Pergamon

Oral Oncol, Eur J Cancer, Vol. 31B, No. 2, pp. 100–105, 1995

0964–1955/95 $9.50+0.00

0964-1955(94)00036-0

Papers

A Clinicopathological and Immunohistochemical Analysis of Primary Oral Mucosal Melanoma

A.W. Barrett, J.H. Bennett and P.M. Speight

Nine cases of primary oral mucosal melanoma in Caucasian patients were reviewed and the tumours analysed for expression of S100, HMB45, NKI/C3, HLA-DR, PCNA, cytokeratin and von Willebrand factor. The clinical, histopathological and immunohistochemical features were quite distinctive and our findings support previous suggestions that oral melanoma should be classified as a separate entity rather than as a sub-type of cutaneous melanoma.

Keywords: melanoma, mouth mucosa.

Oral Oncol, Eur J Cancer, Vol. 31B, No. 2, pp. 100–105, 1995.

INTRODUCTION

PRIMARY ORAL malignant melanoma (OMM) comprises only 0.2–8% of all melanomas [1]. Nevertheless, the aggressive nature of this neoplasm attracts frequent interest and several series have been reported recently [2–5]. OMM differs from its cutaneous counterpart in incidence, geographical distribution and clinical and pathological features. Like melanoma elsewhere, OMM appears to progress from a radial to a vertical growth phase associated with invasion of the underlying tissues [2, 6] but it defies precise classification into any one of the well-recognised categories of cutaneous melanoma.

OMM, like cutaneous melanomas, may be difficult to distinguish from other anaplastic tumours on morphological grounds and so immunohistochemical analysis with S100 and HMB45 may be used to aid diagnosis [7]. However, it is unknown whether other antigens expressed by skin melanomas are also expressed by OMM.

The aim of this report was to document a further 9 cases of OMM. The objectives were to record the natural history, histological features and immunohistochemical profile of OMM and to determine whether the disease conforms to a category of cutaneous melanoma.

MATERIALS AND METHODS

Cases diagnosed as primary OMM in the Joint Department of Oral Pathology, the Eastman Dental Institute and London Hospital Medical College between 1977 and 1992 were reviewed. Those received for a second opinion were not included, nor were cases with a history or clinical evidence of melanoma elsewhere in the body. Clinical and photographic data were obtained from the hospital records. Where necessary, supplementary histopathological and clinical data were obtained from other centres. Biopsies had been fixed in 10% formal saline, routinely processed and embedded in paraffin wax. Five micrometre sections were prepared for histology and routinely stained with haematoxylin and eosin. Sections were also stained using a standard Masson-Fontana or Schmorl's technique for melanin.

Immunohistochemistry

Sections of OMM were dewaxed to alcohol and endogenous peroxidase blocked by immersion in 1% methanolic hydrogen peroxide for 10 min followed by washing in deionised water. The effect of trypsinisation on all antibodies was tested. Sections were warmed to 37°C and then immersed in 0.1% trypsin/$CaCl_2$ (also heated to 37°C and adjusted to pH 7.8) for 10 min followed by three washes in phosphate buffered saline (PBS). Sections were then incubated in a 1/5 dilution of normal swine serum (Dako, High Wycombe, U.K.) for 10 min and incubated with one of the primary antibodies as listed in Table 1. Sections to be tested for HLA-DR expression were immersed in 0.01 M sodium citrate in 0.035 M HCl, pH 6.0, then cooked under pressure for 2 min and allowed to cool. Following incubation with primary antibody, sections were washed three times in PBS, incubated in a 1/200 dilution of biotinylated swine "Multilink" immunoglobulin (Dako) for 30 min, washed a further three times and then incubated with

Correspondence to P.M. Speight.
The authors are at the Joint Department of Oral Pathology, Eastman Dental Institute and the London Hospital Medical College, 256 Grays Inn Road, London WC1X 8LD, U.K.
Received 18 July 1994; provisionally accepted 1 Sep. 1994; revised manuscript received 19 Sep. 1994.

