IPD Project Details

Project ID: IPD2932

Project Title: The salivary proteome profile in women throughout the menstrual cycle characterized by HR-LC-MS/MS

Description: Generally saliva consists of mixture of proteins electrolytes and small organic compounds. This whole saliva stands for secretions from major (submandibular, sublingual and parotid) and minor salivary glands, along with crevicular fluid, bacteria and cellular debris. The secretion of saliva is regulated under the autonomic nervous system via signal transduction systems that couple receptor stimulation to ion transport and protein secretory mechanisms (Dodds, Johnson, & Yeh, 2005). SDS-PAGE is a unidimensional method used to analyze the molecular weights and charges of proteins by separating the proteins into bands, thereby making them visible. The most common use of gel electrophoresis is the qualitative analysis of the complex mixtures of proteins. Schwartz et al. proposed polyacrylamide gel electrophoresis (SDS-PAGE) as an adequate method to evaluate protein content in the human saliva. In this work it was aimed to study the saliva protein composition from different subjects by high resolution liquid chromatography mass spectrometry (HR-LC-MS/MS) on different days, throughout the menstrual cycle and the influence of ovulation.

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Sample Preparation: Sample collection and process The saliva was collected by spitting into a sterile vial, which was immediately kept in an ice box and brought to the laboratory. The duration of collection of saliva was about 10 min and the saliva secretion over the first minute was discarded. The samples were centrifuged at 16000 x g for 15 min to remove insoluble materials and cells, if any. The samples were stored at -80?C until further use. Observation of ovulation The saliva samples were assigned among the three phases, preovulatory (day 6 to 12), ovulatory (days 13 and 14) and postovulatory (day 15 to 26) phases according to salivary hormone fern pattern analysis. Protein precipitation and estimation The salivary proteins were precipitated by trichloroacetic acid (TCA)-acetone precipitation method (Gehrke, 2006). The saliva samples were mixed with TCA-acetone solution (TCA-20% W/V, Acetone-90% V/V) with 1:1 ratio and 20 mM dithiothreitol (DTT) and incubated for overnight at -20°C. After incubation, the

samples were centrifuged at 5000 x g at 4°C for 30 min. The pellets were washed twice with cold acetone and centrifuged at 5000 x g at 4°C for 30 min. Finally, the pellets were air-dried and resuspended in UTC (6M Urea, 3M Thiourea, 8% CHAPS) buffer. The protein concentration was determined adopting modified protocol of Bradford method. 1D – Electrophoresis To resolve the salivary proteins SDS-PAGE was carried out on 12% gel adopting modified by Laemmli (1970), using the standard medium-range molecular weight marker (Low-Range SDS-PAGE Standards, Bio-Rad, Hercules CA) consists Phosphorylase b (97.4 kDa), Bovine serum albumin (66.2 kDa), Ovalbumin (45 kDa), Carbonic anhydrase (31 kDa), Soybean trypsin inhibitor (21.5 kDa), Lysozyme (14.4 kDa). The salivary protein preparation from each volunteer (30 µg) was thoroughly mixed with 1x sample buffer [50 mM Tris-Cl (pH 6.8), 2% SDS, 10% glycerol, 0.1% bromophenol blue, 100 mM ?-mercaptoethanol] and kept for 1 min at 100°C for complete denaturation of proteins, after which the sample was loaded onto the gel. A constant current of 50 V was applied for electrophoresis and the entire setup was maintained at room temperature. Coomassie brilliant blue (CBB) staining After the electrophoresis, the gels were immersed in distilled water for 2 min and subsequently stained with 0.5% Coomassie brilliant blue solution (40% methanol, 10% acetic acid, 0.5% Coomassie brilliant blue R-250 and distilled water) by continuous rocking at room temperature for 2 h. The gels were destained using 40% methanol and 10% acetic acid solution until the background of the gels became clear. Finally, the gels were rinsed with distilled water and stored in 5% acetic acid until used for further analysis.

Peptide Separation: In-gel digestion Trypsin digestion was conducted according to Muthukumar et al. The protein bands were excised using sterile blade and placed in a tube containing 25 mM NH_4HCO_3 and 50% acetonitrile (v/v) in 1:1 ratio and incubated at room temperature, until no color was visible. The destained gels were sliced into small pieces and transferred to a fresh tube. The gels were dried in Speed-Vac and the gel pieces were immersed in 2% ?-mercaptoethanol/25 mM NH_4HCO_3 and incubated in dark for 20 min at room temperature. For cystine alkaylation, equal volumes of 10% 4-vinylpyridine and 25 mM NH_4HCO_3/50% acetonitrile were added. After incubation, the gels were washed with 25 mM NH_4HCO_3 for 10 min, dehydrated and then 100 ng of trypsin (Promega) in 25 mM NH_4HCO_3 was added and incubated overnight for digestion. After enzymatic cleavage the trypsin solution was removed, and proteins/peptides were extracted with 25 mM NH_4HCO_3 and 25 mM NH_4HCO_3/50% acetonitrile, respectively. The extract was dried in Speed-Vac and stored at -20°C until further analysis. Mass spectrometer analysis Tryptic digested samples were subjected to analysis in 6550 i-Funnel QTOF-LC-MS/MS (Agilent Technologies, Santa Clara, California, USA) coupled with 1260 Infinity Nano pump and 1260 Cap pump along with 1260 Chip-cube. The mobile phase consisted solvent A (100% milliQ water + 0.1% Formic acid) and solvent B (90% acetonitrile + 10% Water +0.1% Formic acid). A typical LC linear gradient was set from 97% solvent A to 97% solvent B for over a 100 min runtime at a pump flow rate of 0.3 µl/min. The conventional MS spectrum was acquired at high resolution over the acquisition range m/z 300-3000.

Protein Characterization: The acquired masses spectra were processed by using Xcalibur software (Version 2.0 SR1). The MS/MS spectrum ion scans were searched using, SEQUEST (TURBO) (Gatlin et al., 2000). The protein identification was analyzed against Homo sapiens protein sequence in SWISS-PROT database entries. Modification of cysteine by carbamidomethylation and methionine by oxidation modifications was allowed. The precursor and product mass tolerance set as +/-50 and +/-100 ppm respectively. Two or more unique peptides for each protein were taken for confirmation of the protein present in the sample.

Experiment Type: Gel-based experiment

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Disease free No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: No PTM

PubMed ID: