3153 Elevated Saliva Cortisol Concentration in HIV-infection/AIDS.

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Human immunodeficiency virus-1 (HIV-1) infection is characterized by complex endocrine changes with hypercortisolemia observed in many patients. Previous studies have demonstrated a strong linear correlation ($r \geq 0.9$) between saliva concentration of cortisol and plasma level of the free hormone, a relationship independent of saliva flow rate. We therefore evaluated unstimulated whole saliva concentrations of cortisol in 19 HIV/AIDS subjects (14 males and 5 females, ages 24-48 yr) and 10 age-matched controls. Expectorated saliva was collected at 10.00 to 11.00 hr and cortisol level measured by radioimmunoassay (RIA kit from Sigma Chem. Co. and ^{3}H-cortisol, specific activity 100 Ci/mmol from DuPont NEN). Mean saliva cortisol concentration (nm/L ± SD) in the HIV/AIDS patients was significantly higher ($p < 0.025$) than the control level (23.84 ± 6.22 versus 12.05 ± 2.66). Glucocorticoids are anti-inflammatory and immunosuppressive. Previous studies indicate presence of a corticosteroid-binding protein (CBP) in *Candida albicans*. Additionally, corticosteroid therapy is linked with increased risk of Kaposi's sarcoma (KS) and studies have demonstrated an unusually high level of CBP in AIDS-KS cells, as well as a prominent dexamethasone-induced stimulation of the growth of cultured KS cells which is completely abolished by RU-486, a glucocorticoid receptor antagonist (Guo & Antakly, *Amer J Pathol* 146.727-734, 1995). We conclude that endogenously mediated hypercortisolism, particularly increased saliva content of glucocorticoids, may have some role in the pathogenesis of some common HIV/AIDS-associated oral lesions, especially KS and candidiasis. This study was supported in part by NIH Grant DE-09653.

3154 Salivary and urinary cortisol excretion before dental treatment.

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Blood cortisol levels are modified by stressors, like (anticipation of) dental treatment. The advantage of measuring cortisol in saliva and urine is the non-invasive, stress-free way of collecting samples. We measured cortisol excretion before 5 different categories of dental treatment in adult male patients. Before each treatment, parotid saliva and urine samples were collected. Corah's Dental Anxiety Scale and a Visual Analogue Scale measured Trait and State anxiety resp. Thirtyfive dental sessions were classified: I: Checkup/Diagnosis. II: drilling/filling after local anesthesia (LA). III: Root planing/scaling without LA. IV: Pocket measurements. V: Extractions after LA. Ultracain DS with 1:100 000 epinephrine was used as LA. Cortisol was quantified by RIA. Urinary cortisol was expressed as ratio of creatinine.

Category:		Cortisol Urine (nmol/mmol creat)	Cortisol Saliva (nmol/L)	Dental Anxiety DAS	Dental Anxiety VAS (mm)
I	(n = 12)	30.8 ± 4.1	6.4 ± 0.6	9.9 ± 0.4	32.4 ± 4.2
II	(n = 5)	41.3 ± 8.9	7.0 ± 1.0	9.4 ± 0.7	30.0 ± 8.7
III	(n = 9)	42.4 ± 10.1	11.2 ± 1.8	9.6 ± 0.5	30.9 ± 10.1
IV	(n = 4)	55.1 ± 15.3	11.4 ± 2.7	10.2 ± 0.5	39.7 ± 5.2
V	(n = 5)	52.1 ± 11.1	7.3 ± 1.3	9.4 ± 0.6	49.1 ± 5.7

Data are expressed as mean ± S.E.M.

Mean urinary and salivary cortisol concentrations did not differ significantly between the categories (1-way ANOVA) and were in the physiological range (which is 10-75 nmol/mmol creatinine for urine and 5-20 nmol/L for saliva). Salivary cortisol did not correlate with both dental anxiety parameters. Urinary cortisol correlated significantly with Trait Anxiety (DAS: $r = .35$, $p < 0.05$) but not with State Anxiety (VAS).

