

is contradictory to recent reports on CK7 negativity of BSCC. Low expression rate of BSCC for CK19 contrasts with invariable expression in conventional SCC, and limits the diagnostic utility of CK19 mRNA detection in this peculiar entity.

**610** **Application of infrared microspectroscopy and chemometrics for diagnosis of colon cancer** Poster

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**Background:** Nowadays the application of attenuated total reflectance - Fourier transform infrared (ATR-FTIR) microspectroscopy in the detection of cancer is a newly emerging diagnostic field, which has shown great potential. The applicability of advanced chemometrics techniques such as principal component analysis (PCA), linear discriminant analysis (LDA) and soft independent modeling class analogy (SIMCA) and their capability in interpreting the complex environmental data have been discussed.

**Materials and methods:** Totally 80 Formalin-fixed, paraffin-embedded tissues from patients were prepared from the histopathology division of Shahid Rajaei Hospital. ATR-FTIR studies were performed by a Nicolet<sup>®</sup> instrument. All spectral studies were performed 4000-400 cm<sup>-1</sup> wavenumber region. The measured sites' area was about 50x50 µm<sup>2</sup>. Number of 49 scans and 16 cm<sup>-1</sup> were optimized. Spectroscopic data were processed in total spectral region by this software. Resolution was set as the optimum condition. In order to apply the chemometrics methods, PCA and SIMCA techniques were performed by MATLAB<sup>®</sup> version 7.1 software.

**Results:** Two intensive signals at 1660 cm<sup>-1</sup> and 1540 cm<sup>-1</sup> which are due to amide I and amide II respectively, are indicative of protein structure. Spectral features due to stretching vibrations of PO<sub>2</sub><sup>-</sup> from the nucleic acids and the C-O stretching vibrations from the carbohydrate residues in the glycogen (or collagen) are found in 1240 and 1250 cm<sup>-1</sup>. PCA is an approach that can simplify the data interpretation by reducing the variable dimension of the spectral data matrix down to a very small number of orthogonal principal components that summarize nearly all of the variance in the original data matrix. SIMCA is sensitive to the quality of the data used to generate the principal component models. As a result, there are diagnostics to assess the quality of the data, such as the modeling power and the discriminatory power. After application of cross-validation 3 PCs for each class of sample (normal and cancer) were found. After application of principal components in the model, Q as a residual error for prediction the model was 7x10<sup>-4</sup> and 1.4x10<sup>-3</sup> for cancerous and normal class in calibration set respectively. In SIMCA, an unknown spectrum is identified as a specific group (or other group) by comparing the F-distribution with the 95% confidence interval. Sixty unknown samples (26 normal and 34 cancer) were predicted by SIMCA model. In the prediction step, 4 cancer tissue samples were predicted to be normal wrongly. The accuracy, sensitivity and specificity of the model were 93.3 and 88.2% and 100% respectively.

**Conclusions:** It was tried to demonstrate that ATR-FTIR microspectroscopy in combination with chemometric methods can reliably distinguish malignant colon tissues from healthy ones. It is important to be explored a noninvasive and rapid method for detection of colon cancer biopsies. High accuracy, sensitivity and specificity were obtained in the research.

**611** **Urine carcinoembryonic antigen levels are more useful than serum levels for early detection of Bilharzial and non-Bilharzial urinary bladder carcinoma: observations of 43 Egyptian cases** Poster

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**Background:** Both urinary bilharziasis and urothelial neoplasia are associated with increased production of tissue carcinoembryonic antigen (CEA).

**Patients and methods:** Urine and serum CEA were determined in 43 patients with urinary bladder carcinoma including 22 post bilharzial and 21 nonbilharzial cases, in addition to 10 normal control cases.

