Biomedicine, 1981, *35*, 179-180.

SERUM ALKALINE DEOXYRIBONUCLEASE IN ORAL CANCER AND PREMALIGNANT LESIONS

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SUMMARY

Serum activities of alkaline deoxyribonuclease were assayed in 61 patients with oral squamous cell carcinoma, 23 with oral keratosis (a possible premalignant lesion), and 48 matched controls.

The activity of alkaline deoxyribonuclease was significantly greater in patients with oral cancer than in either those with oral keratosis, or controls. The activities of deoxyribonucleases in keratosis were greater than in controls but not at a significant difference.

RÉSUMÉ

L'activité sérique déoxyribonucléase alkaiine a été mesurée chez 61 patients atteints de carcinome épidermoïde de la sphère O. R. L., dans 23 cas de kératose orale (une éventuelle lésion pré-maligne) et 48 témoins appariés.

L'activité de la déoxyribonucléase était significativement plus élevée chez les porteurs de tumeur que chez les patients atteints de kératose orale ou chez les témoins. L'activité était augmentée dans la kératose par rapport aux témoins, mais sans différence statistique.

Oral cancer accounts for some 40 % of all malignancies in parts of South East Asia (7) and, even in the Western World, accounts for about 3 to 5 % of malignancies (14).

Potential serum markers for oral cancer have received relatively little attention although concentrations of various glycoproteins (16), carcinoembryonic antigen (11), β_2 microglobulin (9), and IgA (1, 3, 5) may be increased. Malignant proliferation is associated with pronounced changes in activity of various enzymes involved in the metabolism of nucleic acids (6, 15). Recently, we have reported an increase in serum activities of deoxyribonucleases (DNases) in patients with breast cancer (12) and other malignant neoplasms (13) but deoxyribonuclease activities do not, however, appear to have been examined in patients with oral cancer or with premalignant lesions.

We have therefore investigated the activity of alkaline DNase in the serum of patients with oral cancer and in patients with possible premalignant oral lesions.

METHODS

The study group consisted of 61 caucasian patients with histologically confirmed oral squamous cell carcinoma; 23 patients with oral keratosis (leukoplakia); and 48 age and sex matched controls. Blood was collected in all instances before definitive treatment was instituted, and serum was separated after clotting at 4°C for 12 to 16 hours. Serum was stored in aliquots at − 20° C until assayed for DNase activities.

Alkaline DNase was assayed in duplicate by a modification of the method of Spandidos *et al* (12), in which 25 µl of test serum were incubated for 15 minutes at 25° C with 100 µg DNA (Calf thymus DNA; Millipore, New Jersey, USA) in 0.1 M Tris-Cl buffer pH 8.0. The reaction was terminated with 2 ml of 1.5 M perchloric acid at 4° C, the mixture centrifuged for 10 minutes at 1,000 g and supernatant separated.

The absorbance of the supernatant was measured at 260 nm against a blank consisting of buffer, DNA and test serum added after the perchloric acid. The DNase activity was calculated from the increase in absorbance at 260 nm; a unit of DNase activity being defined as that causing an increase in absorbance of 0.001 per minute. A DNase standard (DNase I, Sigma Chemical Company, Poole) was run in parallel with the test sera. Intra-assay variation (standard coefficient of variation) of the same sera, tested on 5 cancer patients and 5 controls, was always less than 10 %. The results were analysed by the Mann-Whitney test.

RESULTS

Serum alkaline DNase activities in oral cancer (198 ± standard deviation 170 units/ml) were significantly greater than in those with oral keratosis (105 ± 80 units/ml; $p < 0.01$) or controls (89 ± 40 units/ml; $p < 0.001$; Fig. 1). Nearly one half of the group with oral cancer (26/61) had DNase activities in excess of control mean levels plus 2 standard deviations. Alkaline DNase activities did not differ significantly between patients with oral keratosis and controls.

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DISCUSSION

The results indicate that the serum activities of alkaline DNases (Fig. 1) were significantly increased in patients with oral squamous cell carcinoma over

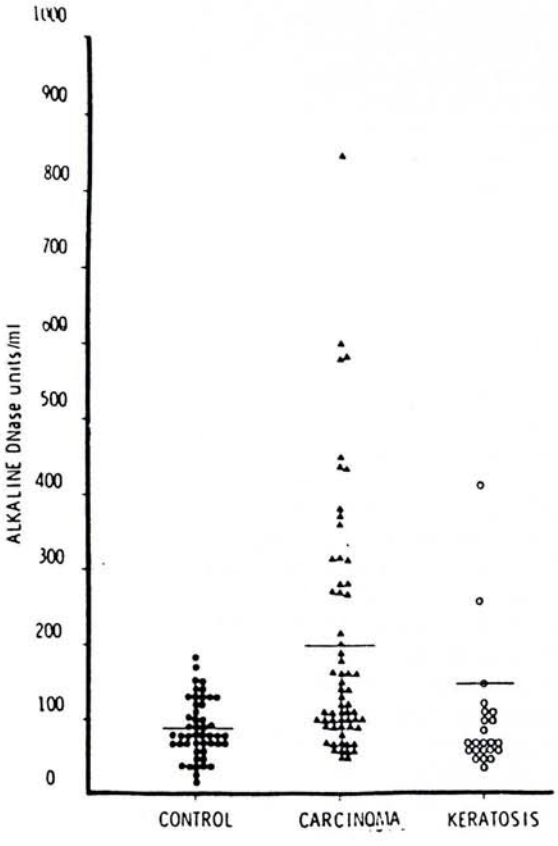

FIG. 1.
Serum alkaline deoxyribonucleases in oral carcinoma, keratosis and controls.

