AADR March 2012 Abstracts (Summer 2011 Students)

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Does a woman's periodontium respond to exercise like a man's?
Thursday, March 22, 2012: 3:30 p.m. - 4:45 p.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

N. ALMUDALLAL, W. LIU, C. FRACCARO, and L. BAHL-PALOMO, Periodontics, Case Western Reserve University, Cleveland, OH

Overall, lower prevalence of periodontitis is noted in patients with an active lifestyle which includes exercise. Regular exercise improves risk variables associated with periodontitis such as inflammatory cytokines and is shown to reduce CRP in postmenopausal women. Little is known about the response to non-surgical therapy in postmenopausal women (where estrogen does not play a role) who exercise, versus age matched men who exercise.

Objective: Do post-menopausal women who exercise respond to non-surgical periodontal therapy differently than age-matched men who exercise?

Method: This study was conducted at Case School of Dental Medicine, with IRB approval in accordance with HIPPA regulations. 59 Postmenopausal women and 42 age-matched men who met the American Heart Association exercise qualification were included in the study. Periodontal parameters including bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) were recorded before and after non-surgical periodontal therapy.

Result: All participants receiving periodontal therapy showed significant differences in each periodontal parameter following treatment ($p<.05$). However, there was no significant difference in the change in any of the parameters after treatment between women and men ($p<.05$).

Conclusion: Although non-surgical periodontal therapy is benefitial for both postmenopausal women who exercise and men who exercise, there is no significant difference in the response to the non-surgical periodontal therapy between women and men. It appears that in the absence of estrogen, women and men who have healthy lifestyles including exercise respond to non-surgical periodontal therapy.

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Patient Recall of Oral Cancer Screening Examinations
Thursday, March 22, 2012: 2 p.m. – 3:15 p.m.

Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

R. APPEL, Case Western Reserve University, Cleveland, OH, C. DEMKO, Community Dentistry, Case Western Reserve University, Cleveland, OH, and J. IANNADREA D.D.S, Oral Diagnosis and Comprehensive Care, Case Western Reserve University, Cleveland, OH

Objectives: To examine patient recall of oral cancer screening examinations (OCS) compared to chart review during an admitting visit to a dental school clinic.

Methods: Seventy-four adult patients were interviewed by one investigator who administered 12 questions and recorded responses on a standardized form. Patients reported recall of OCS, radiographs (RAD), blood pressure(BP), and tobacco use(TOB); responses were compared to charted data for percent agreement. Because the proportion of negative agreement was zero for most procedures, a prevalence adjusted bias adjusted kappa (PABAK) was calculated. Open-ended questions were qualitatively summarized.

Results: On average, patients were 51 years old (±17, range 19-85) and were interviewed 4.2 days (±2, range 1-8) after the admitting visit; 50% of patients were male. Percent agreement was highest for recall of BP (98.6%), RAD (98.6%), and TOB (92%), but lower for recall of OCS(84%). The adjusted kappas (BP, .97; RAD, .97; TOB, .84; OCS, .68) reflected the high negative agreement for the procedures as well the possible failure to chart OCS which patients reported (n=8). Patient recall was not associated with age, sex or time since admit visit. 49% of patients reported the provider using the phrase 'oral cancer' while 23% did not remember; only 32% reported being informed of the results of the OCS, e.g. 'I don't see anything out of the ordinary', while 16% did not remember. Among 19 patients who were smokers, only 6 recalled information about risk factors for oral cancer.

Conclusions: Compared to other procedures during the admit visit, the concordance between patient recall and charting for OCS is lower. OCS performance and findings may not be as routinely charted as needed. Missed opportunities to discuss oral cancer risks among dental patients, especially among smokers, were identified. Better communication to inform patients of the OCS and findings is warranted.

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Using WHIM syndrome-like cells to study hBD3 induced CXCR4 internalization
Saturday, March 24, 2012: 9:45 a.m. - 11 a.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

P. BERMUDEZ, Z. FENG, X. JIA, and A. WEINBERG, Biological Sciences, Case Western Reserve University, School of Dental Medicine, Cleveland, OH

WHIM syndrome is a rare immunodeficiency disorder, characterized in part by disproportionate susceptibility of epithelial cells to human papillomavirus (HPV) induced warts. This susceptibility appears to be associated with a high degree of cellular activation caused by the inability of stromal derived factor 1 (SDF-1), the natural ligand of CXCR4, to promote internalization of the receptor. This, in turn, is due to defined mutations in the C-terminus of CXCR4. We previously showed that human beta defensin 3 (hBD3) can internalize CXCR4 without activating the cell (Feng et al, 2006).

Objective: To determine the effect of hBD-3 on the internalization of CXCR4 truncation mutant, mimicking WHIM syndrome cells.

Methods: CXCR4 was cloned into the pEGFP-N1 mammalian expression vector. CXCR4 mutant was then generated by site directed mutagenesis and verified by DNA sequencing. The CXCR4 mutant was subcloned into the pmCherry-N1 vector. pEGFP-N1-CXCR4 (green) and pmCherry-N1-CXCR4 mutant (red) were co-transfected into Hela cells. After 24 hours, the cells were put into serum free medium containing hBD-3 (20μg/ml), and images were captured. SDF-1 (30ng/ml) and PMA (100ng/ml) were used as positive controls.

Results: Imaging data indicated that while the GFP tagged CXCR4 was internalized by hBD-3, the peptide was unable to induce internalization of the CXCR4 truncation mutant (pmCherry tagged).

Conclusion: Results indicate that hBD3 dependent CXCR4 internalization requires an intact C-terminal tail; i.e., similar to SDF-1 dependent internalization. WHIM-like cells could be used to further study HPV related susceptibility and infection. Supported by NIH/NIDCR P01DE019759 (AW).

This abstract is based on research that was funded entirely or partially by an outside source: NIH/NIDCR P01DE019759

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Receiving Dental Care: A Qualitative Assessment by HIV+ Adults

Thursday, March 22, 2012: 3:30 p.m. - 4:45 p.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

N. APOLLON CHIROUZE, D. WILLENBERG, M. DAVIS, and L.T. VERNON, Case Western Reserve University, Cleveland, OH

Objective: Despite the increased need for dental care and available dental resources, HIV-infected adults have suboptimal rates of dental care use. This qualitative study aimed to discover internal and external barriers that prevent oral health care seeking behaviors among this population by exploring subject's experiences and expectations concerning dental care.

Method: A convenience sample of HIV-infected adult subjects was recruited from three outpatient HIV-medical clinics in Cleveland, Ohio. We performed one-on-one semi-structured face-to face audio-recorded interviews consisting of 9 open-ended questions about medical/dental care experiences. Of these, 2 questions assessed the least comfortable (past) experience and most comfortable imagined (future) experience in a dental care setting. Themes and sub-themes from respondents were identified by one dental student (NAC) using grounded theory. Data saturation was reached at 42 subjects.

Result: Assessing the least comfortable (actual) and most comfortable (ideal) dental experience of 46 subjects, we identified four major themes: 1) communication between dentist and patient, 2) professionalism of dentists and dental staff, 3) fear of dentists and dental procedures, and 4) the atmosphere of the dental office. Amongst each major theme were sub-themes which included: 26% of respondents desired a more relaxing atmosphere within the dental office, 24% wanted dentists to communicate with care and compassion, and 24% wanted prompt service. Additionally, 22% wanted staff to be more pleasant, 17% wanted dentists to explain procedures/expectations, and 15% of subjects felt the dentist did not listen to them. Finally, 15% of subjects had dental fears.

Conclusion: Our results indicate that more effective communication by dentists is desired by HIV-infected adults. More favorable attitudes by patients towards dentists, the staff and the dental setting may translate into improved dental care compliance and improved oral health outcomes in this population.

This abstract is based on research that was funded entirely or partially by an outside source: NIDCR K23 DE15746 and R21 DE21376, Center for AIDS Research AI136219, Dahms Clinical Research Unit of the CTSA UL1 RR024989 and UM01 RR000080, The Case Western Reserve University/Cleveland Clinic CTSA UL1 RR024989

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Periodontal Benefits of Supplementation and Exercise in Post-Menopausal Women

Thursday, March 22, 2012: 3:30 p.m. - 4:45 p.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

C.P. FRACCARO, W. LIU, N. ALMUDALLAL, and L. BAHL-PALOMO, Periodontics, Case Western Reserve University, Cleveland, OH

Objectives: Post-menopausal women (PMW) are shown to lose skeletal bone linking them to periodontitis and tooth loss. Supplementation with Vitamin D, calcium, and bone-sparing medications is beneficial for prevention and treatment of bone loss. Likewise, exercise has been shown to be beneficial. The incidence of periodontitis is said to be lower in patients with a healthy lifestyle, however, the association between a healthy lifestyle, including exercise and supplementation, and response to non-surgical periodontal therapy has not been investigated. Objective: Are the outcomes of non-surgical periodontal therapy better in PMW who use supplements and exercise versus those who do not?

Methods: 42 PMW with mild to moderate periodontitis participated in this IRB approved study. After consenting to participate, all participants received a periodontal exam and a prophy. Periodontal parameters, such as probing depth (PD) and gingival recession were used to calculate clinical attachment level (CAL) for all participants. Based on a questionnaire, the participants were divided into healthy lifestyle and control groups. For the purposes of this study, a healthy lifestyle is defined as including both exercise according to American Heart Association guidelines and use of a physician prescribed supplement regimen including vitamin D, calcium, hormone treatment and/or bone sparing medications. T-tests were used to determine if there was a significant difference between the groups.

Results: There is a statistically significant difference in CAL ($p=.035$) between the groups. The healthy lifestyle group had greater CAL values compared to control groups.

Conclusions: Results suggest regular exercise and supplementation may improve periodontium in PMW. Future investigation is needed to compare the effect of lifestyle in PMW with osteoporotic PMW versus non-osteoporotic controls.

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Quantification of edentulous jaw bones using cone-beam computed tomography images

Saturday, March 24, 2012: 9:45 a.m. - 11 a.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

R. WANG[1], **Y. HUANG**[2], J. FAN[3], and L. LANG[2], [1]Restorative Dentistry, Case Western Reserve University, Cleveland, OH, [2]School of Dental Medicine, Case Western Reserve University, Cleveland, OH, [3]Departments of Epidemiology and Biostatistics AND Psychiatry, Case Western Reserve University, Cleveland, OH

Objectives: Determination of anatomic dimensions and bone quality is an important part for preoperative planning of dental implant treatment. Currently, the quality of jaw bones is subjectively categorized by

clinicians using various imaging methods. The purpose of this study was to quantitatively evaluate jaw bone quality by CBCT images.

Methods: 20 completely edentulous patients were selected for this study. CBCT scans (Hitachi Inc. Japan) were performed using the following technical parameters: 120 KV, 15 MA, 240 mm field of view, and 0.292 voxel of 512x512 pixels for each slice. CBCT images were reconstructed to a three-dimensional model of each mandible and maxilla. Six sites of each patient were studied: upper incisor (UI), upper premolar (UP), upper molar (UM), lower incisor (LI), lower premolar (LP), lower molar (LM). For each site, cortical bone thickness (CBT) and bone mineral density using Hounsfield unit (HU) were measured at 6 locations; 0, 45, 90, 135, 180, 270 degree of each cross section of alveolar bones. Trabecular bone (TB) width, height, and Hu were also measured. Non-parametric statistical tests were applied using Friedman rank-sum test and Kruskal-wallis one-way ANOVA at $p< 0.05$.

