showing buccal and lingual expansion associated with tooth displacement.

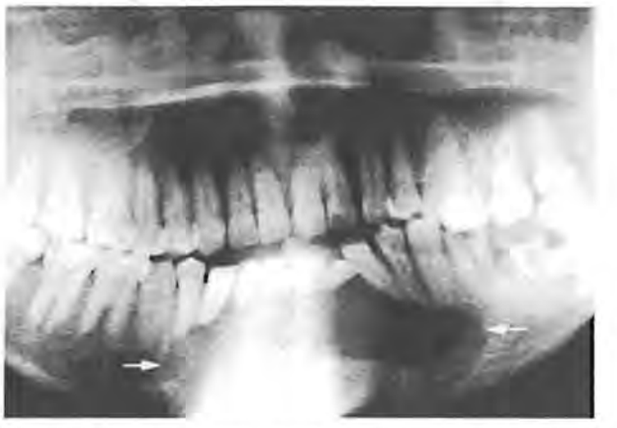

FIGURE 2. Pantomograph exhibiting an unilocular radiolucency (arrows) of the anterior mandible.

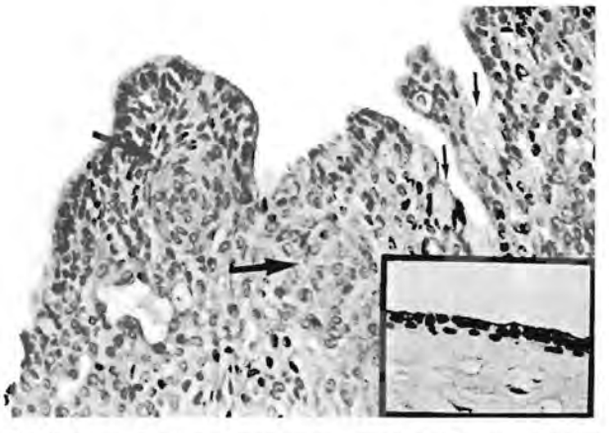

FIGURE 3. Papillary processes associated with epithelial spheres (bold arrows) and superficial mucous cells (fine arrows). Hematoxylin & eosin; original magnification, ×200. Inset: The cyst lining is partly composed of a double layer cuboidal epithelium. Hematoxylin & eosin; original magnification ×200.

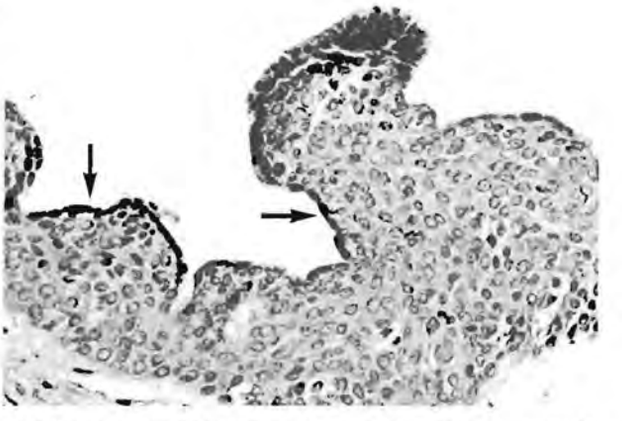

FIGURE 4. Superficial cell layer consisting of small cuboidal cells with hyperchromatic nucleii and eosinophilic cytoplasms (arrows). Note the papillary processes. Hematoxylin & eosin; original magnification, ×200.

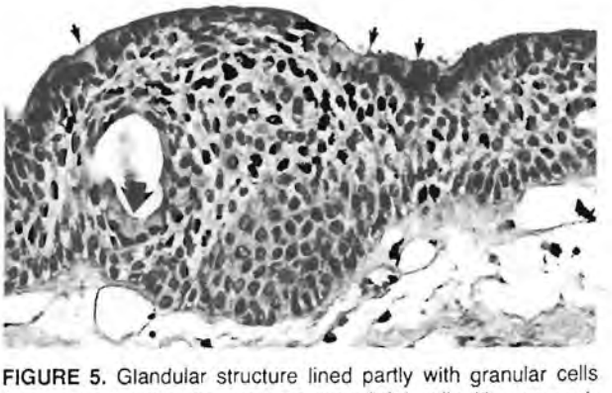

FIGURE 5. Glandular structure lined partly with granular cells (bold arrow). Note the granular superficial cells (fine arrows). Hematoxylin & eosin; original magnification, ×200.

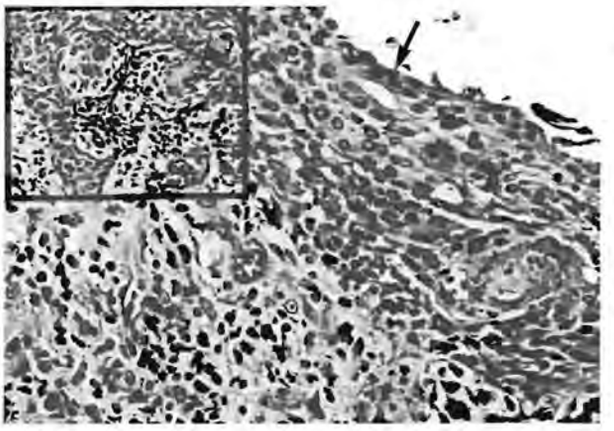

FIGURE 6. Inflammatory induced changes in lining of case 1. A glandular structure is visible (arrow). Hematoxylin & eosin; original magnification, ×200. Inset: Epithelial arcading associated with lymphocytes. Hematoxylin & eosin; original magnification ×100.

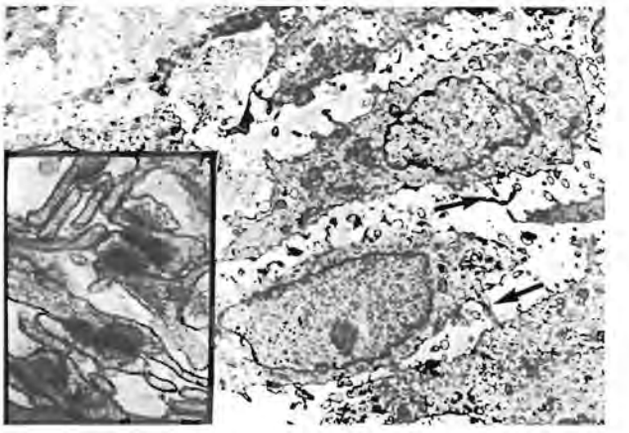

FIGURE 7. Transmission electron micrograph of the inflammed lining revealed widened intercellular spaces containing finger-like protrusions (arrows). Original magnification, ×2600. Inset: Well-formed desmosomes were present between the protrusions. Original magnification, ×8300.

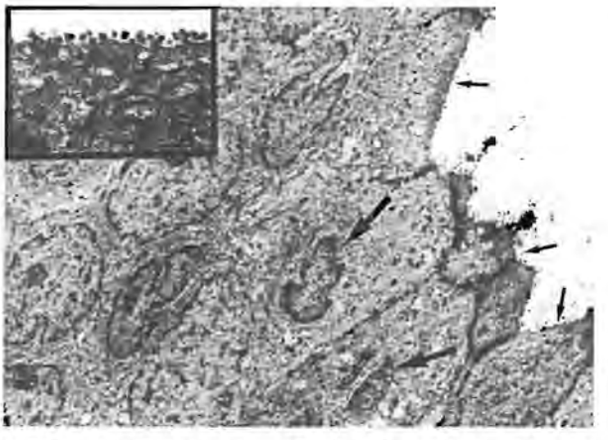

FIGURE 8. Electron micrograph of the lining of case 1 representing the superficial eosinophilic cuboidal cells. Note the smaller, denser nucleoli in the more superficial cells (bold arrows) and the absence of nuclear material in the remainder of the superficial cells (fine arrows). Original magnification, ×3300. Inset: Microvilli on the luminal aspect of the superficial cells. Original magnification, ×10,000.

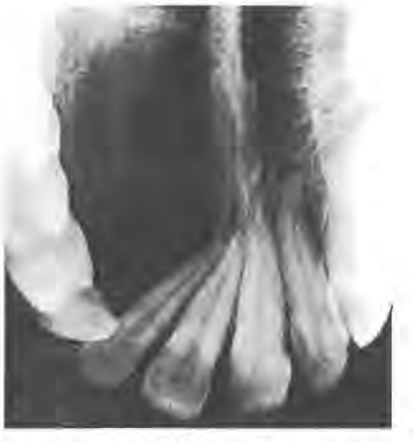

FIGURE 9. Occlusal radiograph revealed a well-circumscribed radiolucency causing root divergence of the lateral and incisor teeth.

stratified squamous. Papillary epithelial processes into the lumen were noted, especially where epithelial thickenings were present. Epithelial spheres consisting of swirled epithelial cells were found occasionally (Figure 3). The superficial cell layer consisted mainly of small cuboidal cells with scanty eosinophilic cytoplasm and hyperchromatic nuclei (Figure 4). Larger cells with an eosinophilic granular cytoplasm and a round nucleus, which was oriented away from the surface, as well as scattered mucous cells were also present in the superficial layer. Ciliated cells were focally seen.