controls. Furthermore, the DNase activities were significantly greater in oral cancer than in patients with oral keratosis. Although the results are preliminary with respect to the number of patients, it will be interesting to examine changes in serum DNase activities in relation to the degree of epithelial dysplasia in the keratosis group, since the patient who had the greatest serum alkaline DNase activity in this group developed oral carcinoma within 6 months, suggesting that high DNase activity in a patient with oral keratosis might be a sign of poor prognosis.

However, it should be noted that not all patients with oral carcinoma had raised activities in the serum of alkaline DNase. Indeed, almost one half of the group had levels within the mean plus or minus two standard deviations. Normal serum activity of DNase does not, therefore, exclude the presence of a tumour.

The increased alkaline DNase activity in the serum of patients with oral cancer might be the result of cell proliferation or aberrant metabolism, or perhaps lysosomal enzyme release (8). Aberrant metabolism in oral cancer is suggested by the increased activity of tissue enzymes of the hexose monophosphate shunt (2). This shunt is utilised for the production of pentoses that are required for nucleic acid synthesis and for changes in the tissue activity of several other enzymes (4).

Serum activities of DNases have recently been reported to be increased in breast cancer (12) and some other tumours but not in any of a wide spectrum of non-malignant disease, except rheumatoid arthritis and polymyositis (13). Preliminary observations in patients with oral cancer suggest that the serum DNase activities may decline after surgical treatment (10) and this assay may therefore prove of some value in the management of patients with oral carcinoma.

REFERENCES

1. Brown A. M., Lally E. T., Frankel A., Harwick R., Davis L. W. & Rominger C. J. The association of the IgA levels of serum and whole saliva with the progression of oral cancer. *Cancer*, 1975, *35*, 1154.
2. Heyden G. Histochemical investigation of malignant cells. *Histochemistry*, 1974, *39*, 327.
3. Hughes N. R. Serum concentrations of G, A and M immunoglobulins in in-patients with carcinoma, melanoma and sarcoma, *J. nat. Cancer Inst.*, 1971, *46*, 1015.
4. Johnson N. W., Evans A. W., Morgan P. R. & Butcher R. G. Biochemical and Histochemical Changes on Oral Premalignancy. Eds MacKenzie I. C., Dabelsteen E. and Squier C. A. P. 132. *In*: Oral Premalignancy. *University of Iowa Press*, Iowa, 1980.
5. Katz A. E., Yoo T. J. & Harker C. A. Serum immunoglobulin A (IgA) levels in carcinoma of the head and neck. *Trans. Amer. Acad. ophthal, otol.*, 1976, *82*, 131.
6. Kit S. Thymidine kinase, DNA synthesis and cancer. *Mol. Cell. Biochem.*, 1976, *11*, 161.
7. Pindborg J. J. Studies in Cancer Epidemiology. 2. Frequency of Oral Cancer. *J. Dent. Res.*, 1963, *42*, 348.
8. Poole A. R. Tumour lysosomal enzymes and invasive growth. P. 303. *In*: Frontiers of Biology., Vol. 29, Lysosomes in Biology and Pathology, Dingle J. T. Ed. *Elsevier*, Amsterdam, 1973.
9. Scully C. Serum B_2 microglobulin in oral malignancy and premalignancy. *J. Oral Path.*, 1981. In Press.
10. Scully C., Ward-Booth P., McGregor I. A. & Boyle P. Serum deoxyribonucleases in oral cancer. *Int. Assoc. Dent. Res.*, 1981, *60 B*, 1164.
11. Silverman M. A., Alexander J. C. & Chretien P. B. CEA levels in head and neck cancer. *Cancer*, 1976, *37*, 2204.
12. Spandidos D. A., Ramandanis G., Garas J. & Kottaridis S. D. Serum deoxyribonucleases in patients with breast cancer. *Europ. J. Cancer.*, 1980, *16*, 1615.
13. Spandidos D. A. & Stathopoulos G. Serum deoxyribonucleases as a biological marker in cancer patients. *Excerpta Medica*, 1980, *5*, 68.
14. Waterhouse J., Muir C. & Correa P. (Ed.) Cancer Incidence in five continents. Vol. III. Lyon-International Agency for Research on Cancer. 1976.
15. Weber G. Enzymology of cancer cell. *New Engl. J. Med.*, 1977, *296*, 486.
16. Wolf C. T., Chretien P. B., Elias E. G., Makuch R. W., Baskies A. M. Spiegel H. E. & Weiss J. F. Serum glycoproteins in head and neck squamous carcinoma. *Amer. J. Surg.*, 1979, *138*, 489.