Results:

Summary of averaged measurements as follows:

	UI	UP	UM	LI	LP	LM
CBHU	305+111	96+61	151+105	643+101	588+143	583+130
CBT (mm)	1.25+0.28	1.16+0.28	1.07+0..16	2.01+0.68	1.91+0.72	1.95+0.62
TBHU	5.48+1.82	3.34+1.42	3.82+0.86	7.90+2.88	6.72+2.75	7.14+2.35

Conclusions: Statistical analyses conclude there were significant differences across 6 sites regarding CBHU, CBT, and TBHU. The rankings are: LI>LM>LP>UI>UP>UM for CBHU; LM>LP>LI>UI>UP>UM for CBT; and LI>LM>LP>UI>UM>UP for TBHU.

Supported by CWRU Summer Research Fellowship Program.

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NOS2 Induction by Human Beta Defensin 3 (hBD3) in Macrophages

Saturday, March 24, 2012: 9:45 a.m. - 11 a.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

J. JUN, Case Western Reserve University, Cleveland, OH

Objectives: Macrophages are a central player in cancer development and progression, where an M2-like phenotype stimulates chronic inflammatory conditions and perpetuates cancer-promoting processes. hBD3 is an antimicrobial peptide found to play an important role in innate immunity particularly at mucosal surfaces, and has recently been implicated in cancer progression by its ability to chemoattract macrophages to oral carcinoma lesions and to promote an inflammatory cytokine profile in situ. NOS2 (iNOS) is an evolutionarily ancient enzyme that synthesizes nitric oxide, is induced by pro-inflammatory mediators (cytokines, hypoxia, etc.), and is involved in a plethora of physiological processes from vasodilation to direct anti-bacterial effects; aberrant NOS2 expression has been implicated in the pathogenesis of a multitude of inflammatory conditions including asthma, arthritis, colitis, and most recently, cancer. Mounting evidence demonstrates a pro-tumorigenic role of NOS2 in multiple cancers, and this study explores the role of hBD3 in promoting a pro-inflammatory tumor microenvironment through induction of NOS2.

Methods: RT-PCR, immunocytochemistry, western blot

Results: RNA and protein data show dose-response induction of NOS2 in human THP-1 macrophages by hBD3 treatment (2-10ug). Immunocytochemistry of macrophages treated with hBD3 in combination with intracellular signaling pathway inhibitors suggests that hBD3-mediated induction of NOS2 occurs via the NF-kB (2.5uM inhibitor), but not the JAK/STAT pathway.

Conclusion: That hBD3 induces NOS2 expression from human macrophages in vitro suggests an important mechanism by which hBD3 may promote cancer progression. hBD3 has so far been implicated in vivo in oral cancer pathogenesis, but recent data demonstrating hBD3 induction in non-oral cancer lesions suggests that hBD3 may play a role in cancer pathogenesis beyond the oral cavity – this project suggests that hBD3 may do so by establishing a cancer-promoting environment through NOS2 induction.

This abstract is based on research that was funded entirely or partially by an outside source: NIH/NIDCR PO1DE019759 (PI: Aaron Weinberg)

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Ontogeny of Salivary Human Beta-Defensin-1 and Lactoferrin
Saturday, March 24, 2012: 9:45 a.m. - 11 a.m.
Location: East Hall (Tampa Convention Center)
Presentation Type: Poster Session

A. KENNEDY, Case Western Reserve University, Cleveland Heights, OH, S.K. GHOSH, Biological Science, Dental School, Case Western Reserve University, Cleveland, OH, C. DEMKO, Case Reserve University, Cleveland, OH, and A. WEINBERG, Dept of Biosciences, Case Western Reserve University, Cleveland, OH

Antimicrobial peptides (AMPs), an important component of innate immunity in the oral cavity, provide a first line of host defense against bacterial, viral and fungal challenges. These peptides may play a vital role in saliva, where the oral microbiota present constant and varied challenges to the oral mucosa.