3155 Studies on the Susceptibility of *Candida* Species to Lactoferrin.

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Although lactoferrin is well recognised as a constituent of saliva, its antifungal properties have received little attention. Hence the main aim of this study was to examine the antifungal activity of lactoferrin against six oral isolates each of *C. albicans (CA), C. glabrata (CGl), C. krusei (CK), C. parapsilosis (CP), C.tropicalis (CT)* and *C. guilliermondii (CGu)* (total, 36 isolates). A secondary aim was to study the ultrastructural topographical features of the yeasts so treated. The yeasts grown in brain heart infusion broth (Oxoid, England) for 18 h at 37°C were washed once with potassium chloride buffer (0.05 mM, pH 7.0) and reconstituted to yield a suspension of 10^4 yeasts/ml. The effect of both human (HLF) and bovine lactoferrin (BLF; Sigma Chemicals, USA) on the viability of *Candida* species was investigated as described in our previous studies (Archs Oral Biol 1993; 38: 1057-63). Experiments withe a single isolate of *C. albicans* indicated that the fungicidal effect of both HLF and BLF was dose dependent ($p < 0.05$), but not time dependent. When the relative potency of BLF in killing six isolates each of the foregoing *Candida* species was examined *C. tropicalis* was the most susceptible and *C. glabrata* the least, although there were variations in susceptibility to BLF within a given species. Thus the interspecies hierarchy of susceptibility of the organisms were CT > CK > CA > CGu > CP > CGl. Ultrastructural studies, under Cryo-SEM revealed cell surface damage and ballooned, degenerated cells treated with BLF. Hence we conclude that salivary lactoferrin may regulate both qualitatively and quantitatively, the yeast populations in the human oral cavity.

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3156 Proteinase production by *Candida albicans* in HIV infection and its attenuation by antimycotics.

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Extracelluar proteinase is increasingly attributed as a virulence factor of *C. albicans*. Therefore proteinase production by 36 oral *Candida albicans* isolates from patients with and without HIV infection, and the effect of sub-minimal inhibitory concentrations (sub-MICs) of nystatin (Ny), amphotericin B (Am), clotrimazole (Cl) and miconazole (Mi) on proteinase production were investigated using the method of bovine serum agar proteolysis, described by Staib (*Sabouraudia* 1965; 4: 187-93). Proteinase production of *C. albicans* from patients with HIV infection was significantly greater than those from individuals without infection ($p = 0.002$). All 18 isolates from HIV-infected individuals produced proteinase, as opposed to 56% isolates from uninfected individuals. Pre-exposure of *C. albicans* isolates to 1:4 and 1:16 MICs of Ny, Am, Cl, Mi, resulted in decreased proteinase production in 14 isolates tested, and this phenomenon was seen to a significantly greater extent in isolates from uninfected individuals compared to those with HIV disease ($p < 0.05$). Further, when the relative concentration effect of antimycotics on proteinase production was compared, isolates from HIV-free group demonstrated a significant dose response relationship compared with the HIV-infected. We conclude that *C. albicans* from patients with HIV infection are significantly more proteolytic than from those without the infection, and the polyenes and imidazoles curtail the proteolytic activity of all *C. albicans* isolates.

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3157 Demonstration of transglutaminase reactive proteins on hyphal surfaces of *Candida albicans*.

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Candida albicans is an opportunistic fungal pathogen that colonizes the GI tract in health but causes serious disease in patients such as those that are iatrogenically immunosuppressed or those with AIDS. The processes of persisting on the oral mucosa during health and adherence and invasion during infection require the presence of surface structures which interact with host tissues. Our research focuses on proteins expressed on *C. albicans* cell surfaces and in recent results, we identified a protein specific to hyphal surfaces (hwp-1). The primary amino acid sequence of hwp-1 had features similar to those of transglutaminase (TG) substrates, an important observation because of the existence of TG on buccal epithelial cell surfaces of the oral mucosa. To determine if *C. albicans* expressed TG substrates, we incubated organisms in the presence of a biotinylated primary amine and soluble transglutaminase purified from guinea pig liver. TG reactive sites were localized to hyphal but not yeast surfaces by an avidin-FITC conjugate and fluorescence microscopy. Cell bound buccal epithelial transglutaminase also cross linked the biotinylated primary amine to its surface, confirming the presence of TG on oral mucosa. Taken together, these results suggest that buccal epithelial cell TG mediated cross linking to C. albicans hyphae may be important for host parasite interactions on the oral mucosa. This work was supported by NIH Grant DE 11375.