**Results:** A significant increase was detected in both urine and serum CEA levels with bladder carcinoma compared to control cases. Urinary CEA was significantly elevated in 86% of bilharzial, versus 62% in nonbilharzial bladder carcinoma. Only 10.5% of control cases had urinary CEA elevation. The mean urinary CEA in bilharzial, was higher than that of nonbilharzial carcinoma, but the difference was not statistically significant. There was a definite relationship between urine CEA and the stage of malignancy; the higher the stage, the higher the level of urine CEA. No

relationship could be detected between the stage of malignancy and serum CEA, or between the grades of malignancy and urine or serum CEA levels.

**Conclusion :** Urinary CEA is more useful than serum CEA in the early detection of urothelial carcinoma particularly if provoked by bilharziasis. Its level is also correlated with the tumor stage.

**612** **Effectiveness of COX2 inhibitor treatment in patients with dysplastic oral leukoplakia** Poster

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**Background:** Oral premalignant lesions include leukoplakias, which develop along epithelial surfaces that have been chronically exposed to carcinogens such as tobacco and alcohol. These lesions are markers for field cancerization because patients with oral premalignancy can develop squamous cell carcinoma at the site of the lesion as well as at other sites in the upper aerodigestive tract. Approximately 6% of all oral leukoplakias become malignant. An effort is being made to identify surrogate endpoint biomarkers (SEBs) for HNSCC. **Objective:** Study effectiveness of COX2 inhibitor treatment in patients with dysplastic oral leukoplakia, and also analyze COX2 as potential SEB in leukoplakia tissue samples by means of qPCR. **Materials and Methods:** Expression levels of COX2 gene, in 24 unique freeze samples from 24 patients with leukoplakia, were measured. From each patient, 3 samples were obtained: opposed lateral oral mucosa, leukoplakia mucosa before and after treatment with COX2 inhibitors. As control, a pool of healthy human oral mucosa from healthy donors (n=4) was used. qPCR experiments were performed on a LightCycler 480 Instrument (Roche) using LightCycler 480 SYBR Green I Master (Roche). A constitutively expressed gene, HPRT, was used as internal control. **Results:** The highest levels of COX2 expression were obtained in leukoplakia samples not only before treatment with COX2 inhibitors but also after it. 41.66% of samples showed lower COX2 expression levels in leukoplakia samples after the treatment and 37.5% of samples showed higher expression levels after it. Only 20.84% of samples showed higher expression levels in opposed lateral oral mucosa from patient. COX2 expression was always lower in the pool of healthy human oral mucosa from healthy donors than in opposed lateral oral mucosa from patient, which can be explained by means of the "field cancerization" phenomenon. **Conclusions:** Real Time PCR assays confirmed that COX2 is up-regulated in premalignant oral epithelial lesions, indicating that COX2 may contribute to carcinogenesis. 41.66% of patients treated with COX2 inhibitors showed a decrease in COX2 expression levels. The results showed a correlation with clinical response to treatment with COX2 inhibitors (Celecoxib). Therefore COX2 could represent an attractive SEB to target to in leukoplakias. **Support:** S. Díaz Prado is beneficiary of an Isidro Parga Pondal contract from Xunta de Galicia (Spain).

**613** **Ki-67 and survivin immunostaining for evaluation of cervical precancer lesions** Poster

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**Background:** Numerous molecular biomarkers have been suggested for early verification of cervical precancer. Ki-67 immunostaining is usually used as pattern of cellular proliferation. Survivin, a novel member of the family of apoptosis inhibitors, plays an important role in cell cycle regulation. Aim of this study was to examine whether a combination of immunostaining for Ki-67 and survivin could be used to evaluate the further progression of cervical intraepithelial neoplasias (CIN) to cervical cancer (CC).

**Materials and methods:** The study included 30 CIN cases, 14 squamous-cell CC stage 1A (microcarcinomas) and 16 invasive CC cases. High risk HPV (HPV16 and 18) were demonstrated in 83% of CIN and 80% of CC cases, correspondently. Ki-67 and survivin were used for immunostaining of paraffin sections with monoclonal (MIB-1) and polyclonal antibodies correspondently. To detect an early genetic abnormality in cervical pathogenesis the loss of heterozygosity at 6p21.3 (HLA region) was tested