Objective: To explore the association of salivary AMPs, hBD-1 and lactoferrin, with age in a cross-sectional study. Methods: A 4 ml whole, unstimulated saliva sample was obtained from each of 183 healthy subjects (age 8 to 78). Periodontal measures of the 6 Ramjford teeth were measured on adult subjects. Decayed/Missing/Filled Teeth (DMFT) and gingival indices were obtained on all subjects. Levels of hBD-1 were measured by Sandwhich ELISA following the method of Ghosh et al (2007) using antibody pairs from Peprotech (NJ, USA). Recombinant hBD-1 peptide (Peprotech, NJ) was used as standard. Levels of lactoferrin were measured using an ELISA kit from Hycult Biotech (Plymouth Meeting, PA) following the vendor's instructions. Concentrations of the AMPs were normalized with total salivary protein. Spearman rank-order correlation coefficient was used to examine AMPs and between AMPs with age.

Results: Median levels of hBD-1 and lactoferrin (ng/mg protein) standardized by total protein were 195.0 (32.0 – 965.6) and 2619.4 (88.8 – 14,641.4), respectively. We found no direct or inverse coreelation between hBD-1 and lactoferrin with age, and only modestly for hBD-1 with periodontal measure.

Conclusions: Unlike other AMPs, such as SLPI ,calprotectin, hBD-2 and LL37 [Shugars et al, Gerontology, 2001 47(5):246-53; VanYperen et al, Abstract# 860 and Demko et al, Abstract# 863, IADR 2011, San Diego, CA] that were found to correlate with age, hBD-1 and lactoferrin did not.

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This abstract is based on research that was funded entirely or partially by an outside source: NIH/NIDCR

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Beta-defensin 3 and Cathelicidin-mediated Chemokine Induction in Monocytes and Macrophages

Friday, March 23, 2012: 10:45 a.m. - 12:15 p.m.
Location: Room 18 (Tampa Convention Center)
Presentation Type: Oral Session

V. PETROV, and S. SIEG, Case Western Reserve University, Cleveland, OH

Background: Chemokines attract various cell types to tissues and are important for modulating inflammation and immune responses. Antimicrobial peptides are produced at epithelial barriers. These molecules have chemotactic properties and can activate antigen-presenting cells such as monocytes, thereby, contributing to both innate and adaptive immune responses.

Objectives: To investigate the ability of antimicrobial peptides-i.e., human beta defensin-3 (hBD-3) and cathelicidin (LL-37), to modulate co-stimulatory molecule expression and chemokine production by monocytes and macrophages *in vitro.*

Methods: Peripheral blood was obtained from 6 healthy adult volunteers. Monocytes were purified to achieve >85% purity. Cells were incubated overnight in medium alone, in medium plus hBD-3 (20ug/ml), LL-37 (20ug/ml) or PAM3CSK4 (500ng/ml) as a positive control. Culture supernatants were frozen at -20^{o}C until assessed by infrared array technology for various chemokines, cytokines and angiogenic factors. Cells recovered at the end of the culture period were examined by flow cytometry for expression of co-stimulatory molecules, CD80 and CD86. Monocytes from 4 subjects were cultured for 7 d with M-CSF (100 ng/ml) to induce maturation into macrophages. Cells were stimulated as above and assessed for cell phenotype and chemokine production.

Results: HBD-3 induced surface expression of both CD80 and CD86 on monocytes, whereas LL-37 only induced CD86 expression on monocytes. hBD-3 induced a variety of chemokines in monocytes including monocyte-derived chemokine, monocyte-chemotactic protein-1 and macrophage inflammatory protein-β as well as angiogenesis factors, GROα and vascular-endothelial growth factor (p values <0.05). LL-37 also induced chemokines, but generally at lower levels than hBD-3. Similar results were obtained with monocyte-derived macrophages.

Conclusion: By modulating chemokine production in monocyte/macrophages, hBD-3 and LL-37 can influence the cellular and inflammatory milieu at sites of microbial challenge. These data support a role for antimicrobial peptides in immune regulation and tissue repair.

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