Proton HR-MAS MR Spectroscopy of Oral Squamous Cell Carcinoma tissues: A metabolic and Multivariate Approach to Distinguish Malignant Tissues

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Introduction: Oral Squamous Cell Carcinoma (SCC) represents more than 90% of all head and neck cancers with the worldwide incidence of more than 350,000 new cases per year. The histopathology, a gold standard technique cannot give explanation why certain tumors metastasize while other tumors grow to larger size and remain restricted. Therefore, newer methods are required for understanding of intracellular features of post-surgical tumor tissues along with negative margins, including third dimension that is bed of the tumor. Therefore, an *ex vivo* HR-MAS NMR spectroscopic studies have been performed on resected human (n= 36) oral Squamous Cell Carcinoma (SCC) biopsies, its neighboring margins and bed tissues (n=159) to understand their altered metabolic profile.

Materials and Methods: Tissue specimens (n=159) were obtained from 36 patients (30 males and 6 females) who were enrolled with a written consent to participate in the study that was approved by the ethics committee. The stored tissues at -80°C were thawed and washed with D_2O prior to NMR analysis, to remove blood and other impurities. The tissues were then dissected and the inner-core (30-40 mg of wet-weight) of the tissues was taken for HR-MAS NMR experiments. Typically, the sample was packed into a 4 mm ZrO_2 rotor of 50 µl capacity; a volume of 20µl of D_2O having 0.03% TSP was used as a chemical shift reference. The sample-rotor-setup was then transferred into the HR-MAS NMR probe for NMR analysis on Bruker Avance 400 MHz NMR spectrometer. Proton NMR spectra with water suppression were acquired using one-dimensional NOESY CPMG spectra at 8.0 °C. A representative class of CPMG proton MR-spectra (1.80 – 4.25 ppm) obtained from muscle , salivary glands and oral SCC tissue specimens along with histopathology are shown in Figure 1(a), (b) and (c). On few sample COSY and HSQC were also recorded for resonance assignments. All tissue specimens were further subjected for histopathology. The data from 27 patients (120 tissue specimens) were subjected for training set for OSC-filtered PLS-DA model using the software 'The Unscrambler X' Software package (Version 10.0.1, Camo ASA, Norway) and the rest nine patients tissue specimens (n=39) were classified according to the model.

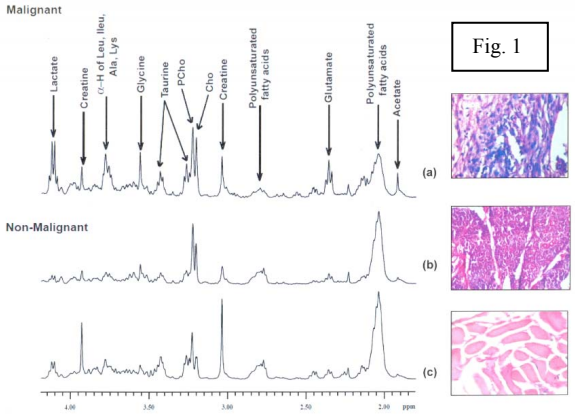

Fig. 1

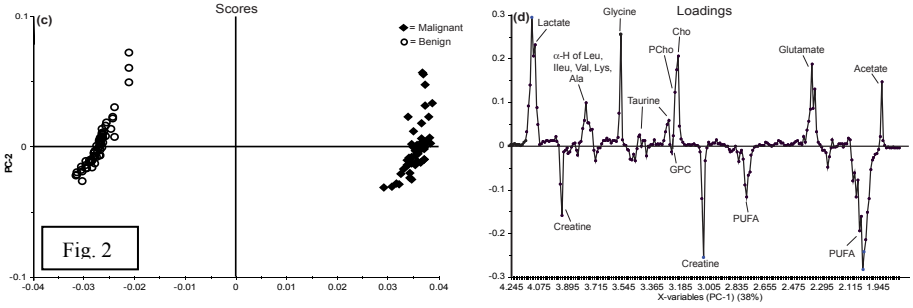

Fig. 2

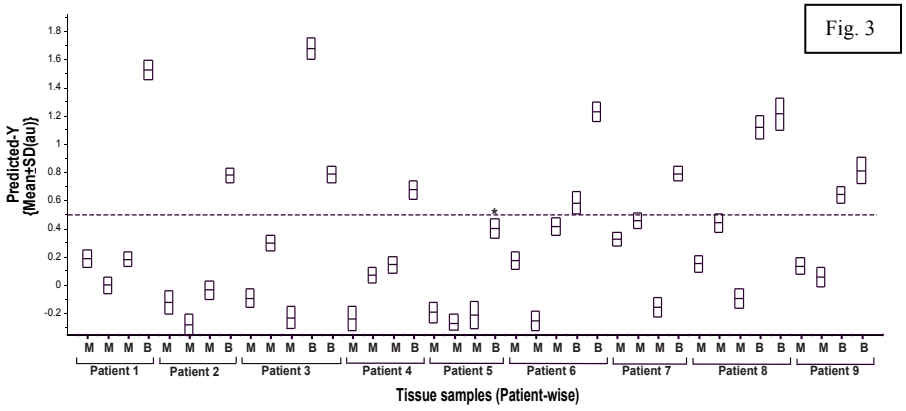

Fig. 3

Results and Discussion: The OSC-filtered PCA model indicated that malignant tissues had higher levels of glutamate, choline, phosphocholine, lactate, acetate, taurine, glycine, leucine, lysine, isoleucine and alanine, and lower levels of creatine and PUFA, representing altered metabolic processes (lipidogenesis, protein synthesis, and volume regulation) during tumor progression as observed in the PCA 1 loading plot (Fig 2). The regression coefficient OSC-filtered PLS-DA model was then generated for classification of unknown sample. The unknown tissue specimen's proton HR-MAS spectra were correctly classified in its respective histological categories with 97.4% diagnostic accuracy (Fig 3) with 100% sensitivity and 92.3% specificity. The overall representation of metabolic shift in this prospective ^{1}H HR-MAS NMR study goes hand-in-hand with the tumor biology, which is explained by the results of multivariate statistical analyses. In other words, the metabolic fingerprints are easily identifiable for oral tissues having SCC. Thus, simultaneous analysis of metabolic changes with ^{1}H HR-MAS NMR, in conjunction with subsequent histopathology of the same tumor specimen will ultimately improve accuracy in diagnosing, characterizing and evaluating tumor progression.

Conclusions: The proton HR-MAS MR spectroscopy could efficiently identify the metabolic derangements of malignant tumor from non-malignant bed and margins tissue specimens, which may be helpful in understanding the extent of tumor penetration in neighboring tissues.