3158 Identification of a secretory-protein activating enzyme in *Candida albicans*.

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The secretory pathway of *Candida albicans* mediates transport of lytic enzymes and cell wall components into the extracellular space. The lytic proteins include aspartyl proteinases that are thought to contribute to the degree of invasiveness of the organism, and the cell wall components several β-glucanases that define cross-linking patterns of structural peptidoglycans. Prior to secretion from the organism, some of these proteins are modified by proteolytic cleavage adjacent to a pair of basic amino acids, ostensibly to convert them from a catalytically inactive to active form. This type of processing is reminiscent of that noted for one of the mating pheromones of *Saccharomyces cerevisiae*, where a prepropeptide precursor is converted into biologically active α-factor by a series of *kex2* proteinase-mediated cleavages. To establish whether *C. albicans* shares a similar pathway, a *kex2* strain of *S. cerevisiae* was transformed with *C. albicans* DNA and assayed for the ability to produce α-factor. Plasmids that complemented the *kex2* mutation were characterized, and shown to contain a gene homologous to *KEX2*. Based on Southern analysis using the cloned gene, it appears that it exists as a single copy per haploid *C. albicans* genome. In common with the Kex2 protein, the predicted *Candida* homologue has a hydrophobic amino-terminal leader sequence, a propeptide ending with a lysine-arginine motif, a catalytic domain characteristic of the subtilisin family of serine proteinases, a serine/threonine-rich stretch that may regulate its retention in the Golgi compartment, and a transmembrane region. We conclude that *C. albicans*, which does not have a sexual phase, nonetheless shares a component of the *S. cerevisiae* mating pathway. Conversely, we hypothesize that the product of this gene plays a role in proteinase secretion by *C. albicans*, a pathway not present in *Saccharomyces*. This study was supported by NIH grants 1RO1AI-33317 and PO1-DE-07946, and a University of California Wide AIDS Research Program Grant from the State of California.

3159 Oral Carriage and Antifungal Susceptibility of Candida in Immunocompetent Adults.

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Objective: To determine the rate of carriage of yeast in dental plaque and their antifungal susceptibility using the Etest® strip method in immunocompetent adults. Methods: Pooled supra- and subgingival plaque was collected from 35 adults with gingivitis and no history of antibiotic or antifungal use in the preceding 6 months and cultured on selective media. Five to 6 isolates per subject (if present) were examined for germ tube production in fresh serum. Carbohydrate assimilation was determined for representative strains using API 20C. Antifungal susceptibility was determined using Etest® strips with continuous concentration gradients of antifungal agents fluconazole (FL) and amphotericin B (AP)0.016-256 μg/ml; itraconazole (IT), ketoconazole (KE), and 5-fluorocytosine (5FC) 0.002-32 μg/ml. Strips were applied to 2 types of media, MOPS buffered- RPMI 1640 agar supplemented with 2% glucose and Casitone agar after seeding with a light inoculum of each isolate and control strain Candida albicans ATCC 90028. MIC end points were determined by visual inspection of 80% growth inhibition for FL, IT and KE and 90% for AP and 5FC compared to non-inhibited growth. Results: 17/35 subjects were culture positive for yeast yielding 94 isolates. All were germ tube positive and identified as C. albicans. Carbohydrate assimilation tests confirmed the identification of 22 representative strains. MICs of isolates from 4 subjects were as follows; FL, IT and KE 1.5-2, 0.012 and 0.094-0.19 μg/ml respectively on Casitone medium. AP and 5FC MIC on RPMI medium were 0.045-1.5 and 0.25-0.38 μg/ml respectively. Trailing end points were common on both media making precise MIC determination difficult but discernible within 1 dilution. Conclusions: C. albicans was the sole yeast species isolated from the dental plaque of 30% (17/35) of immunocompetent adults with gingivitis. All isolates were uniformly sensitive to FL, IT, KE, AP and 5FC. The Etest® strip method was simple to perform but required experience and judgment to read MIC end points. #DE10592.

3160 Typing System for Tracing Origin of Yeast Isolates in Blood.

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Saccharomyces cerevisiae (*Sc*), which is a yeast recovered from the oral cavity, is associated with infections in immunocompromised patients secondary to cancer chemotherapy, HIV-infection and alcoholism. In this study *Sc* was isolated from the root canal and blood stream of a patient during endodontic therapy of a tooth with *asymptomatic apical periodontitis*. Phenotypic and genetic characterization of the clinical strains and the type strain of *Sc* was performed to examine if *Sc* in the blood originated from the root canal. *Sc* strains were cultured aerobically in duplicate on Sabouraud dextrose agar plates at 37°C for 24 h. Biochemical and antifungal susceptibility tests of *Sc* strains were performed by using the bioMérieux databased system (*Endod Dent Traumatol* 11:142-49,1995). The profiles of *Sc* whole-cell soluble proteins were provided through SDS-PAGE electrophoresis. DNA restriction patterns and corresponding ribotypes of the *Sc* isolates were also examined. Purified chromosomal DNA was digested by *BglI*, *EcoRI*, and *HindIII*. The restriction fragments were separated by electrophoresis in 0.7% agarose gel in TBE buffer and transferred (Southern blotting) to nylon membranes. The hybridization was carried out with a digoxigenin-labelled cDNA probe obtained by reverse transcription of *Escherichia coli* 16S + 23S rRNA. The biochemical and antifungal susceptibility tests, SDS-PAGE of proteins, DNA restriction patterns and ribotypes showed identical characteristics of the isolates recovered from root canal and blood. The results strongly suggested that *Sc* isolated from blood originated from the root canal. The present typing system appeared well suited for tracing the origin of yeast isolates in the blood stream.