Table 1. Antibodies used in this study

Antibody	Supplier	Specificity	Dilution	Incubation time at 37°C
Rabbit anti-bovine S100*	Dako†	Wide range of normal and neoplastic tissues, including melanoma	1/2000	1 h
Mouse anti-human HMB45	Dako	Active melanocytes and melanoma cells	1/100	1 h
Mouse anti-human NKI/C3	Euro-path Ltd‡	Melanoma cells	1/100	1 h
Mouse anti-human HLA-DR	Dako (TAL 1B5)	33 kDa alpha chain subunit of HLA-DR	1/100	1 h
Mouse anti-human PCNA	Dako (PC10)	Cells in S-phase of cell cycle	1/200	18 h
Mouse anti-human von Willebrand factor*	Dako	Endothelial cells	1/20	1 h
Mouse anti-human cytokeratin	Dako (MNF116)	Broad pattern of activity within epithelial tissues	1/200	1 h

*Polyclonal antibody; †High Wycombe, Bucks, U.K.; ‡Bude, Cornwall, U.K.

streptavidin–biotin–peroxidase complex (Dako) for a further 30 min. The sites of antibody binding were visualised with diaminobenzidine (DAB). Sections were counterstained with Harris' haematoxylin. All antibodies were diluted in PBS and all reactions carried out at room temperature unless otherwise stated.

Where the OMM contained melanin, sections were bleached [8] either prior to the endogenous peroxidase block, or following visualisation with DAB. Alternatively, cobalt chloride was added to the DAB solution to a concentration of 1% in order to turn the reaction product grey-blue [9]. In these slides the sections were counterstained with neutral red.

Care was taken when analysing the immunohistochemical results to differentiate tumour from stromal cells. Positive cells were scored, using a subjective method previously described for melanoma [10], as: negative; occasional cells positive (+); minority of cells positive (++); approximately half the cells positive (+++); majority of cells positive (++++); almost all cells positive (+++++). Where staining was not uniform, it was recorded as variable in intensity or focal in distribution. Numbers of tumour cells containing melanin were quantified in a similar manner.

RESULTS

Clinical features of OMM

9 cases of OMM were retrieved. 5 patients had more than one oral biopsy, in 3 cases because an excision followed an incisional biopsy and in 2 (cases 1 and 5) because of recurrent OMM. All patients were Caucasian. The principal clinical signs and symptoms are summarised in Table 2. 7 cases presented on the palate and the degree of pigmentation varied considerably between patients. 3 cases presented with macular lesions (Fig. 1a), 2 with solitary pigmented nodules (Fig. 1b) and 4 with features of both. Two of the macules subsequently developed into nodular lesions.

Case 1 was a much younger individual and suffered from multiple endocrinopathies, including Addison's disease. Case 6 had rheumatoid arthritis, medication for which was originally thought to be the cause of pigmentation. 2 patients were former smokers, 2 (1 of whom was a smoker) wore a maxillary denture where palatal melanomas subsequently occurred. The smoking and denture-wearing histories were unknown in the remaining cases, thus preventing any valid conclusions to be drawn regarding these factors. The other medical histories were unremarkable. Cases 1 and 5 developed recurrent OMM. Case 4 is still alive 18 months after diagnosis but, other than case 9, no other patient survived beyond 3 years.

Histological features

The characteristic histological pattern was a combination of radial and vertical growth phases. Only one specimen showed *in situ* features alone, the remaining eight all had infiltrative vertical growth phase lesions. Two lesions showed vertical growth phase only, but one of these (case 8) had clinical evidence of widespread pigmentation suggestive of a radial growth phase elsewhere.

Histologically, the radial growth phase showed junctional activity with nests of naevoid cells often associated with acanthosis and rete hyperplasia (Fig. 2). Upward migration was often present but pagetoid islands of clear, epithelioid melanocytes, were only seen in one case. Although present in 8/9 cases, melanin varied in amount from sparse to abundant. With the latter, there was pigmentary incontinence and many melanophages in the superficial corium (Fig. 3a).

Although the overlying epithelium was atrophic and, in 6 cases, ulcerated, all nodular lesions of the vertical growth phase showed a cuff of adjacent mucosa with a rim of hyperplastic epithelium similar to that seen in nodular lesions of the skin. All nodular lesions were both exo- and endophytic and infiltrated to depths of between 3 and 8 mm. The infiltrating cells were epithelioid and polygonal, or spindle-shaped. In 7 cases, the epithelioid component was predominant. Such cells had large nuclei with prominent nucleoli and were arranged in nests, or in fascicles enclosed by fibrous septa which coalesced to form multinodular masses or sheets. The frequency of mitotic figures ranged from two per high power field to one in five high power fields. Some nodular lesions had only focal deposits of melanin, whilst others had a more diffuse distribution. The spindle cells formed bundles irregularly dispersed among the epithelioid sheets, occasionally producing a storiform or sarcomatous pattern (Fig. 4). In cases 1 and 6, maxillary bone was extensively infiltrated by tumour.