Intra-epithelial glandular structures, filled with an eosinophilic, mucicarmine-positive material were present, the majority located in the superficial half of the epithelium (Figure 5). These glandular spaces were lined mainly by granular cells, although mucous cells were focally present. No mitotic figures were noted. Palisading of the basal cells were focally seen, and no maturation changes of the epithelial cells were noted. Cleaving between the epithelium and connective tissue was focally observed. The underlying connective tissue consisted of dense fibrous tissue with a few vascular spaces. No epithelial islands nor calcifications were noted.

A diagnosis of a glandular odontogenic cyst

was made, and the cyst lining was enucleated under general anesthesia. The wound was closed primarily and healing was uneventful. Small fragments of the lining were fixed separately in 3% gluteraldehyde for electron microscopic examination. Light microscopic examination of the enucleated material revealed a dense, chronic inflammatory cell infiltrate consisting mainly of lymphocytes in the subepithelial connective tissue and neutrophils in the epithelium. The epithelial lining had lost most of the features described in the incisional biopsy material. Epithelial hyperplasia and proliferation into the underlying connective tissue with an arcading effect were present. The eosinophilic cuboidal superficial cell layer as well as glandular structures in the epithelium were focally present (Figure 6).

Electron microscopic examination revealed widened intercellular spaces with numerous fingerlike protrusions that attached adjoining epithelial cells by well-formed desmosomes (Figure 7). As the biopsies taken for electron microscopy were not representative of all epithelial types as seen in the sections of the incision biopsy, a small fragment was then removed from the wax block of the noninflammed biopsy specimen and processed for electron microscopy. This epithelial lining consisted of tightly aggregated cells with well-formed desmosomes. Microvilli-like projections were present on the luminal aspect of the superficial cells, the majority of which contained no nucleii. Their cell volume seemed to be decreased, resulting in a closer association of the desmosomes (Figure 8). The cells immediately underneath the superficial cells contained a denser nucleus, and signs of nuclear fragmentation were present.

CASE 2

A 14-year-old boy presented with swelling of the right upper lip. Oral examination revealed a firm buccal and palatal swelling involving the right maxillary canine area. Radiographs showed a well circumscribed, unilocular lytic lesion in the globulo-maxillary area causing root divergence of the lateral and incisor teeth (Figure 9). A biopsy was taken, and microscopic examination showed a cyst lining with similar features as described in case 1 (Figure 10). A diagnosis of a glandular odontogenic cyst was made. The patient did not return for treatment.

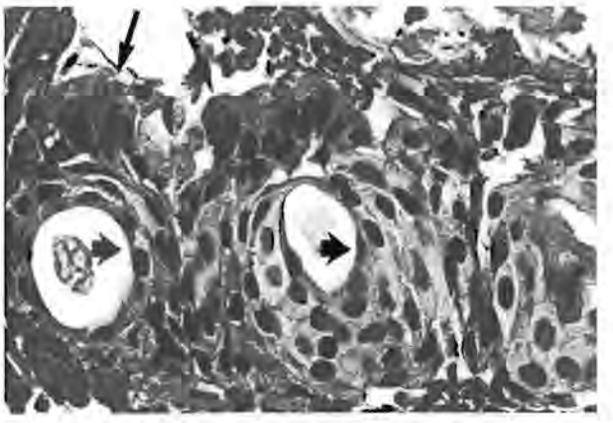

FIGURE 10. Glandular structures were present in the lining epithelium (short arrows). The superficial cells had a granular appearance (long arrows). Hematoxylin & eosin; original magnifications, ×400.

DISCUSSION

There are sufficient criteria to regard GOC as a distinct entity and not a variant of any other cyst. The unique features include the presence of eosinophilic cuboidal and larger granular superficial cells, intraepithelial glandular structures lined by granular and mucous cells, and papillary processes protruding into the lumen. Epithelial spheres are also found in both lateral periodontal cysts and dentigerous cysts.[3] The presence of numerous mucous cells alone does not warrant the diagnosis of GOC. Browne has shown that mucous metaplasia is fairly common in dentigerous cysts, and can be found in the majority of jaw cysts.[4]

The widened interepithelial cell spaces and the finger-like protrusions found in inflammed GOC tissue on electron microscopic examination are also present in inflammed as well as noninflammed radicular and follicular cysts.[5] The spinous cells of odontogenic keratocysts, however, show a close intercellular relationship with desmosomes rarely detected.[5] The tissue fragment removed from the wax block for electron microscopy study contained superficial eosinophilic cuboidal cells. It is tempting to speculate that the superficial cells undergo a process similar to apoptosis. This will explain the eosinophilic light microscopic appearance of the superficial cells with hyperchromatic nucleii, although the microvilli-like projections seen on electron microscopy are too small to represent apoptotic bodies.[6]

The prevalence of GOC is low. The two cases reported in this study are the only GOCs in our

collection of 152 jaw cysts that were diagnosed during an 8-year period. A contributory factor to this low prevalence may be the difficulty in identifying the characteristic features of a GOC in inflammed tissue, especially if only material from an incisional biopsy is available. The changes brought about by an inflammatory process were evident in the excised tissue in case 1.

