2681 Clinical Characteristics of HIV Seropositive Children Undergoing Antiretroviral Therapy.

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Vertical transmission of HIV to children presents a unique challenge to developing oral structures and some studies have shown a relationship to the variety of oral complications associated with suppression of the immune system. Antiretroviral therapy in pediatric patients has shown promise in stabilizing disease progression, however, little is known about any developmental impact of these interventions. An investigation has been undertaken to compare and characterize oral developmental and clinical oral/dental findings in 15 HIV seropositive children (Group A) and 15 age, race and sex matched HIV seronegative controls (Group B). The following clinical characteristics were considered: height; weight; tooth eruption trends, presence, absence or history of caries, gingival inflammation, fungal infection; current and previous antiretroviral medications. A matched pairs t test revealed no statistically significant differences related to height, caries experience, or gingival inflammation between the groups. Weight was found to be at or below the 50th percentile for the HIV seropositive group. Presence of fungal species was found to be higher in Group A with identification of *Candida dubliniensis, Candida albicans* and *Candida glabrata*. Analysis of eruption patterns revealed significant findings with 56% of Group A and 12% of Group B exhibiting over-retention of primary teeth. Nucleoside analog drug therapy was directly related to delays in natural exfoliation. These results indicate that immune compromise may inhibit development in the oral cavity. Further investigation is underway to investigate the potential relationship between antiretroviral medications and altered apoptosis patterns in the dental follicle. Study supported by NIH DE11373.

2682 Presence of *Candida dubliniensis* in HIV- Seropositive Pediatric Patients.

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The combination of an immature immune system and suppressed cellular immunity in children with HIV infections provides optimal conditions for rapid disease progression. As a result, pediatric AIDS has become a major epidemiological challenge. Oral fungal colonization remains one of the most common opportunistic infections observed in both adult and pediatric HIV patients. Although *C. albicans* is the most frequently isolated opportunistic fungal species, a recently characterized *Candida* species, *C. dubliniensis* (Cdub), has gained considerable attention due to its almost exclusive association with HIV seropositive individuals. The purpose of this study was to prospectively screen for the presence of Cdub among pediatric HIV+ patients. Oral samples taken from 27 children were cultured for the presence of yeast. Isolates were screened for the presence of Cdub by use of tests for germ tube and chlamydospore production, detection of inability to grow at 45°C, by colony color on CHROMagar Candida medium, coaggregation with *Fusobacterium nucleatum* ATCC 49256 and by the results of sugar assimilation testing with the API 20C AUX yeast ID system. Of the 27 patients tested, 3 patients were found to harbor Cdub, one of which also grew *C. glabrata*; 12 patients were colonized with *C. albicans*, while the remaining 12 patients were negative for yeast. Identification of the 3 Cdub isolates was genetically confirmed by electrophoretic karyotyping. All 3 Cdub isolates were found to be susceptible to fluconazole (MIC ≤ 0.25ug/ml). These results confirm the presence of this novel species among the pediatric HIV seropositive population in the United States and support the need for further investigation into the prevalence and pathogenesis of *C. dubliniensis*. Supported by NIH grantDE11373.

2683 The Genetic Diversity in Superficial and Systemic Isolates of *Candida parapsilosis*

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Candida parapsilosis, a form species of the fungi imperfecti, is an emerging pathogen gaining recognition as an opportunistic agent, especially in the immunocompromised. The application of randomly amplified polymorphic DNA (RAPD) technology for strain delineation of medically important yeasts has proven to be a valuable tool for epidemiological studies of *Candida* species. Therefore, 15 clinical isolates of *C. parapsilosis* obtained from oral, cutaneous and systemic infections, were typed by RAPD analysis using seven different, custom synthesized primers designated RSD 6 to RSD12 (Gibco BR, Hong Kong) based on the method of Akopyanz et al. (*Nucleic Acids Res.* 1992; *20*: 5137-5142). The primers RSD6 and RSD9 elicited seven genotypes each, whereas primers RSD 7 and RSD 12 revealed six and ten genotypes, respectively. When the data were correlated a greater degree of genomic heterogeneity in systemic isolates was noted compared with the oral and cutaneous isolates, which shared similar RAPD profiles. However, a single oral isolate (P5), and two systemic isolates (P13 and P15) elicited radically divergent profiles, dissimilar to their counterparts. RAPD study of the latter two isolates with three additional primers RSD8, RSD10 and RSD11 confirmed the observed genomic disparity. These data substantiate the observations on the genomic heterogeneity in *C. parapsilosis* and points to genetic shifts associated with ecodiversity, as well as the possible existence of distinct genetic groups within this form species. Supported by the Research Grant Council, and the Committee for Research and Conference Grants of the University of Hong Kong, Hong Kong.