Immunohistochemical features

All antibodies reacted in wax-embedded sections. Trypsinisation adversely affected the reaction with the antibodies to HLA-DR and PCNA, but heat treatment was essential to retrieve the HLA-DR antigen although nuclear morphology was compromised. The results of immunohistochemistry are shown in Table 3.

Table 2. Summary of the age, sex, presenting features, treatment, metastatic sites and survival time of the 9 cases of OMM in this series

Case	Age (years)	Sex	Site and clinical features	Histological appearance	Treatment	Metastasis	Survival (years)
1	26	F	Sought treatment for pigmented epulis between 25 and 26; pigmentation also present on hard palate	Vertical/radial growth phase/pagetoid infiltration	Local resection	Widespread; peritoneum, lung, liver, omentum*	2.5
2	77	F	Sought treatment for epulis in maxillary left quadrant	Vertical growth phase	Palliative	Not known†	0.75
3	72	F	Soreness for 6 months; pigmentation of left tuberosity	Radial growth phase	Limited local resection and palliative	Not known†	2.0
4	78	M	Incidental finding of polyp left buccal mucosa with adjacent pigmentation	Vertical/radial growth phase	Palliative	None yet found	1.5 (alive)
5	85	F	Soreness for 6 months; speckled pigmentation of palate	Vertical/radial growth phase	Local resection and radiotherapy	Spine, lung	2.75
6	61	M	Soreness; extensive pigmentation of the hard palate. Subsequently a nodular lesion developed	Vertical/radial growth phase	Local resection	Neck, lung and liver	2.6
7	50	M	9 day history of poorly fitting denture; polyp on hard palate with mucosal pigmentation	Vertical/radial growth phase	Local resection and fast neutron therapy	Neck, lung	2.9
8	56	F	2 week history of poorly fitting denture; diffuse pigmentation of the palate with nodular lesion	Vertical/radial growth phase	Local resection	Lung	<2.0‡
9	75	M	Incidental finding; pedunculated polyp on hard palate	Vertical/radial growth phase	Local resection	Cervical lymph nodes	>5.0‡

*Consent for a post-mortem examination was refused so a complete assessment of her metastatic disease was not possible. During the course of her disease she gave birth by caesarian section and notes on the distribution of the metastatic deposits are based on observations made at the time.
†These patients were given terminal care and palliation in a hospice setting. Extensive investigations were not performed and consent for post-mortem examination was refused.
‡Exact date of death unknown.

S100 was the only antigen which consistently survived bleaching before incubation of the section with the primary antibody (Fig. 3b). All OMM were S100+ regardless of whether they were in radial or vertical growth phase. The staining was cytoplasmic and nuclear, but variable in intensity in most tumours. Cells at the advancing front of a vertical growth phase were often more strongly positive than superficial cells and epithelioid cells were more strongly positive than spindle cells.

HMB45 staining was granular and mainly cytoplasmic and/or nuclear (Fig. 5), but membrane staining was occasionally observed. Seven OMM were HMB45+ with, in 3 cases, prominent expression in the vertical growth phase and in superficially migrating melanocytes. Two tumours in radial growth phase showed an increase in HMB45 expression as they progressed to vertical growth phase.

The seven cases that were HMB45+ also reacted with NKI/C3. The latter produced a granular reaction which predominantly stained the cell membrane. In two cases, NKI/C3 stained many dendritic cells within stratified squamous epithelium overlying a vertical growth phase. Three tumours showed increased intensity of expression towards the deeper aspects of the vertical growth phase. Epithelioid cells stained more intensely than spindle cells but overall, NKI/C3 staining consistently produced more uniform reactions (in terms of intensity and distribution) than S100 and HMB45.

HLA-DR+ dendritic cells, deemed to be monocytic inflammatory cells, permeated the stroma between tumour cells in 8 cases. However, in four OMM the tumour cells also expressed HLA-DR. 8 cases expressed PCNA, with more than half the tumour cells positive in 7. The reaction product was granular, intranuclear, of uniform intensity and diffusely distributed throughout the lesion.

