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Predominant Cultivable Subgingival Flora of Renal Transplant Recipients.

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INTRODUCTION

Progression of renal disease from inflammation, fibrosis and atrophy into end-stage renal failure is a multi-factorial process. Alteration in intra-renal haemodynamics and hence increase in proteinuria and subsequent release of vasoactive and inflammatory substances would lead to worsening of the kidney condition (Remuzzi & Bertani 1998). Renal transplantation offers the best opportunity for rehabilitation of individuals suffering from end-stage renal failure (Carpenter & Lazarus & 1998). Renal replacement therapy for patients with end-stage renal failure was introduced in the Hong Kong public hospital system some 24 years ago (Chan 1997). Immunosuppression therapy by various combinations of: glucocorticoids, azathiopirne, cyclosporin, tacrolimus, monoclonal antibody OKT3, or antilymphocyte globulin were often used to reduce graft rejection in imperfectly matched donor-receptors cases. It was shown that immunosuppression agents such as cyclosporin A could improve survival rate of transplant recipients (Sketris et al. 1995). A recent study reported the long-term 10-year allograft survival rate of a cohort of renal recipients in Hong Kong was 53% (Tang et al. 1999). This study implied that with continuously improving medical care and survival of this patient group, increased oral health care demand will be a natural consequence.

The present report is part of a project which focused on the oral health status and the corresponding treatment needs of post-operative, stable renal transplant recipients. The prevalence rate of gingival overgrowth in the local sample was at a high level (53%) (Chu et al. 2000) compatible to reports from other parts of the world (King et al. 1993, Spratt et al. 1999, Thomason et al. 1996). Including pseudo-pockets, up to 80% of the local cohort had probing depth ≥ 3.5 mm (Chu et al. 2000). This study investigated the impact that renal transplant therapy had on periodontal status and the subgingival microflora.

MATERIALS AND METHODS

Subjects:

- 38 renal transplant recipients (16 females, age 21 – 68 years; ≥ 6 months post-transplant) from Nephrology Clinic, Department of Medicine, HKU were recruited for the study.

Clinical/Laboratory investigations:

- Clinical examination by Florida Probe® and paralleling periapical radiography
- Subgingival plaque samples (one/subject)
- Gram-stain smear
- Anaerobic culture on enriched Columbia blood agar
- Selective culture on MacConkey and Sabouraud's dextrose agars
- Identification and quantification using: RapID ANAII, API 20 Strep, API Staph, API 20E, and API 20C AUX kits

RESULTS

Table 1. **Demographic data, post-transplant duration and types of immunosuppressant used.**

Group	Sex		Sample Sites[a]				Age (year) (mean ± SD)	Post-transplant Duration (year) (mean ± SD)	Transplant type			Immunosuppressant used		
	Male	Female	Incisors/ Canines	Premolars	Molars	With gingival Overgrowth (Prevalence, %)	Probing Depth (mm) (mean ± SD)		Cadaveric	Living Not genetic related	Living genetic related	Cyclosporin A	Tacrolimus	Nil
Healthy	2	2	4	0	0	0	2.2-3.2 (2.5±0.5)	30-41 (35.4±4.6)	0.5-6 (2.7±2.4)	3	1	0	3	1	0
Shallow Pocket	7	7	5	2	7	29	3.6-5.4 (4.3±0.8)	31-55 (41.0±6.1)	0.5-12 (5.0±3.8)	10	1	3	12	1	1
Deep Pocket	9	4	4	0	9	23[b]	5.8-10.0 (7.1±1.4)	40-67 (49.9±9.2)[c]	1-13 (5.0±3.6)	11	0	2	12	1	0
Pseudo-Pocket	4	3	2	2	3	100	5.8-7.2 (6.2±0.5)	21-68 (38.3±14.7)	1-7 (3.3±2.5)	5	1	1	6	1	0
Total	22	16	15	4	19	42[d]	2.2–10.0 (5.5 ± 1.9)	21-68 (42.2±11.3)	0.5-13 (4.4±3.4)	29	3	6	33	4	1

[a] One sample site per subject.
[b] Out of 46% of the subgroup subjects showed gingival overgrowth clinically.
[c] Significant different ($P < 0.05$, Fisher's PLSD) of data value between: Deep Pocket vs Healthy, Deep Pocket vs Shallow Pocket and Deep Pocket vs Pseudo-Pocket groups.
[d] Out of 50% of all subjects showed gingival overgrowth clinically.