2684 Antifungal Profile of Clones of *C. albicans* Isolated on Sequential Visits in a HIV Infected Cohort

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The increased frequency and severity of candidal infections in human immuno-deficient virus (HIV) infected individuals has prompted the wide use of antifungals such as amphotericin B, ketoconazole, and fluconazole resulting in the emergence of drug resistance strains of *C. albicans*. To study this phenomenon in an Asian cohort we isolated clones of *Candida* from the oral cavities of six HIV infected patients on sequential visits over a period of 12 months. Oral rinses (Samaranayake et. al., 1986, Journal of Oral Pathology, 15:,251-254) were collected from this cohort on intermittent antifungal therapy, and processed in the laboratory for the isolation of *Candida* species in a standard manner. All isolates were tested for their susceptibility to amphotericin B, ketoconazole and fluconazole using an agar diffusion method using the Etest (AB Biodisk; Dalvägen, Solna, Sweden). All tested isolates demonstrated variable susceptibility to amphotericin B, ketoconazole and fluconazole. The minimum inhibitory concentration (MIC) of the isolates for amphotericin B, ketoconazole and fluconazole ranged from < 0.002 – 1.5 μg/ml, < 0.002 - 0.047 μg/ml and < 0.016 – 12 μg/ml, respectively. Sequential isolates of a few patients demonstrated decreased susceptibility to antifungals over time while no such trend was seen in the vast majority of the isolates. Interestingly, variation in susceptibility to antifungals was also noted in isolates obtained from the same patient on a single visit. The results show that, in HIV disease, ecological pressure exerted by antifungals may not necessarily lead to adaptive changes in sensitivity profiles in sequential oral isolates of *C. albicans*. This study was supported by the Research Grants Council, and the Committee on Research and Conference Grants of the University of Hong Kong, Hong Kong.

2685 The Post Antifungal Effect of Nystatin and Amphotericin B on *Candida* Species in Different Media.

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Post-antifungal effect (PAFE) is the suppression of growth that persists following limited exposure of yeasts to antimycotics and subsequent removal of the drug. Sabouraud's dextrose broth (SDB) and RPMI broth are the commonly used recommended media for testing antifungal susceptibility of different drugs. The aim of this study was to compare the PAFE of oral isolates of *Candida* belonging to six different species (*C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*) following limited exposure to nystatin and amphotericin B in either SDB or RPMI broth. The yeasts were examined for the presence of the PAFE after one hour exposure to the minimum inhibitory concentration (MIC) of nystatin and amphotericin B growing in SDB or RPMI broth. The PAFE was determined as the difference in time (hours) required for the growth of the drug-free control and the drug-exposed test cultures to increase to a specific absorbance level following removal of the drug by two cycles of washing.

		nystatin		amphotericin B	
		SDB	RPMI	SDB	RPMI
C. albicans	n = 5	1.88 (0.46)	3.09 (1.32)	4.36 (2.10)	4.55 (4.99)
C. glabrata	n = 4	2.63 (0.42)	3.39 (1.29)	3.13 (2.42)	2.70 (1.29)
C. guilliermondii	n = 4	3.70 (1.86)	1.71 (1.07)	5.71 (5.36)	1.11 (0.70)
C. krusei	n = 4	4.87 (0.89)	6.89 (1.63)	3.40 (1.14)	7.01 (3.72)
C. parapsilosis	n = 4	3.18 (0.99)	5.64 (4.61)	7.51 (3.00)	4.48 (3.13)
C. tropicalis	n = 5	3.14 (1.34)	0.66 (0.45)	10.98 (5.36)	0.97 (1.06)

As different *Candida* species exhibit varying growth patterns and PAFE in SDB and RPMI broth, researchers should pay attention to this phenomenon when investigating these attributes of this opportunist pathogen.

2686 *Candida albicans* Triggers Interleukin-1β Responses by Oral Epithelial Cells.