Glandular odontogenic cysts are considered to be aggressive. One of the cases reported by Padayachee and van Wyk[2] recurred, and recurrences were present in two of the eight cases described by Gardner et al.[1] Although no recurrence was present in case 1, after 2 years, the eroded cortical plates suggested aggressive behavior.

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PIGMENTED NEUROECTODERMAL TUMOUR OF INFANCY

W F P van Heerden & E J Raubenheimer

Keywords: pigmented neuroectodermal tumour

Pigmented neuroectodermal tumour of infancy (PNTI) is an uncommon neoplasm occurring primarily in young children, 82% are 6 months or less in age while 92% are under 12 months[1]. The tumour has a predilection for the anterior maxilla but has also been reported in the mandible[1], epididymis[2], mediastinum and brain[1]. Clinically, PNTI presents as a soft tissue mass, 1-3 cm in size with a firm consistency. It is frequently associated with rapid growth and a stretched, non ulcerated overlying mucosa or skin. Radiographic features include an ill-defined radiolucent lesion causing local destruction and displacement of the developing teeth. Despite the tumour's rapid growth and tendency to invade bone the majority of cases are successfully treated with conservative therapy (local excision and curettage of underlying bone). Recurrences develop in about 15% of patients[1] while metastases and cellular malignant change have been documented in a few cases[3].

The purpose of this article is to present a case of PNTI with exceptional clinical features.

MATERIALS AND METHODS

A 7-month old female patient was referred to the Garankuwa Hospital with a large soft tissue of the right maxilla present since birth (Fig.1). The tumour had grown rapidly since then and caused severe disfigurement. Two areas of ulceration was present on the skin. An incisional biopsy was performed and diagnosed as a PNTI.

The tumour was excised and the post operative healing uneventful. The excised tumour measured 18 cm in the longest diameter. The consistency was firm and fibrous and the specimen had a blue-black colour on cut surface (Fig.2). It appeared to be well demarcated. Microscopically, it was composed of non encapsulated dense fibrovascular tissue with large epithelial-like melanin producing cells arranged either in strands or clusters and often forming the lining of small cleft-like and alveolar spaces. Smaller non pigmented cells resembling neuroblasts were present in the alveolar spaces or as isolated nests in the stroma (Fig.3). No mitotic figures were present. Immunohistochemical examinations using paraffin embedded tissue revealed focal positivity for neuron specific enolase (NSE) in both the pigmented and small cells. Vimentin was focally expressed in the pigmented cells. Both cell types were negative for S100-protein and cytokeratin.

DISCUSSION

This PNTI was the largest tumour of its kind described in the literature up to date. PNTI was first described in 1918 by Krompecher under the term congenital melanocarcinoma[4]. Difficulty in deciding the cellular origin has led to a variety of terms describing this lesion such as melanotic ameloblastoma, melanotic prognoma, retinal anlage tumour and pigmented epulis of infancy[5]. The concept of a congenital melanoma failed to explain the presence of the primitive neuroblast-like cells as well as the benign clinical course. The odontogenic theory was prompted by the predilection of this tumour for the maxilla but does not take into consideration the extragnatic sites where there are no odontogenic rests. The tumour cells also bear no resemblance to any cell involve in odontogenesis. The association between PNTI and the developing retina is highly unlikely because the retina is well developed in the embryo before the anlage of the maxilla and mandibles develops[6]. Electron microscopy and histochemical studies however have establish the neural crest as the most likely origin[7]. This will explain the presence of melanin and neuroblast cells, the distribution of the lesions as well as the few tumours associated with increased levels of vanillylmandelic acid[6].
The immunohistochemical findings in the present case was in large in agreement with similar studies in the literature[8]. Cytokeratin however is positive in the pig-

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mented cells in other studies. The absence of cytokeratin in our case was probably the result of different types of cytokeratin used as the primary antibody.

There was no evidence of the tumour recurring in the 6 months of follow-up examinations. It was found that incomplete surgical removal of this tumour is not necessarily associated with recurrences[9]. The debulking effect as well as the removal of stimulatory cells influencing the invading peripheral tumour cells are possible explanations.

Few other lesions would present in this age group and in the typical location. PNTI must be differentiated from congenital epulis as well as malignancies of early childhood such as neuroblastomas and rhabdomyosarcomas.

