Nuclear morphometry in canine acanthomatous ameloblastomas and squamous cell carcinomas

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The aim of this study was to evaluate whether morphometrical analysis can be of diagnostic value for canine acanthomatous ameloblastoma. We calculated, by means of an automated image analyser, some morphometric nuclear parameters, in particular: mean nuclear area (MNA), mean nuclear perimeter (MNP), maximum and minimum diameters (MDx and MDm) coefficient of variation of the nuclear area (NACV), largest to smallest dimension ratio (LS ratio), and form factor (FF), in 8 canine acanthomatous ameloblastomas, and we compared these morphometric data to those of 13 squamous cell carcinomas of canine gingiva. The results indicated a progressive increase of the MNA, NACV, MNP and MDm proceeding from acanthomatous ameloblastomas (MNA: 42.11±8.74; NACV: 28,36±7,23; MNP: 24.18± 2.68; MDm: 5.69±0.49) to squamous cell carcinomas (MNA:49,69±9,10; NACV: 30,89±7,75; MNP: 25.63±2.54; MDm: 6.64±0.73). On the contrary, the LS ratio and the FF resulted greater in acanthomatous ameloblastomas (LS ratio: 1,63±0,12; FF: 1,13±0,002) than in SCCs (LS ratio: 1,40±0,12; FF:0.91±0.38). Moreover, the MNA, MNP,MDx and MDm resulted similar (MNA: p=0.89; MNP: p=0,65; MDm: p=0,16; MDx: p=0,13) in a subset of four acanthomatous ameloblastomas with cellular atypia (MNA:49,01±6,88; MNP: 26,28±1,99; MDm: 6.08±0.41; MDx: 10.18±0.88) and in squamous cell carcinomas (MNA:49.69±9,10; MNP: 25.63±2.54; MDm: 6.64±0.73; MDx: 9.26±1.05). While the NACV values resulted higher in typical acanthomatous ameloblastoma (29,99±6,06) than in atypical acanthomatous ameloblastoma (26,74±8,84) and similar to those of the SCCs (30,89±7,75). These results seem to confirm that acanthomatous ameloblastoma is a malignant or potentially malignant lesion and emphasizes that nuclear morphometry analysis can be an useful diagnostic and prognostic method in canine oral pathology.

Key words: acanthomatous ameloblastoma, dog, nuclear morphometry, squamous cell carcinoma.

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Paper accepted on April 4, 2006

European Journal of Histochemistry
2006; vol. 50 issue 2 (Apr-Jun):125-130

Epulides are a common group of oral lesions which account for 40 per cent of all oral neoplasms in dog.

There is considerable confusion about the use of the term epulis in veterinary literature. It simply refers to localized gingival enlargment and encompasses both non-neoplastic reactive and neoplastic lesions (Bostoch, 1987; Dubielzig, 2002).

Canine epulides differ strikingly in clinical behaviour. Fibromatous epulis of periodontal ligament origin, also called peripheral odontogenic fibroma by Gardner and Baker (1991) and ossifying epulides are considered inflammatory and hyperplasic lesions. On the contrary, acanthomatous ameloblastoma (Gardner, 1993; Head, 2003), previously called acanthomatous epulides because origins from odontogenic epithelial cells found near the tooth or in the gingival epithelium (Dunne, 2001), despite its benign histological appearance, usually shows a malignant biological behaviour (Yoshida, 1999, b).

Acanthomatous ameloblastoma is considered an aggressive tumor of the canine jaw, characterized by an irregular verrucous masses adjacent to the tooth, consisting of sheets of no keratinizing odontogenic epithelium, with peripheral palisading epithelium and abundant central acanthocytes with prominent intercellular bridges. (Head, 2003). Characteristically, epithelial cells have an infiltrative growth and invade the deep sub mucosa. These lesions are in fact locally invasive, with repeated recurrences following surgery (Backer, 1993; Gardner, 1993) and can change into malignant tumors when they invade bone and after irradiation (Thrall, 1981; Thrall, 1984). Moreover, recurrent tumors are more aggressive and anaplastic than primary tumour, and have a biological behaviour similar to that of squamous cell carcinoma (Head, 2003).

Several studies, in veterinary medicine, have considered nuclear morphometric analysis as an useful diagnostic predictor in various cancers (mast cell

tumors, (Maiolino, 2005; Strefezzi, 2003), squamous cell carcinomas of skin, (Maiolino, 2002), seminomas, (Maiolino, 2004), mammary tumors (De Vico, 1997). For many years, the degree of nuclear differentiation of neoplastic cells, so-called *nuclear grade*, has been the only method to determine the presence of pre- or malignant lesions and the risk of progression in cancer, but often lacks objectivity and, in the hands of different pathologists, reproducibility (Baak, 1983). Computerized nuclear morphometry has enabled to quantify and standardize this parameter (Baak, 1982). The purpose of this study was to compare nuclear morphometric features of acanthomatous ameloblastomas (typical and atypical) to those of squamous cell carcinomas, and to evaluate the diagnostic usefulness of morphometrical method in acanthomatous ameloblastomas with probable risk of malignant transformation.

Materials and Methods

Samples

Twenty-three gingival samples were examined. All samples were formalin-fixed, and paraffin wax-embedded, and sections (4 μm) were stained with haematoxylin and eosin (HE). These included two normal gingival samples, eight acanthomatous ameloblastomas, (Dubielzig, 2002) and thirteen squamous cell carcinomas arising from canine gingiva.

Morphometric analysis

Nuclear morphometric analysis was performed on HE-stained sections by means of an automated image analyser (Mono, Images and Computer System, Milan, Italy), connected to a Nikon Eclipse E-400 microscope (Tokyo, Japan), with the Image Pro Plus Program (Media Cybernetics, Inc., Silver Spring, MD). For each specimen, 10 images of cells fields with a 40X objective lens, including areas with high and low cellularity, were randomly captured by the operator, who moved the microscopic field across the specimen from the surface to its margin. Images were stored in the digital memory and displayed on the monitor screen. A total of 100 sampled nuclei from selected areas were measured by outlining their profiles with a computer *mouse*. In each case, the mean nuclear area (MNA; μm^2), the coefficient of variation of the nuclear area (NACV), the mean nuclear perimeter (MNP; μm), the mean nuclear diameter maximum (MDx), and the mean nuclear diameter minimum (MDm) were calculated (Maiolino, 2004). The coefficient of variation of the nuclear area (NACV) was calculated expressing the variation in size in an individual case (Nagashima, 1998; Sekine, 2003). To determine the variation in shape, the nuclear form factor (perimeter^2/4π area) (FF) and the largest to the smallest dimension ratio (LS ratio) were calculated. Generally, in a round circle the values of LS ratio correspond to 1; if the object is elliptic the LS ratio is higher than 1 (Nagashima, 1998; Sekine, 2003).

Statistical analysis

One-way analysis of variance (ANOVA), followed by t-test was carried out to study the significance of differences of the tested parameters (MNA, NACV, MNP, MDX, MDN, FF,LS ratio) between acanthomatous ameloblastoma and SCCS. The accepted level of significance was $p<0.01$.

Results

In our study, squamous cell carcinomas, which arose more frequently from maxillary canineteeth (69%; 9/13), were characterized by erosive, plaque-like lesions comprising islands, cords and trabeculae of neoplastic epithelial cells, showing variable degrees of squamous differentiation and karyomegaly, nuclear hyperchromatism, enlarged and prominent nucleoli (Figure 1). Numerous mitotic figures and *horn-pearl* formation were observed. These tumors have always resulted in bone invasion.

In contrast to SCCs, acanthomatous ameloblastomas arose from the epithelial surface, outside the bone, and were continuous with the surface gingival epithelium; their gross morphology consisted usually of masses with cauliflower-like growth, that, in our study, according to Yoshida *et al.,* (Yoshida, 1999, b), occurred more frequently on the gingiva around the maxillary and mandibular canine (75%; 6/8).

The distinction between acanthomatous ameloblastoma and SCCs was performed on the basis of defined histological criteria. Acanthomatous ameloblastomas were histologically characterized by islands, cords and nests of no keratinizing epithelial cells with prominent intercellular bridges (Figure 2). The nuclei of the basal cells were distinctly palisaded, with their long axis arranged at right angles to the basement membrane.

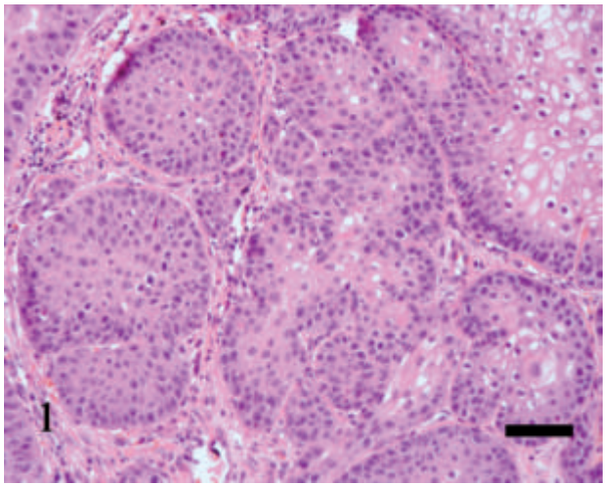

Figure 1. Gingiva; dog. Canine squamous cell carcinoma. Islands of neoplastic epithelelial cells with squamous differentiation, showing nuclear hyperchromatism and enlarged and prominent nucleoli. HE. Bar = 50 μm.