DISCUSSION

The sex and age range of this series of OMM (Table 2) is generally in keeping with previous reports [2–6, 11–14] with the exception of case 1, who was unusually young when she contracted OMM. This patient also had Addison's disease, an endocrinopathy associated with oral hyper-pigmentation, but a condition which has never before been reported in combination with OMM. The signs, symptoms, anatomical distribution and dismal prognosis of the patients in this series are also consistent with earlier studies, with the exception of one group who reported a 64% 5-year survival rate [4].

Cutaneous melanoma is classified as lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous

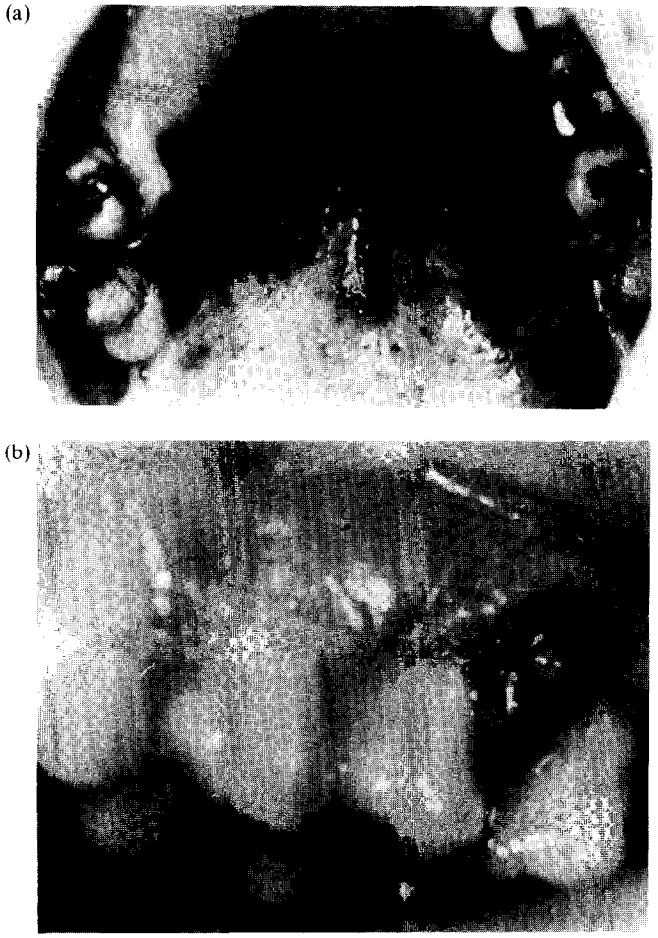

Fig. 1(a) Diffuse macular pigmentation of palate in case 6. This patient subsequently developed extensive, nodular OMM; (b) pigmented epulis adjacent to 25 and 26 on maxillary gingiva of case 1.