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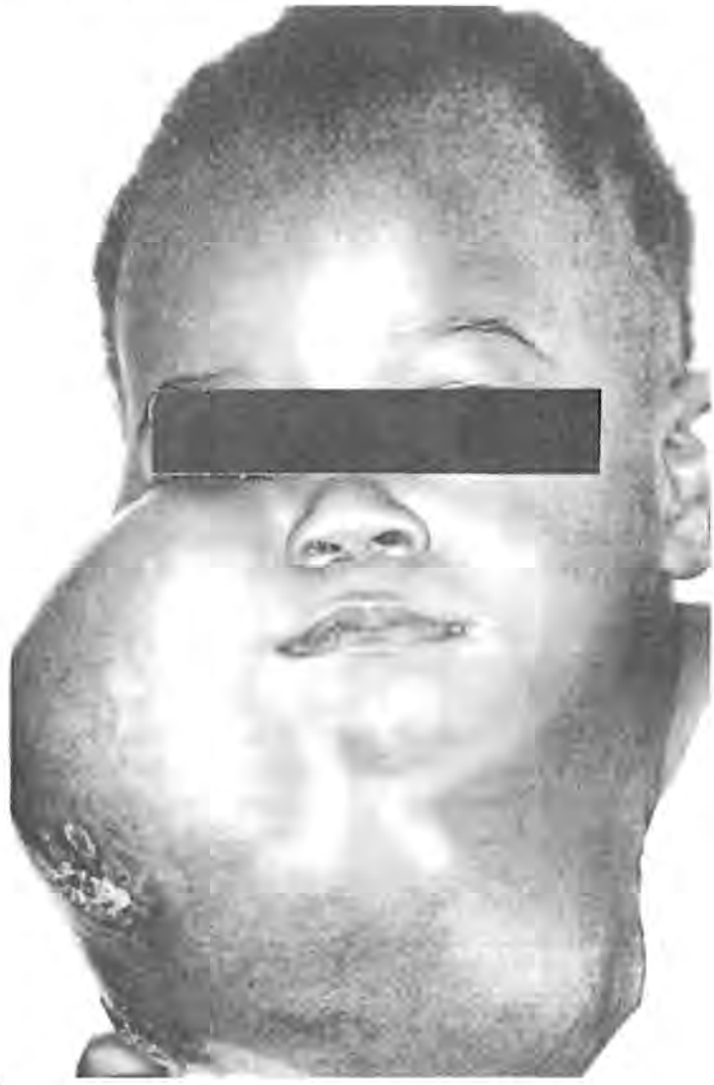

Fig. 1. The 7-month old female patient with the PNTI involving the whole right side of the face.