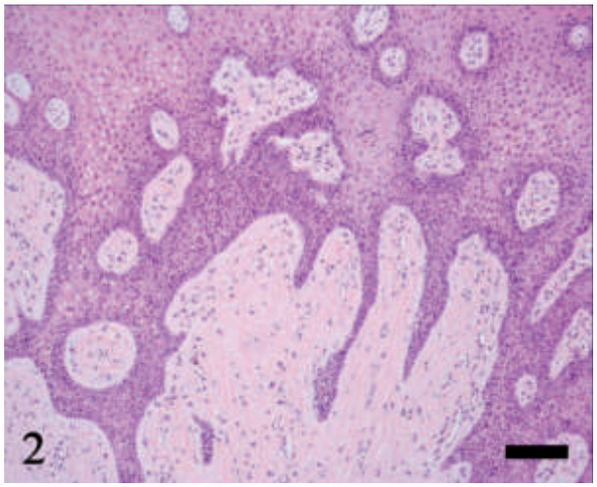

Figure 2. Gingiva; dog. *Typical* canine acanthomatous ameloblastoma . Proliferation of prickle cells, uniform in size, with prominent intercellular bridges. The nuclei of the basal cells are palisaded. HE. Bar = 50 μm.

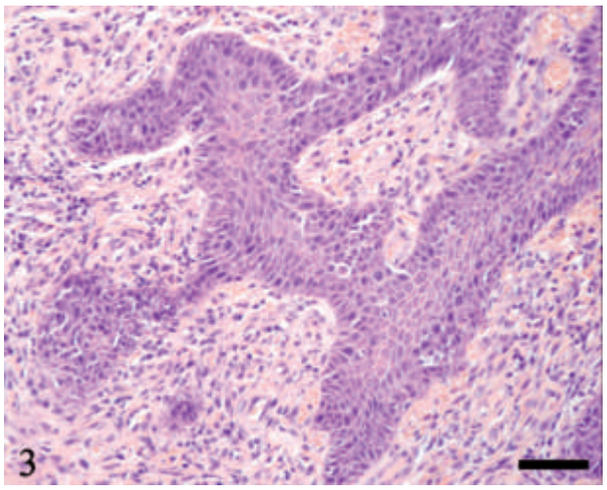

Figure 3. Gingiva; dog. Acanthomatous ameloblastoma with evident atypical features. Irregular epithelial stratification and loss of polarity of the basal cells. Cells with evident atypical features. HE. Bar = 50 μm.

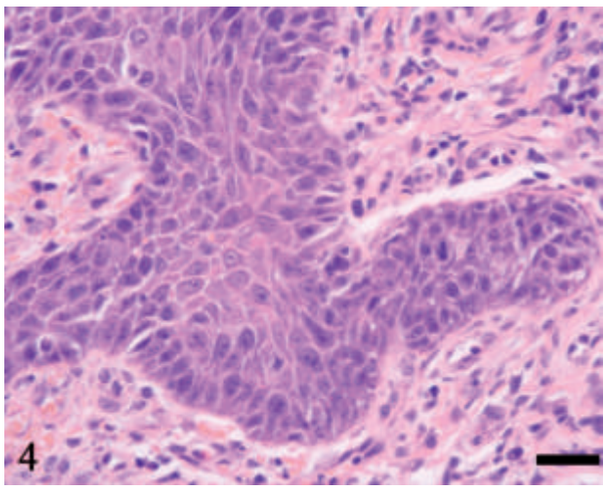

Figure 4. Gingiva; dog. Acanthomatous ameloblastoma with atypical features. Squamous cells showing increased nuclear-cytoplasmic ratio, nuclear hyperchromatism, enlarged and multiples nucleoli and presence of mitotic figures. HE. Bar = 20 μm

Moreover, the proliferating cells showed no cellular atypia and mitotic figures were uncommon, except in four *atypical acanthomatous ameloblastomas,* which exhibited irregular epithelial stratification (more than one cell layer having a basaloid appearance) and loss of polarity of basal cells (Figure 3). Furthermore, these atypical acanthomatous ameloblastomas showed cellular and nuclear polymorphism, increased nuclear-cytoplasmic ratio, nuclear hypercromatism , enlarged nucleoli and the presence of mitotic figures (Figure 4).