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Oral *Candida albicans* (*Ca*) infections are emerging as a serious health problem as the number of patients immunocompromised by disease or treatment is rising in recent years. Production of proinflammatory cytokines, such as Interleukin-1β (IL-1β), by oral mucosal cells in response to *Ca* can be expected to play a major role in the initiation of an effective immune response as well as in the immunopathology of the developing oral lesion. The purpose of this investigation was to determine whether *Ca* can trigger secretion of IL-1β by oral epithelial cells *in vitro* and further investigate mechanisms of host cell-*Ca* interactions that trigger such responses. The KB oral epithelial cell line (ATCC) as well as primary gingival epithelial cell cultures were used in this study. Cell cultures were seeded at $5X10^5$ cells/well in 96 well plates and allowed to adhere overnight. Subsequently, stationary phase viable *Ca*, strains SC5314, ATCC28366 or ATCC32077, were suspended in fresh media and added to human cell cultures at yeast:oral cell ratios ranging from 100:1-0:1. Yeast were allowed to germinate for up to 48 h and supernatants were analyzed for IL-1β content by ELISA. A germination-deficient mutant, otherwise isogenic to strain SC5314, was also used to assess the effects of germination on the IL-1β responses of these cells. In order to ascertain whether direct contact of *Ca* with host cells is required to trigger cytokine production, epithelial cells were separated by yeast cells using cell culture inserts. Both cell line and primary cultures responded with a considerable increase in IL-1β secretion after 6 hours of coculture with *Ca*. Loss of fungal viability by fixation abolished all cytokine responses. Strain ATCC28366 was the most potent cytokine stimulator followed by SC5314, whereas strain ATCC32077 did not have an effect at any dose. Direct host cell-yeast contact was required for IL-1β secretion, since separation using culture inserts abolished all cytokine responses. However, yeast germination was not required. In conclusion *C. albicans* triggers IL-1β responses by oral epithelial cells *in vitro*. These responses are contact-dependent, strain-dependent, and require yeast viability but not germination into hyphae. Supported by the SDOS, Columbia University.

2687 *Antifungal drugs and virulence properties of C. albicans in diabetic patients.*

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This study investigated the influence of nystatin and fluconazole on virulence properties of *C. albicans*. A total of 108 diabetic patients participated in the study. Eighty-eight patients had clinical oral candidosis. Drug therapy was given at 6 hourly intervals for nystatin or daily with fluconazole for a maximum of 2 weeks. Adhesion of *C. albicans* to buccal epithelial cells was determined using an autologous adhesion assay prospectively over 6 months. Phospholipase production was estimated using an agar plate method. The data analysis included paired student's t-test and calculation of correlation coefficients. Unlike nystatin, treatment with fluconazole reduced the ability of *C. albicans* to colonise the buccal mucosa up to 8 weeks post-treatment. There was a significant difference in the number of isolates of *C. albicans* producing phospholipase in diabetic patients with oral candidosis compared to diabetic patients who were candidal carriers, but had a clinically healthy oral mucosa. Treatment with fluconazole reduced the production of phospholipase by *C. albicans* isolates from diabetic patients with oral candidosis.

In addition to being antifungal, fluconazole affects phospholipase production, and modifies buccal epithelial cells, reducing the adhesion of *C. albicans* for up to 8 weeks post-treatment.

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2688 Susceptibility of Oral Bacteria and Yeast to Mammalian Cathelicidins.

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Cathelicidins are a novel group of antimicrobial peptides found in secondary granules of neutrophils. While preliminary reports from our laboratory show expression of FALL-39 in gingival keratinocytes and in saliva, the antimicrobial effects of this human cathelicidin and other mammalian cathelicidins against oral bacteria are not known. The goal of this study was to survey the antimicrobial properties of several mammalian cathelicidins against a group of oral bacteria and yeast. Three cathelicidins, human (FALL-39), sheep (SMAP-29) and rabbit (CAP18) were tested against *Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Streptococcus sanguis, Candida albicans, Candida tropicalis and Candida krusei.* Cells were grown to early log phase, harvested and adjusted to a final concentration of $2 X 10^5$ CFU/ml. Peptides were used in concentrations ranging from 0.1ug/ml to 100ug/ml in a 10mM sodium phosphate buffer pH 7.4 with 100mM NaCl and TSB. Incubations were carried out for 0 to 3 hours, after which aliquots were removed at five time points, diluted and plated on respective agar plates. CFU/ml were assessed after appropriate 72-hour incubation. Our results showed differential antimicrobial activity of the three peptides against the panel of bacteria and yeast tested in this study (100% to 0% kill). SMAP-29 was consistently the most bactericidal at concentrations ranging from 10-100μg/ml ($p<0.05$). In conclusion, killing of a limited number of oral organisms was shown for three different mammalian cathelicidins. Ongoing studies in this area will assist in determining future potential for these antimicrobial peptides in prevention and treatment of oral infections. This study was supported by NIH/NIDCR P30 DE10126-09.