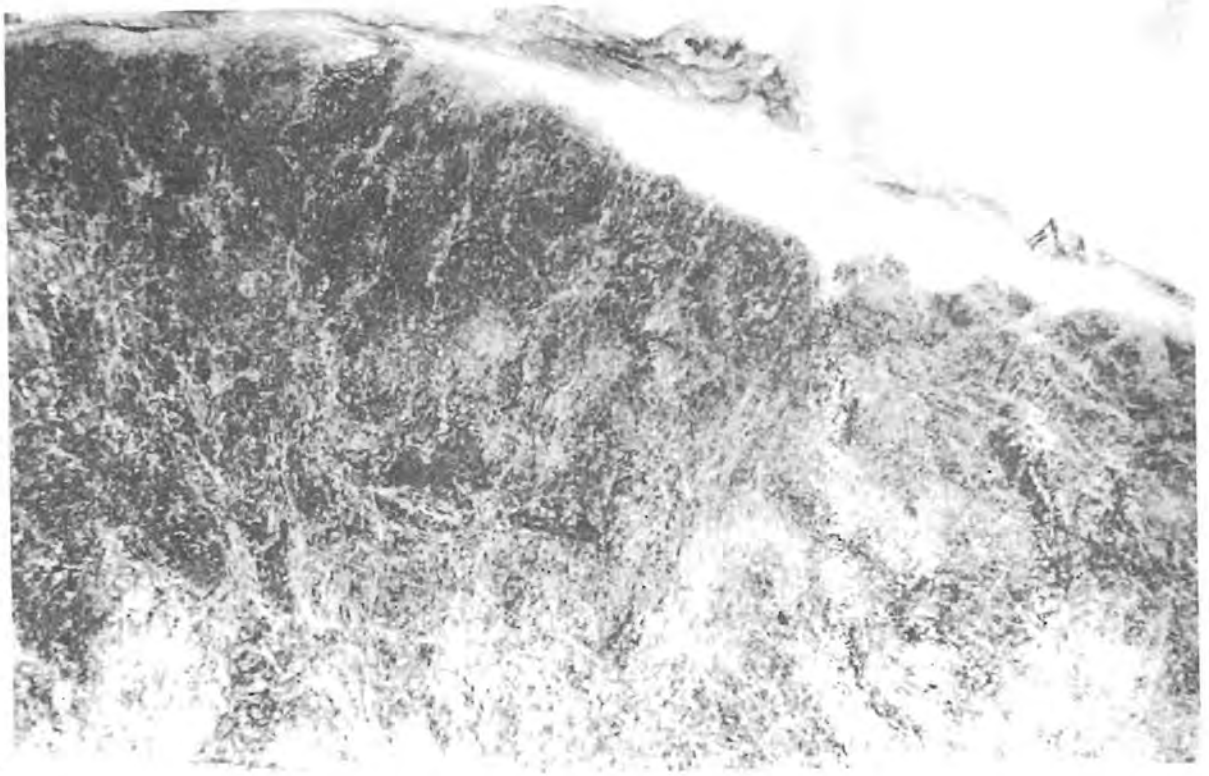

Fig. 2

Fig. 2. Cross section of the tumour showed a firm black fibrous tumour.

Fig. 3

Fig. 3. Microscopic examination showed cleft-like spaces lined by pigmented cells (thin arrows) as well as smaller cells resembling neuroblasts (bold arrows). (H & E x200).

Epstein-Barr virus prevalence in Burkitt's lymphomas in a South African population sample

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Running title: *EBV prevalence in Burkitt's lymphomas*

Abstract

The Epstein-Barr virus (EBV) is no longer thought to be the sole cause of Burkitt's lymphoma (BL) but is still accepted as a cofactor in the pathogenesis of this neoplasm. According to the National Cancer Registry of South Africa, BL represented less than 0,04% of all malignancies reported for the period of 1993-1995. No data is available on the association of EBV with BL in South Africa and it was decided to perform a pilot study in the Gauteng province of this country. Twenty-four cases of BL were retrieved from the archives of the Departments of Oral Pathology and Anatomical Pathology of Medunsa and the Universities of Pretoria and Witwatersrand. All cases were divided into two groups according to the primary site of the tumour: the Oral-Maxillo-Facial (OMF) group (14 cases) and the Non-Facial (NF) group (10 cases). *In situ* hybridisation for EBV encoded RNAs (EBERs) was performed on paraffin sections and the proliferation index (PI) of each case was determined by Ki-67. Fifty percent of the BL cases in this study were positive for the virus. The site of the primary tumour did not significantly influence the EBV status of the tumour ($p=0,88$). The mean PI was 87,5% and the EBV positive cases had a close to statistically significant higher PI than the negative ones ($p=0,05$). Larger studies should be conducted to evaluate the cytogenetics and possible role of HIV in this disease in South Africa.

Introduction

The role of viruses in oncogenesis has been a major research field during the last few decades, and it is now estimated that viruses cause approximately 15% of all human tumours[1]. The Epstein-Barr virus (EBV), a DNA virus, was first discovered in a line of explanted lymphoblasts from Burkitt's lymphoma in an Ugandian child[2], becoming the first virus to be related to human neoplasia. The virus appears to have at least two natural target cells, B-lymphocytes and epithelial cells[3]. EBV infection of B-lymphocytes results in the proliferation and transformation of some B-lymphocytes into immortal lymphoblastoid cells each carrying multiple copies of the EBV genome[4]. Once infected, individuals become life-long virus carriers[5]. The EBV genome encodes for more than a hundred genes, but only a restricted set of gene products, the six Epstein-Barr nuclear antigens (EBNA-1, -2, -3A, and –3B, -3C, and -LP), two latent membrane-associated oncoproteins (LMP-1 and -2) and two small non-polyadenylated nuclear RNA's, EBER-1 and EBER-2, are associated with latent infection[6]

Burkitt's lymphoma (BL) includes endemic BL (eBL) and sporadic BL (sBL)[7]. Of all the B-cell lymphomas EBV is most strongly associated with eBL in equatorial Africa[8]. This form of BL is recognised as the most common malignancy amongst children in tropical Africa[9, 10]. The clinical hallmark of eBL is a rapidly growing jaw tumour in a child[11]. The sporadic form of this disease has identical histological features to eBL[12], occurs worldwide, but is characteristically more prevalent in Europe and the USA[13]. Sporadic BL most commonly presents with a rapidly growing abdominal mass (70-90%), frequently involving the terminal ileum[14]. The occurrence of head and neck tumours is low in sBL[15], with jaw lesions reported in less than 9% of cases[14].