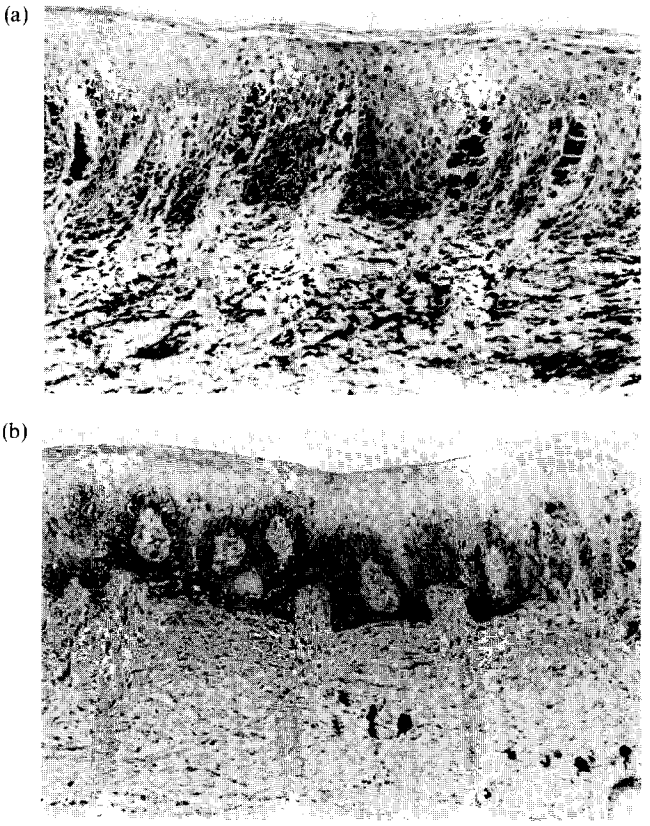

Fig. 3(a) Radial growth phase of OMM shown in Fig. 1(b) showing junctional activity with nests of pleomorphic, hyperchromatic melanocytes at the tips of the rete pegs, and pigmentary incontinence and melanophages in the superficial corium. Haematoxylin and eosin, original magnification ×25. (b) Section from same specimen bleached and stained with anti-S100 antibody. There is heavy basal and suprabasal staining, with positive superficially migrating cells. Avidin–biotin–immunoperoxidase, haematoxylin counterstain. Original magnification ×25.

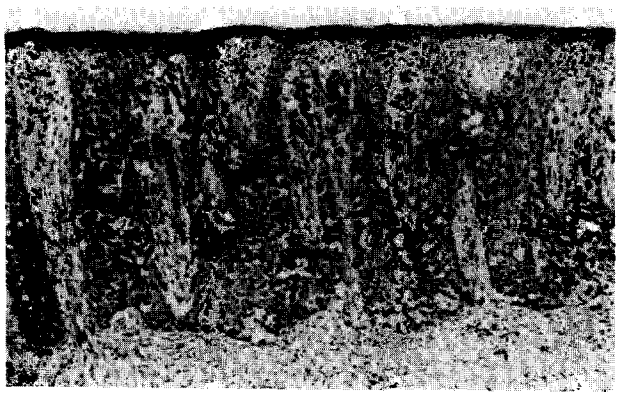

Fig. 2. Radial growth phase of OMM showing junctional activity with nests of naevoid cells, acanthosis and rete hyperplasia. Haematoxylin and eosin, original magnification ×16.

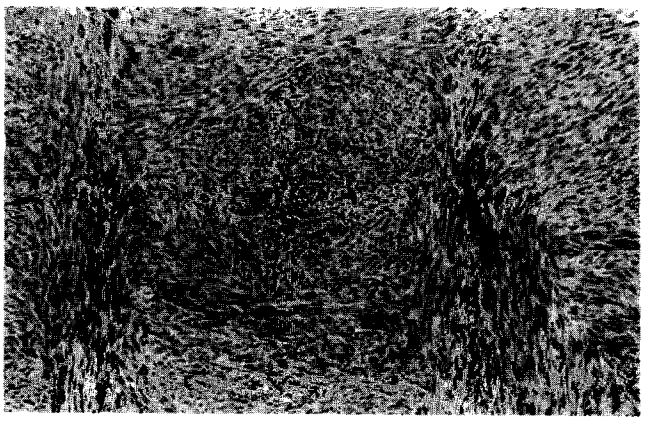

Fig. 4. Storiform arrangement of sheets of spindle cells. Haematoxylin and eosin; original magnification ×25.

melanoma or nodular melanoma [15]. OMM does not easily fit into any one of these categories and we found features of each type within individual tumours. The histological pattern may be obscured or complicated by factors such as ulceration, late presentation or inadequate excision [13, 16]. Some of the histological features, together with the poor prognosis and early metastasis, have prompted others [4, 17] to conclude that OMM most closely resembles acral lentiginous melanoma,

Table 3. Immunophenotypic profile of primary OMM with grading of melanin content based on staining by Schmorl's or Masson-Fontana methods. All OMM were negative for cytokeratin and von Willebrand factor

Case number	S100	HMB45	NKI/C3	HLA-DR	PCNA	Melanin
1	+ +	+ + +	+	negative	negative	+ + + +
2	+ + + + var	negative	negative	negative	+ + + var	+
3	+ + + + +	negative	negative	negative	+ + + +	+ + + +
4	+ + + + + var	+ + + +	+ + + +	+ +	+ + + + var	+ focal
5	+ + + +	+ + + var	+ + + + +	negative	+ + +	negative
6	+ + + + var	+ + + +	+ + + +	negative	+ +	+ + + +
7	+ + + + var	+ + + + var	+ + + + var	+	+ + + var	+ + focal
8	+ + + + var	+ + focal	+ + + + + var	+	+ + +	+
9	+ + + + var	+ + var focal	+ + + + var	+ +	+ + +	+ focal

Var = variable intensity of staining; focal = focal distribution of positive cells.