The results of cell measurements in total acanthomatous ameloblastomas and SCCs are shown in Table 1 and Figure 5. Acanthomatous ameloblastomas (MNA: 42.11±8.74; NACV: 28.36±7.23; MNP:24.18±2.68) showed lower mean values of nuclear area, coefficient of variation of the nuclear area and perimeter than SCCs (MNA:49.69±9.10; NACV: 30.89±7.75; MNP:25.63±2.54) but these differences were not statistically significant (p=0,07; p=0,47; p=0,23, respectively).

The mean values of minimum diameter increased significantly (p=0.004) from acanthomatous ameloblastomas (MDn: 5.69±0.49) to SCCs (MDn: 6.64±0.73); while, the mean values of maximum diameter proceeding from acanthomatous

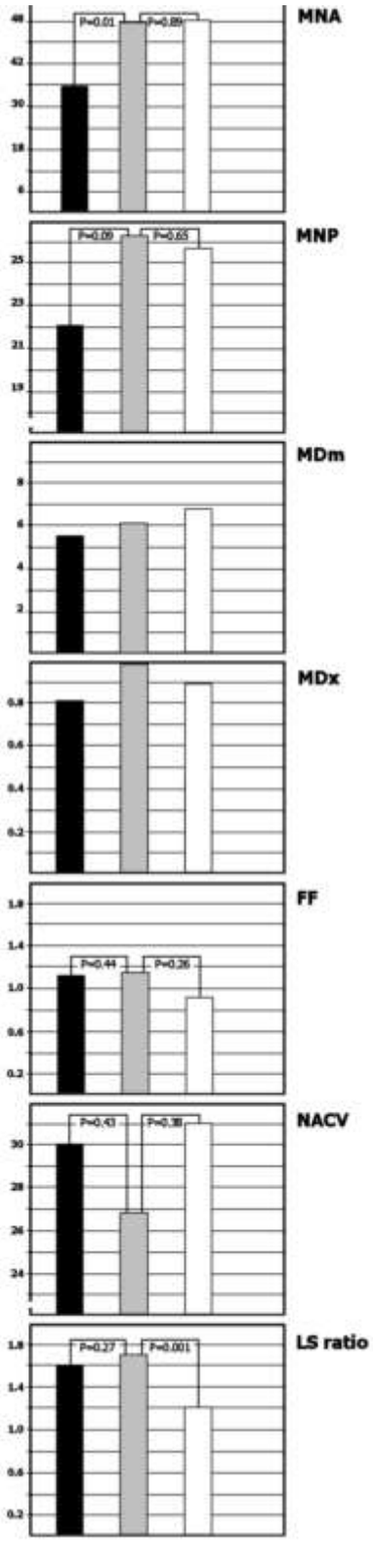

Figure 5. The mean values of the nuclear area (MNA) and perimeter (MNP), the minimum diameter (MDm), the maximum diameter (MDx), the form factor (FF), the coefficient of variation of nuclear area (NACV), and the largest to smallest dimension ratio (LS ratio) are presented. Black columns: typical ameloblastomas; grey columns: atypical ameloblastomas; white columns: carcinomas.

ameloblastomas to SCCs (MDx 9.27±1.19 and MDx 9.26±1.05 respectively) tended to be equal (p=0.98). The LS ratio and the form factor resulted greater in acanthomatous ameloblastomas (LS ratio: 1,63 ± 0,12; FF: 1.13± 0.02) than in SCCs (LS ratio: 1,40 ± 0,12; FF: 0.91± 0.38), but these values were statistically significant in LS ratio (p=0,0008) and not in form factor (p=0.12).

Table 2 shows the results of measuring the cells in the four acanthomatous ameloblastomas with atypical features (Figures 3 and 4), compared with other acanthomatous ameloblastomas (4) and squamous cell carcinomas. The mean values of nuclear area, perimeter and minimum diameter were greater in acanthomatous ameloblastomas with atypical features (MNA: 49.01±6.88; MNP: 26.28±1.99; MDm: 6,08±0,41) than in other acanthomatous ameloblastomas (MNA: 35,22±2,07; MNP: 22,08±1; MDm: 5,30±0.05) and similar to that of the SCCs (MNA: 49.69±9.10; MNP: 25.63±2.54; MDm: 6.64±0.73). In fact the differences between atypical acanthomatous ameloblastomas and SCCs were not significant (MNA: p=0.89; MNP: p=0,65; MDm: p=0,16). However, the acanthomatous ameloblastomas with cellular atypia showed mean values of nuclear maximum diameter greater (MDx: 10.18±0.88) than that of the SCCs (DMx 9.26±1.05), with no significant differences (p=0,13). Unexpectedly, typical acanthomatous ameloblastomas showed the NACV values greater (29,99±6,06) than in acanthomatous ameloblastomas with atypical features (26,74±8,84) and similar to those of SCCs (30,89±7,75). About the LS ratio and the FF values, they were greater in acanthomatous ameloblastomas with atypical features (LS ratio: 1,69±0,06; FF: 1,14±0,005) than in other acanthomatous ameloblastomas (LS ratio: 1,58±0,15; FF: 1,12± 0,03) and than in SCCs (LS ratio: 1,40± 0,12; FF: 0,91±0,38).