Although controversial[16], a third form of BL, Burkitt-like lymphoma, is described in the context of the acquired immunodeficiency syndrome (AIDS)[17]. Burkitt-like lymphoma involves peripheral lymph nodes, the central nervous system and bone marrow[18], with less frequent extranodal, gastrointestinal tract or jaw involvement[17]. Although these three types of

BL are morphologically indistinguishable from each other, there is sufficient clinical, immunophenotypic, genotypic and virological differences to consider them as distinct clinicopathological entities[19]. The most distinct differences are seen between eBL in equatorial Africa and sBL in the United States. Other geographic-regions are described to exhibit clinical and virological features intermediate between those of eBL and sBL[20].

The association of EBV with BL varies greatly in different parts of the world. Classic eBL is associated with EBV in more than 95% of cases[8] while sBL is less often associated with this virus, ranging from 5-15% in cases in Europe and the United States of America, 50-80% in the Middle East, South America and India, 13% in Japan and 28% in Hong Kong[21]. Interestingly, approximately 85% of tumours in North Africa contain EBV DNA, although the clinical characteristics of BL in this area are more in keeping with those of sBL[7]. The reasons for this are uncertain but might reflect the early EBV seroconversion in developing countries[22]. Despite the fact that most patients with AIDS carry a large burden of EBV, the EBV genome is detected in only 30-50% of lymphomas in patients with AIDS[23]. Although EBV is no longer thought to be the sole cause of BL, it is still accepted as a cofactor in the tumour pathogenesis by stimulating B-lymphocyte proliferation, which increases the likelihood of a selection of cells with mutation of the *c-myc* gene[24], an essential component of the pathogenesis of BL[25].

According to the National Cancer Registry of South Africa, forty cases of BL were reported during the period of 1993-1995, representing less than 0,04% of all malignancies[26]. Only one study on the possible nature or type of BL seen in South Africa could be found in the literature. In 1989, Hesseling[27] reported the clinical features, incidence, and seasonal occurrence of BL for the period 1977 to 1986, as consistent with typical eBL. The authors determined the serological EBV status in only two of the twenty-two patients in their study, but did not examine any of the tumours for the presence of the EBV-genome. Other than this study, which is not considered a true South African sample because it also included cases

from Namibia, no data is available on BL or its association with EBV in South African patients. Macdougall reported early EBV seroconversion in black South African children[28], results similar to those reported for other developing countries where eBL is strongly associated with EBV[22].

A pilot study was done in the Gauteng Province of South Africa in order to evaluate the prevalence of EBV in BL cases in a South African population sample. The possible effect of EBV on the proliferation index (PI) of the tumour cells and whether the anatomical site of the lymphoma influenced the presence of EBV was investigated.

Materials and Methods

Case selection

A retrospective study was done on BL cases diagnosed at the Departments of Oral Pathology and Anatomical Pathology of Medunsa, and the Universities of Pretoria and Witwatersrand, 1972 to 1998 inclusive. Criteria for inclusion in this study were a confirmed histological diagnosis of BL according to the criteria of the World Health Organisation[29], available clinical information including the site of the primary tumour as well as available paraffin-embedded tissue blocks for analysis by immunohistochemistry and RNA *in situ* hybridization. In order to determine if the anatomical site of the lymphoma influenced the presence of EBV, cases retrieved were arbitrarily divided into two groups according to the primary site of the tumour. The Oral-Maxillo-Facial (OMF) group included BL of the jawbones, Waldeyer's ring, supra-clavicular neck lymph nodes and oral soft tissues, and a Non-Facial (NF) group involving any infra-clavicular site. Patients with tumours at both sites were excluded from this study, as it was not possible to determine the primary site of the tumour as opposed to disease dissemination.

Immunohistochemistry

The immunophenotype of all tumours was confirmed by demonstration of B-lineage using a standard immunoperoxidase technique with L26, a pan-B-cell antigen (CD20, DAKO, Carpinteria, CA). The PI of each tumour was determined using prediluted antibody (DAKO) directed towards the Ki-67 nuclear antigen. Antigen enhancement was performed in a microwave using a pressure cooker and citric acid buffer. The lymphoid follicles in a normal reactive palatine tonsil were used as a positive control for the B-cell marker and the proliferation index of each tumour was determined by counting the number of positively stained nuclei per thousand tumour cells using a calibrated eyepiece. The number of Ki-67 positive nuclei was expressed as a percentage. Any degree of dark brown to black nuclear staining was considered positive for the Ki-67 antigen but whenever nuclear staining was doubtful, it was noted as negative.

In situ hybridisation (ISH).