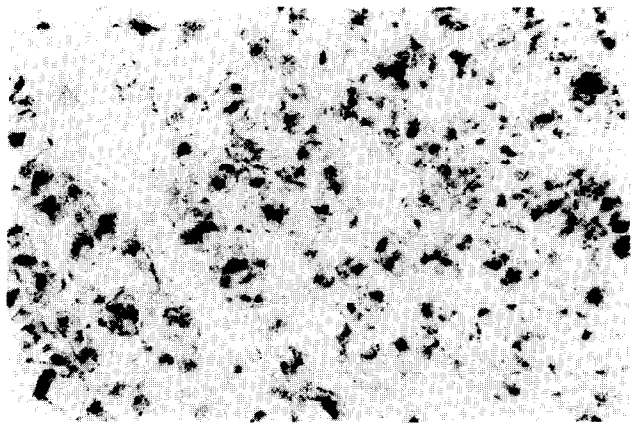

Fig. 5. HMB45 + OMM cells showing cytoplasmic and nuclear staining of variable intensity. Streptavidin–biotin–immunoperoxidase, original magnification × 63.

which, like the examples in our series, can have an *in situ* component or arise as nodular lesions *de novo* [15]. However, the vertical component of acral lentiginous melanoma is often characterised by spindle cells [15], and, although our cases contained spindle cells, the epithelioid component was predominant. Only 1 case in this series showed the pagetoid growth pattern characteristic of superficial spreading melanoma, and this was only in a small, localised area. Hence, the heterogeneous morphology of OMM precludes its categorisation as a variant of cutaneous melanoma, and we agree with others [6] that the presence and extent of the radial and vertical growth phases are the critical observations to be made.

The immunohistochemical profile of OMM (Table 3) was similar to that of cutaneous melanoma, with the exception that no OMM was positive for cytokeratin. Whilst all tumours expressed S100, it is noteworthy that HMB45 and NKI/C3 were negative in two which were strongly positive for S100. Neither S100, HMB45 nor NKI/C3 are melanoma-specific, and some cutaneous melanomas fail to stain. Nevertheless, HMB45 and NKI/C3 are regarded as showing greater specificity for melanoma than S100 [18–20] and may, therefore, be useful in distinguishing OMM from anaplastic carcinomas [21] or tumours of ectomesenchymal or mesodermal origin which are S100+ [22].

HLA-DR expression was seen in four of nine OMM as compared to six of 25 cutaneous melanomas [23]. Such expression is not thought to have any diagnostic or prognostic significance and, in the case of OMM, may merely reflect the presence of inflammatory mediators consequent to ulceration. That most OMM express a high level of PCNA and therefore contain a high proportion of cells which are actively cycling is, together with the observation of frequent mitoses, consistent with an aggressive neoplasm.

In this series, only 1 patient survived longer than 3 years and eight of nine tumours were in the vertical growth phase at first presentation. This supports the view that OMM is a particularly aggressive neoplasm. The poor prognosis, however, may also be due to lack of awareness of the lesion which then proceeds, unobserved by the patient, for a long period. Clinicians need to be aware of this factor and vigilance is required in the management of oral pigmented lesions, particularly those affecting the palate. Clinical, histopathological and immunohistochemical features, though perhaps subtle on their own, indicate that OMM are distinctive and suggest that they should be regarded as a separate entity rather than as a sub-group of cutaneous melanoma.

1. Pliskin ME. Malignant melanoma of the oral cavity. In Clark WH Jr, Golman LI, Mastrangelo MJ, eds. *Human Malignant Melanoma*. New York, Grune and Stratton, 1979, 125–137.
2. Ohashi K, Kasuga T, Tanaka N, Enomoto S, Horiuchi J, Okada N. Malignant melanomas of the oral cavity: heterogeneity of pathological and clinical features. *Virchows Archs A (Pathol Anat)* 1992, **420**, 43–50.
3. Smyth AG, Ward-Booth RP, Avery BS, To EWH. Malignant melanoma of the oral cavity—an increasing clinical diagnosis? *Br J Oral Maxillfac Surg* 1993, **31**, 230–235.
4. Umeda M, Shimada K. Primary malignant melanoma of the oral cavity—its histological classification and treatment. *Br J Oral Maxillofac Surg* 1994, **32**, 39–47.
5. Waal RIF van der, Snow GB, Karim ABMF, Waal I van der. Primary malignant melanoma of the oral cavity: a review of eight cases. *Br Dent J* 1994, **176**, 185–188.
6. Rapini RP, Golitz LE, Greer RO, Krekorian EA, Poulson T. Primary malignant melanoma of the oral cavity: a review of 177 cases. *Cancer* 1985, **55**, 1543–1551.
7. Gazit D, Daniels TE. Oral melanocytic lesions: differences in expression of HMB45 and S100 antigens in round and spindle cells of malignant and benign lesions. *J Oral Pathol Med* 1994, **23**, 60–64.
8. McGovern J, Crocker J. The effect of melanin pigment removal on the peroxidase–antiperoxidase immunoperoxidase technic. *Am J Clin Pathol* 1987, **88**, 480–483.
9. Yamamoto Y, Okato S, Takahashi H, Takeda K, Magari S. Distribution and morphology of macrophages in palatine tonsils. *Acta Otolaryngol (Stockholm)* 1988, **454** suppl, 83–95.
10. Bishop PW, Menasce LP, Yates AJ, Win NA, Banerjee SS. An

immunophenotypic survey of malignant melanomas. *Histopathology* 1993, **23**, 159–166.
11. Chaudhry AP, Hampel A, Gorlin RJ. Primary malignant melanoma of the oral cavity: a review of 105 cases. *Cancer* 1958, **11**, 923–928.
12. Trodahl JN, Sprague WG. Benign and malignant melanocytic lesions in the oral mucosa. An analysis of 135 cases. *Cancer* 1970, **25**, 812–823.
13. Liversedge RL. Oral malignant melanoma. *Br J Oral Surg* 1975, **13**, 40–55.
14. McCaffrey TV, Neel HB, Gaffey TA. Malignant melanoma of the oral cavity: review of 10 cases. *Laryngoscope* 1980, **90**, 1329–1335.
15. McKee PH. *Pathology of the Skin*. Philadelphia, Lippincott, 1989, 13.24–13.32.
16. Gutman M, Inbar M, Chaitchik S, *et al*. Malignant melanoma of the mucous membranes. *Eur J Surg Oncol* 1992, **18**, 307–312.
17. Clark WH Jr, Bernardino EA, Reed RJ, Kopf AW. Acral lentiginous melanomas including melanomas of mucous membranes. In Clark WH Jr, Golman LI, Mastrangelo MJ, eds. *Human Malignant Melanoma*. New York, Grune and Stratton, 1979, 109–124.
18. Vennegoor C, Calafat J, Hageman PH, *et al*. Biochemical characterisation and cellular localization of a formalin-resistant melanoma-associated antigen reacting with monoclonal antibody NKI/C-3. *Int J Cancer* 1985, **35**, 287–295.
19. Gown AM, Vogel AM, Hoak D, Gough F, McNutt MA. Monoclonal antibodies specific for melanocytic tumours distinguish sub-populations of melanocytes. *Am J Pathol* 1986, **123**, 195–203.
20. Leong ASY, Milios J. An assessment of a melanoma-specific antibody (HMB45) and other immunohistochemical markers of malignant melanoma in paraffin-embedded tissues. *Surg Pathol* 1989, **2**, 137–145.
21. Drier JK, Swanson PE, Cherwitz DL, Wick MR. S100 protein immunoreactivity in poorly differentiated carcinomas. Immunohistochemical comparison with malignant melanoma. *Archs Pathol Lab Med* 1987, **111**, 447–452.
22. Barrett AW, Scully C. S100 protein in oral biology and pathology. *J Oral Pathol Med* 1994, **23**, 433–440.
23. West KP, Priyakumar, Jagjivan R, Colloby PS. Can HLADR expression help in the routine diagnosis of malignant melanomas? *Br J Dermatol* 1989, **121**, 175–178.

Acknowledgements—The authors are grateful for the invaluable technical assistance of Mr Paul Darkins and Mrs Bushra Ahmed, and the co-operation of the following Consultant Oral and Maxillofacial Surgeons for their assistance in providing clinical and follow-up information to support this study: Mr P. Bathard-Smith, Eastbourne District General Hospital, Sussex; Mr J. Eyre, St Mary's Hospital, London W2; Mr T. Redpath, Southampton General Hospital, Hampshire, and Mr R.A. Peebles, Kingston Hospital, Surrey.