Table 2. **Differential cell counts from Gram-stained smears[a].**

	Healthy	Shallow Pocket	Deep Pocket	Pseudo-Pocket
Gram-positive microorganisms[b]				
cocci[c]	1.5 – 50.5 (40.0, 33.0)	0 - 27.5 (0, 5.4)	0 - 4.5 (0.5, 0.7)	0 – 35.0 (0, 5.3)
rods	0 – 10.5 (4.0, 4.6)	0 – 24.0 (0, 3.5)	0 – 2.5 (0, 0.4)	0 – 6.5 (0, 0.9)
Total[c]	1.5 – 5.7 (46.0, 37.6)	0 – 51.5 (0, 8.9)	0 – 5.0 (0.5, 1.2)	0 – 41.5 (0, 6.2)
Gram-negative microorganisms				
cocci	0 – 11.5 (8.0, 6.9)	0 – 10.0 (2.3, 3.6)	0.5 – 17.5 (2.5, 6.0)	0 – 10.5 (2.5, 3.6)
rods	25 – 86.5 (43.3, 49.5)	26.0 – 73.5 (56.3, 56.5)	22.0 – 88.5 (68.0, 60.8)	47.5 – 75.0 (70.5 – 62.6)
fusiforms	0 – 7.0 (1.5, 1.5)	0 – 5.0 (1.3, 1.4)	0 – 2.5 (1.5, 1.3)	0 – 3.5 (1.0, 1.3)
curved rods[d]	0 – 1.5 (1.0, 0.9)	0 – 6.0 (3.0, 2.9)	0.5 – 4.5 (1.5, 1.8)	0.5 – 4.5 (2.5, 2.4)
filaments	0 – 0.5 (0, 0.1)	0 – 26.5 (0.5, 2.4)	0 – 2.5 (0.5, 0.7)	0 – 5.0 (1.5, 1.6)
spirochetes[e]	0 – 5.5 (1.3, 2.0)	0 – 66.5 (26.3, 24.1)	0.5 – 63.0 (24.0, 28.2)	0 – 44.0 (16.0, 22.1)
Total[c]	43.0 – 98.5 (54.0, 62.4)	48.5 – 100.0 (99.5, 90.9)	95.0 – 100.0 (99.5, 98.8)	58.5 – 100.0 (100.0, 93.8)
Fungi[f]				
yeast form	0 (0, 0)	0 – 2.0 (0, 0.1)	0 (0, 0)	0 (0, 0)

[a] Data shown are percentage range, median and mean (in parenthesis).
[b] No gram-positive filaments observable.
[c] Significant different ($P < 0.05$, Fisher's PLSD) of data value between: Healthy vs Shallow Pocket, Healthy vs Deep Pocket and Healthy vs Pseudo-Pocket groups.
[d] Significant different ($P < 0.05$, Fisher's PLSD) of data value between: Shallow Pocket vs Healthy, and Shallow Pocket vs Deep Pocket groups.
[e] Significant different ($P < 0.05$, Fisher's PLSD) of data value between: Healthy vs Shallow Pocket, and Healthy vs Deep Pocket groups.
[f] No mycelial form observable.

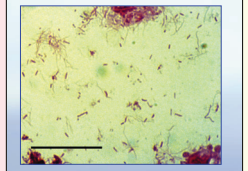

Fig. 1. **Photomicrograph of Gram-stained smears specimen prepared from a sample from the Deep Pocket group. Spirochetes of various sizes were present in abundance. Other morphotypes like gram-negative rods and curved rods were easily observable. Bar = 25μm.**

Table 3. **Prevalence of microbes isolated and the corresponding median and mean percentage isolation from subgingival plaque samples of renal transplant recipients[a].**