Discussion

Previous studies showed that the acanthomatous ameloblastoma has a proliferative potential equivalent to that of malignant tumors, but lower than the squamous cell carcinoma (Yoshida, 1999, a), and also indicated that these lesions differ from other types of epulides in biological and morphological features and has a poor prognosis (Yoshida, 1999, b).

The use of morphometry as a means of empha-

sizing the diagnostic and prognostic *weight* of particular cellular features, has been suggested (Baak, 1982). Several authors have demonstrated the use of nuclear morphometric parameters in predicting human epithelial neoplastic transformation (Appel, 2003; Bacus, 1999; Dunne, 2001; Sowter, 1990; Wolberg, 1999). The general disturbance of the epithelium is designated dysplasia, according to the WHO criteria (Pindborg, 1997), and the potential for developing invasive carcinoma increase with the severity of dysplasia.

More prominent or more numerous the epithelial histological changes are, more severe is the grade of dysplasia. The relationship between dysplasia and the subsequent development of cancer has not been fully clarified. However, it is generally believed that severe dysplasia indicates that there is a very high risk of the development of cancer (Pindborg, 1997).

Not all these changes are necessarily seen in the same case and there is a considerable subjectivity involved in their interpretation. In the assessment of dysplastic and neoplastic lesions a great emphasis is placed on the changes in nuclear size and shape. Nuclear shape, nuclear size and their variability are considered of primary importance for diagnosis of dysplasia/hyperplasia and can be of diagnostic value for lesions with a high risk of malignant transformation. A study in man demonstrated that nuclear size is an important prognostic factor for patients with intra-oral squamous cell carcinoma, but an experienced pathologist is required to assess these parameters, which are, to some extent, inherently subjective. Computerized nuclear morphometry has the advantages of objectivity, reproducibility, and it can be quickly performed by means of conventional microscopical analysis (Baak, 1983).

For this reasons, in this study, we analyzed by computer some nuclear parameters including mean nuclear area and mean nuclear perimeter for size evaluation, maximum and minimum diameter, LS ratio and form factor, for characterizing nuclear shape, and NACV value for anisonucleosis in order to asses the diagnostic and prognostic usefulness of nuclear morphometry in acanthomatous ameloblastoma.

In our study we observed an increase in size of the nuclei proceeding from acanthomatous ameloblastoma to squamous cell carcinoma, and we also indicated that acanthomatous ameloblastomas with atypical features showed mean values of the area and perimeter greater than in other acanthomatous ameloblastomas and similar to that of the SCCs, suggesting a close morphological similarity of the nuclei between the acanthomatous ameloblastomas and SCCs. The increase in nuclear area from acanthomatous ameloblastomas to carcinomas in this study may be a reflection of the increase in DNA synthesis. A proportional increase in the nuclear volume and DNA content was found in the cells of melanocytic tumours of dogs and cats (Roels, 2000).

In a previous study of advanced human gastric cancer there was a significative correlation between the nuclear area of cancer cells and the expression of P53, the Ki67 labelling index, and the DNA ploidy of tumors (Ikeguchi, 1999). Moreover, breast cancer in women is known to progress through multiple genomic changes and these accumulating changes result in nuclear alterations. (Mariuzzi, 2002).

Our data also indicated that the maximum diameter, the form factor and the LS ratio of acanthomatous ameloblastoma nuclei was greater than those in SCCs, although not all were statistically significant, suggesting, according to Sekine *et al.* (2003) that *the malignant nuclei grow large and the shapes become elliptic*. The nuclei observed, in fact, are elongated and polarized against the basement membrane.

It is known that increasing severity of dysplasia there is a marked progressive increase in the nuclear size, in nuclear shape and their variability. (Pindborg, 1997). For these reasons canine acanthomatous ameloblastoma should be considered as premalignant lesions, and post-surgical surveillance should be intensified when they are present. In conclusion, the results of this study suggested that nuclear morphometry analisys can be a useful additional method for diagnostic and prognostic evaluation of acanthomatous ameloblastoma.

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