IPD Project Details

Project ID: IPD8273

Project Title: Analysis of low molecular weight protein profile in whole saliva with special reference to ovulation

Description: Whole saliva is contains major, minor and gingival crevicular fluid/secretions and it plays vital role in maintaining the oral health, three pair of major salivary glands consist of parotid, submandibular and sub-lingual glands. Saliva is a good biological fluid to explore health and disease status of human. Further, the saliva secrets from major salivary glands and have various biomolecules such as, proteins, enzymes and ions originated from serum and saliva is easily accessible through a safe and noninvasive method. In contrast, the concentrations of biomolecules in saliva are generally one tenth to one thousand of the levels in blood. Ovulation is an exclusive process through the mature (pre-ovulatory) ovarian follicles reacts to the surge of luteinizing hormone (LH) and burst to discharge fertilizable oocytes. The LH surge initiates a flow of proteolytic actions with the aim of control ovulation. Presently, many salivary proteins are have been showed as a biomarker such as Sjogrenâ€™s syndrome, Lung cancer, oral and Systemic diseases, HIV-seropositive patients, dental pellicle development, and hyperglycemia. However, there is no prominent marker protein was reported for monitoring ovulation process in human saliva. It is important prediction of fertile period in women for various applications like assisted reproduction and invitro fertilization.

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Sample Preparation: The whole saliva samples were collected at 8.00 to 9.00 AM from 50 healthy female volunteers (age mean = 28) as per spitting method. Prior to study the participants were informed by written assent. The samples were collected in a sterile vial, immediately kept in ice box and brought to the laboratory. The saliva collection period was about 10 min and first 1 min saliva secretion was discarded. The samples were centrifuged at 12000 rpm for 15 min to remove insoluble materials and cells. The saliva sample was categorized into three phases Preovulatory (6th to 12th days), Ovulatory (13th and 14th days) and Postovulatory phases (15th to 26th days) by ferning pattern in saliva. The samples were stored in -80?C for until it use. Sample preparation

Salivary proteins were precipitated by Trichloroacetic acid (TCA)-Acetone precipitation method. The saliva samples mixed with 10% TCA-Acetone and 10mM Dithiothreitol (DTT) and incubated for 1 h at -20°C after the incubation samples were centrifuged for 20 min at 5000g in 4°C. The pellet washed twice with cold Acetone and centrifuged at 5000g for 20 min 4°C. Finally, pellets were air dried and resuspended in 10mM Tris buffer. Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) To resolve salivary proteins the 12% SDS-PAGE was carried out and the standard medium range protein marker used for molecular weight reference. Coomassie brilliant blue (CBB) staining After the electrophoresis done, the gels were immersed in distilled water for 2 min and subsequently stained with 0.5% Comassie brilliant blue solution (40% Methanol, 10% Acetic acid, 49.5% Distilled water, 0.5% Coomassie brilliant blue R-250) by continuous rocking at room temperature for 2 h. Then gels were destained by using 40% Methanol and 10% Acetic acid solution until gels background became clear. The protein band intensity were measured by gel documentation system based on the pixel area occupied by the proteins.

Peptide Separation: In-gel trypsin Digestion The differentially expressed protein band were excised by sterile blade and placed in a tube contains 100µl of 25 mM NH_4HCO_3, 50% acetonitrile (v/v) in 1:1 ratio and incubated for 30min at room temperature. This process was continued until no visible stain for complete detstaining. The destained gel were sliced into small pieces and transferred in a fresh tube. Gels dried in Speed-Vac (Savent) and gel pieces are immersed in 100µl of 2% ?-mercaptoethanol/25 mM NH_4HCO_3 and kept in dark room for 20 min incubation at room temperature. For cystine alkaylation, an equal volume of 10% 4-vinylpyridine, 25 mM NH_4HCO_3/50% acetonitrile was added. After incubation, the gels were washed with 25 mM NH_4HCO_3 for 10 min, and gels pieces were dehydrated and then 100 ng of Trypsin with 25 mM NH_4HCO_3 added and incubated overnight for digestion. After enzymatic cleavage the trypsin solution were removed from gel, and protein peptides were extracted with 25 mM NH_4HCO_3 and 25 mM NH_4HCO_3/50% acetonitrile, respectively. The extraction were dried in Speed-Vac and stored at -20°C until further analysis. Mass spectrometry analysis For mass spectrometry analysis was performed using ESI TOF- MicroTOF-Q-II (Bruker Daltonics) coupled with Nano LC (Dionex Ultimate 3000). The LC gradient was performed by set from 90% water to 90% acetonitrile in a linear gradient over a 50 minute runtime, at a flow rate of 0.2 ml/min. During the LC-MS run the spectra were averaged over four scans.

Protein Characterization: The acquired MS/MS data were processed for further identification. The protein identification was performed in MASCOT search engine (http//www.matrixscience.com). The MASCOT search scores were significant ($p<0.05$). Detected primary sequence of salivary protein was retrieved from NCBI.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: Carbamidomethyl, Oxidation

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