



Significance of serum butyrylcholinesterase levels in oral cancer

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RESEARCH

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Abstract

Background

Oral squamous cell carcinoma (OSCC) is a relatively common epithelial malignancy, and thus represents a significant public health problem. Early detection improves quality of life for affected patients. Identification of molecular markers (or biomarkers) which can predict disease progression is necessary for better management of these disorders. A correlation of cholinesterase with tumorigenesis, cell proliferation and cell differentiation has been observed. Butyrylcholinesterase (BChE; pseudo-cholinesterase) has been shown to be a biochemical marker for cervical cancer which is also an epithelial malignancy. In this study, we sought to estimate and compare serum BChE levels in healthy controls and patients with biopsy-proven oral squamous cell cancer (also an epithelial malignancy) before definitive therapy as radiotherapy or chemotherapy may alter the levels of BChE and may act as a confounding variable.

Method

After obtaining consent from biopsy proven oral cancer patients (n= 39) (before onset of any definitive treatment), and from age- and sex-matched healthy controls (n = 20), 2ml of blood was collected. After clot formation samples were centrifuged, serum was collected for estimation of BChE.

Results

Pre-treatment serum BChE levels were significantly elevated ($p < 0.0001$) in oral cancer patients compared to that of controls. BChE levels showed a significant increase ($p = 0.005$) with advancing stage in oral cancer patients.

Conclusion

Our results show there could be a role for serum BChE in determining the prognosis of oral cancer.

Key Words

Butyrylcholinesterase; oral carcinoma; prognosis

Background

Oral cancer is the eighth most common cancer worldwide¹ and its prevalence is high among men. In India, the age standardised incidence rate of oral cancer is 12.6 per 100,000 population.² The term 'oral' cancer includes cancers of the lip, tongue, gingiva, oral mucosa, oropharynx and hypopharynx.³

Clinical and histological features alone cannot always accurately predict whether potentially malignant disorders of the oral mucosa will remain stable, regress or progress to malignancy.⁴ Identification of molecular markers (or biomarkers) which can predict disease progression (prognostic markers) is necessary for better management of



these disorders.⁴ Markers such as glutathione S-transferases, N-acetyltransferases, serum dipeptidyl peptidase have been associated with various epithelial malignancies and have been shown to influence the susceptibility for cancer and outcome of treatment.⁵⁻⁹ A correlation of cholinesterase with tumourigenesis, cell proliferation and cell differentiation has been observed.¹⁰⁻¹³ Studies have shown that levels of butyrylcholinesterase (BChE; pseudocholinesterase) can be used as a biochemical marker in the management of head, neck and cervical cancer.¹⁴ So we sought to estimate and compare serum BChE levels in healthy controls and biopsy proven oral cancer patients before definitive therapy and evaluate their role in predicting the progression of oral cancer.

Method

The study was carried out after obtaining approval from the institutional ethics committee. The subjects were oral squamous cancer cases of either sex admitted under the Department of Radiotherapy and Oncology, Kasturba Hospital, Manipal from June 2009 to June 2010. Age and sex matched healthy controls were local people from Manipal. Both the controls and cases were included in the study after obtaining informed consent.

Study design: Prospective, case control study

Inclusion criteria

For cases: patients of either sex between 26–75 years with biopsy proven OSCC stage 2–4.

For controls: healthy subjects between the ages of 26 and 75 years, who underwent an oral examination to rule out all possible benign or malignant oral diseases.

Exclusion criteria

For cases: oral cancers which are not squamous cell carcinomas; post-surgery, post chemotherapy or post radiotherapy patients of oral cancer; recurrent cases of squamous cell carcinoma; patients with malignancy other than OSCC; patients with serious medical and surgical illness; patients on long-term medication.

For control: subjects with coexisting diseases; subjects on long-term medications.

We collected 2ml of blood in a vacutainer with no anticoagulant from controls and from cancer patients (before they underwent radiotherapy). The sample was allowed to clot for 30 minutes. After centrifugation at 3,000rpm for five minutes, the clear supernatant serum was used for the determination of BChE levels. BChE levels of samples were measured by using Genesis 10 UV (Thermo Electron Corporation). For maintaining the reaction temperature at 37°C, an automated water bath obtained from Rotek was used. Cyber scan 510 pH metre (Elico Ltd) was used. Sartorius balance was used for weighing chemicals.

BChE assay

Principle:

Acylthiocholine is hydrolysed by BChE to corresponding fatty acid and thiocholine. The rate of formation of thiocholine can be monitored by continuous reaction of thiol group with 5, 5'-dithio-bis-(nitro-benzoic acid) – DTNB to form a yellow anion that can be measured spectrophotometrically at 410nm.¹⁵

Procedure:

In a clean dry cuvette, 2.9ml of 5, 5'-dithiobis 2-nitrobenzoate (DTNB) solution was taken and to this 0.1ml of butyrylthiocholine solution was added and then to this mixture, 20µl of serum sample was added. After mixing by inversion, absorbance was read at wavelength 410nm. Enzyme activity was calculated by absorption coefficient of the product of chemical reaction, 5-thio-2-nitro-benzoate ($1.36 \times \text{mmol}^{-1} \times \text{min}^{-1} \times \text{cm}^{-1}$).

Statistical analysis: Anticipating a mean difference in values for serum BChE of 9,000 units with standard deviation of 1,400 units for a power of 80 %, 38 subjects are required in each group at 5% level of significance. The data analysis was done by using SPSS statistic analyser software version 15. Data was summarised as median and interquartile range since it was skewed. Comparisons between the stages were done using Kruskal Wallis test. Pairwise comparisons was



done using Mann Whitney test with Boneferroni correction for type I error.

Results

In this study, cases and controls were aged between 25 and 75 years of either sex. Of them, 39 were biopsy proven oral cancer patients (stage 2, 3 and 4) and 20 were healthy controls. Thus, a total of 59 subjects participated in this study. Oral cancer patients received radiotherapy as definitive treatment. The control group comprised of age and gender-matched healthy subjects. Among oral cancer patients, 31 were males and 8 were females.

Among the oral cancer patient group, pre-treatment serum BChE levels were significantly elevated ($p \leq 0.0001$) compared to those of controls (Table 1). Pre-treatment serum BChE levels showed an increase in with advancement of oral cancer (Table 2). On stage-wise comparison of pre-treatment serum BChE levels using Kruskal Wallis test, a significant ($p = 0.005$) difference was observed. Pair-wise comparison between stages showed a significant difference in serum BChE levels of stage 2 and stage 3 as compared to that of stage 4 ($p = 0.005$ and $p = 0.002$ respectively, Table 2).

Table 1: Comparison of pre-treatment serum BChE levels of oral cancer patients with healthy controls

Groups	Number	Pre-treatment serum BChE (U/l) Median (IQR)
Controls	20	1725.5 (1400.3, 2266.5)
Cases	39	6956 (5892, 8344)
*P value		< 0.0001

*Mann-Whitney Test

IQR - Interquartile Range

Table 2: Comparison of pre-treatment serum BChE levels in between stages of oral cancer patients

Groups	Number	Pre-treatment serum BChE (U/l) Median(IQR)	*P value
Stage 2	8	5526.5(4031.8, 6863.5)	–
Stage 3	10	6091 (4990.8, 7061.3)	0.477 ^a
Stage 4	21	8115 (6943, 9252)	0.005 ^b
			0.002 ^c

*Mann-Whitney Test

a- P value vs Stage 2- not significant

b- P value vs Stage 2- significant

c- P value vs Stage 3- significant

Bonneferoni correction for alpha error is used for multiple pair wise comparisons.

IQR - Interquartile Range

Discussion

Extensive biochemical studies have been carried out on tumour tissue and peripheral blood to explore the aetiology of cancers and to establish tumour markers as an adjunct for establishing the diagnosis and prognosis of disease.¹⁶ Biochemical changes in the tissue provide a better understanding of the chemical processes responsible for malignancy. In this regard, many studies have shown^{14, 17-21} serum alkaline phosphate, serum amylase, serum lactate dehydrogenase, CEA, serum calcium, serum magnesium, serum copper, serum zinc, and the copper/zinc ratio in various malignancies as possible diagnostic and prognostic biochemical markers

OSCC is relatively common and represents a public health problem. Its early detection helps to provide a good quality of life for patients. During the past years, several studies have identified potential biomarkers of OSCC progression and prognosis.²²



Cholinesterase has a role in cellular proliferation, differentiation and a possible involvement in tumorigenesis.²³ Studies have shown that serum BChE levels which were low in all patients with epithelial malignancies like head and neck and uterine cervix cancers started increasing after radiotherapy. It was observed that patients with no detectable/visible disease activity at six months follow-up had BChE values in the normal range. Thus, serum BChE can be used as a prognostic biochemical tumour marker in the management of these cancers.¹⁴ Abnormal expression of both BChE and AChE, and in vivo amplification of their genes have been observed in intracranial neoplasms such as meningioma, glioma and acoustic neurinomas, lung cancers, megakaryocytopenic disorders and leukemias and ovarian tumours.²⁷ AChE and BChE also modulate cell adhesion in human neuroblastoma cells.²³

In a variety of human tumours, BChE genes are amplified, mutated and/or aberrantly expressed.²⁴⁻²⁶ BChE affects cell proliferation by virtue of its antiapoptotic effects which may support early stages of tumorigenesis. It also plays a role in the later stages of transformation by enhancing anchorage independent cell growth which helps in cancer metastasis.²⁷ We also found increasing levels of serum BChE with advancing cancer

Limitations: The findings of our study cannot be generalised as the sample size is very small. Group characteristics included in the exclusion criteria are confounders for serum BChE levels. So, if these subjects were included in the study, there could have been a possibility of a different result from the present. One has to continue this study in a larger sample to further substantiate our findings. Since the study period was limited to one year we could not get a larger sample to suit our inclusion and exclusion criteria.

Conclusion

In this study, a significant increase in pre-treatment BChE levels in oral cancer patients as compared to controls was

observed. In the oral cancer patients, pre-treatment BChE levels increased with increasing stages of tumour. Thus, BChE may have a role as a prognostic marker of oral cancer. But this has to be ascertained by doing prospective studies in large samples. Serial estimations of BChE during the course of treatment and during the follow-up period after completion of treatment may prove more valuable in further ascertaining the role of this enzyme in the management of oral cancer.

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CONFLICTS OF INTEREST

We all authors solemnly affirm that there is no conflict of interest.

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ETHICS COMMITTEE APPROVAL

Given by Institutional Ethics committee, Manipal University.