To detect expression of the EBV-encoded small nuclear RNA's (EBER-1 and EBER-2), ISH with fluorescein-conjugated oligonucleotide probes was used. The probes were obtained commercially and consisted of a mixture of both EBER-1 and EBER-2 (Novocastra, Newcastle upon Tyne, UK). Probes were labeled with fluorescein isothiocyanate (FITC). Detection of hybridised probes was done with rabbit F(ab') anti-FITC conjugated to alkaline phosphatase. All glassware was treated with DEPC (di-ethyl pyrocarbonate) to prevent endogenous RNase activity. The ISH was done using the OmniSlide System (Hybaid, Teddington, Middlesex, United Kingdom). Sections of the EBV-infected cell line P3HR-1 processed to paraffin wax, was used as positive control and a section of human brain served as negative control. A dark brown to black granular stain within the nucleus of the cells was regarded a positive signal.

Statistical Analysis

Stringent testing of normality was determined by the chi^2-test. To determine the differences between the mean values of Ki-67 positivity the Student's t-test was used if the data was normally distributed, and the Mann-Whitney Test, if the data was not normally distributed. The differences between EBV positivity of the various groups were determined by the chi^2-test.

Results

Twenty-four cases complied with the inclusion criteria for this study. *Table I* demonstrates the number of patients, the EBV-positive cases and mean PI in each group.

Fifty percent of all BL cases in this study were EBV-positive and the mean PI of all twenty-four cases was 87,5%. When comparing the PI of EBV-positive cases with those of EBV-negative cases in both groups, a close to statistical significantly higher PI was found in the EBV-positive cases ($p = 0.055$). The primary site of the tumour, as arbitrary divided into the OMF and the NF groups, was also correlated with the EBV-status of the tumour tissue. The results showed that the site of the primary tumour did not significantly influence the EBV status ($p=0,88$) of such a tumour. Because of the retrospective nature of the study with some of the cases dating back to the early seventies, the HIV-status of only seventeen of the twenty-four cases was known. One of the patients in the OMF group was HIV-positive, ten were negative and four of the patients in this group had an unknown HIV-status. Two of the patients in the NF group were HIV-positive, four were negative and the HIV-status of six was unknown. The HIV-status did not significantly influence the EBV-status of the tumours in these groups.

Discussion

The three types of BL differ in their clinical presentations and EBV association, with the most distinct differences seen between eBL in Africa, and sBL in the United States of America. South Africa differs from equatorial Africa, both ethnically and environmentally. It has a divergent socio-economic status as well as several culturally different population groups living in geographical regions in which both eBL and sBL could occur. Burkitt's lymphoma accounted for approximately 0,04% of all malignancies in South Africa reported for the period 1993-1995[26]. Although EBV is no longer thought to be the sole cause of BL, the association of the virus with BL is still widely recognized. It was decided to perform a clinico-pathological study on a true South African population sample to determine the association of EBV with BL in this region.

To associate EBV with any tumour, identification of EBV genomes or gene products in the tissue is essential. Several monoclonal antibodies specific for EBNA-1, EBNA-2, LMP-1 and LMP-2A have become available for detection of latent EBV gene expression [30]. With the exception of EBNA-1, however, these viral proteins are not invariably expressed. EBER-1 and EBER-2 are consistently expressed in large numbers in all forms of EBV latency[31] and are ideal targets for ISH [32]. This method allows for the detection of viral nucleic acids in formalin-fixed paraffin-embedded tissue sections [3] and has become the most frequently used technique, accepted as the standard approach for diagnostic purposes[33]. Fifty percent of the BL's in our study showed nuclear staining as described in the literature[34, 35]. This degree of EBV positivity is at an intermediate level between the more than 95% EBV association in eBL cases in Africa [8], and 5-15% EBV association reported for sBL in the United States[21].

BL is one of the most rapidly growing tumours known, and will, when labeled with an appropriate proliferation marker, give a PI of close to 100%[16]. The Ki-67 protein is a nuclear protein required for DNA synthesis and is expressed in all phases of the cell cycle[36]. The

mean PI of tumour cells in this study was slightly lower than usually reported (87,5% vs. almost 100%). This can be ascribed to the probable sub-optimal tissue processing of the older tissue blocks with resultant poor antigen retrieval. The proliferation indices of EBV-positive tumours were compared with those of EBV-negative tumours and interestingly, the EBV-positive cases had a close to statistical significant higher proliferation index than the negative ones (p=0,055). This is in contradiction with other studies[37, 38] and it might be of value to investigate this phenomenon in larger study groups of BL.

The results from this study indicated that EBV still played an important role in the pathogenesis of BL in developing countries like South Africa. Our results also contradicted those of Hesseling *et al* who reported BL in South Africa and Namibia to be consistent with typical eBL[27]. Future research on the possible type of BL in South Africa should include cytogenetic studies as well as evaluation of the influence of HIV on the incidence, clinical features, EBV-status and molecular rearrangements of this lymphoma.

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Table I

	OMF Group	NF Group
Number of patients	14	10
EBV-positive cases	6	6
Mean PI (%)	88,6	86,1