	Healthy	Shallow Pocket	Deep Pocket	Pseudo-Pocket
Gram-positive				
Facultative anaerobic cocci				
Gemella haemolysans	25 (0, 1.3)[b]	7.1 (0, 0.5)	7.7 (0, 2.6)	14.3 (0, 1.9)
Gemella morbillorum	25 (0, 6.1)	50.0 (0.5, 6.3)	30.8 (0, 2.6)	28.6 (0, 1.6)
Leuconostoc spp.[c]	0 (0, 0)	28.6 (0,4.3)	0 (0, 0)	0 (0, 0)
Staphylococcus epidermidis	25 (0, 9.2)	0 (0, 0)	7.7 (0, 0.1)	0 (0, 0)
Staphylococcus spp.[d,e]	25 (0, 7.6)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Streptococcus acidominimus	0 (0, 0)	21.4 (0, 0.7)	7.7 (0, 1.4)	0 (0, 0)
Streptococcus constellatus[f,g]	25 (0, 2.6)	7.1 (0, 0.2)	0 (0, 0)	42.9 (0, 7.2)
Streptococcus intermedius	25 (0, 9.5)	0 (0, 0)	7.7 (0, 1.3)	0 (0, 0)
Streptococcus mitis biovar 1	50 (0.9, 3.8)	7.1 (0, 0.7)	30.8 (0, 2.0)	14.3 (0, 0.9)
Streptococcus oralis	25 (0, 1.3)	7.1 (0, 0.3)	0 (0, 0)	0 (0, 0)
Streptococcus pneumoniae	0 (0, 0)	0 (0, 0)	15.4 (0, 4.3)	0 (0, 0)
Streptococcus sanguis	25 (0, 2.6)	7.1 (0, 0.1)	7.7 (0, 0.4)	14.3 (0, 3.3)
Streptococcus salivarius salivarius[g]	0 (0, 0)	0 (0, 0)	15.4 (0, 1.5)	42.9 (0, 5.7)
Anaerobic cocci				
Peptostreptococcus anaerobius	0 (0, 0)	0 (0,0)	15.4 (0, 3.2)	14.3 (0, 3.1)
Peptostreptococcus micros	50 (4.1, 7.5)	7.1 (0, 0.8)	46.2 (0, 9.6)	14.3 (0, 0.5)
Peptostreptococcus prevotii[g]	0 (0, 0)	7.1 (0, 0.2)	15.4 (0, 1.0)	42.9 (0, 3.3)
Facultative anaerobic rods				
Actinomyces georgiae/gerencseriae	0 (0, 0)	28.6 (0, 3.2)	15.4 (0, 1.9)	0 (0, 0)
Actinomyces naeslundii	0 (0, 0)	21.4 (0, 2.2)	0 (0, 0)	28.6 (0, 4.7)
Actinomyces viscosus	25 (0, 3.1)	0 (0, 0)	7.7 (0, 0.1)	0 (0, 0)
Arachnia propionica	25 (0, 0.9)	35.7 (0, 5.9)	15.4 (0, 2.4)	14.3 (0, 1.5)
Lactobacillus acidophilus	0 (0, 0)	7.1 (0, 3.9)	15.4 (0, 1.0)	14.3 (0, 0.7)
Lactobacillus jensenii[g]	0 (0, 0)	14.3 (0, 2.2)	23.1 (0, 1.3)	14.3 (0, 7.0)
Rothia dentocariosa[f]	0 (0, 0)	0 (0, 0)	0 (0, 0)	28.6 (0, 5.9)
Anaerobic rods				
Actinomyces israelii	0 (0, 0)	21.4 (0, 4.3)	15.4 (0, 3.0)	0 (0, 0)
Mobiluncus spp.[c,d,e]	25 (0, 25.0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Gram-negative				
Facultative anaerobic rods				
Kingella kingae	0 (0, 0)	0 (0, 0)	15.4 (0, 1.1)	0 (0, 0)
Pasteurella pneumotropica/haemolytica[d,e]	25 (0, 1.0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Anaerobic rods				
Campylobacter gracilis	25 (0, 1.4)	28.6 (0, 3.2)	38.5 (0, 7.4)	28.6 (0, 9.3)
Campylobacter rectus	0 (0, 0)	28.6 (0, 6.1)	15.4 (0, 1.1)	0 (0, 0)
Prevotella buccae	0 (0, 0)	7.1 (0, 1.0)	15.4 (0, 1.8)	0 (0, 0)
Prevotella corporis	0 (0, 0)	42.9 (0, 2.3)	23.1 (0, 0.6)	14.3 (0, 5.3)
Prevotella intermedia	25 (0, 1.5)	14.3 (0, 0.8)	23.1 (0, 1.4)	0 (0, 0)
Prevotella loescheii	0 (0, 0)	0 (0, 0)	15.4 (0, 3.3)	0 (0, 0)
Prevotella oralis	0 (0, 0)	0 (0, 0)	15.4 (0, 1.8)	14.3 (0, 1.0)
Facultative fusiforms[h]				
Capnocytophaga gingivalis	25 (0, 0.5)	21.4 (0, 2.5)	23.1 (0, 5.4)	14.3 (0, 2.0)
Capnocytophaga ochracea	0 (0, 0)	35.7 (0, 3.8)	7.7 (0, 0.8)	0 (0, 0)
Capnocytophaga sputigena[d,e]	25 (0, 3.5)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Capnocytophaga spp.	0 (0, 0)	21.4 (0, 2.8)	0 (0, 0)	0 (0, 0)
Anaerobic fusiforms[h]				
Fusobacterium nucleatum[d,e]	25.0 (0, 1.8)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Non-oral[i]	25.0 (0, 25.0)	57.1 (5.8, 11.7)	61.5 (3.5, 9.9)	42.9 (0, 10.3)
Lost/unidentified spp.	75.0 (7.7, 9.9)	85.7 (29.2, 25.5)	69.2 (4.5, 25.7)	57.1 (7.1, 22.4)

[a] Only species with frequency of isolation > 15% in any one group are included.
[b] Data shown are percentage prevalence; median and mean percentage proportion (in parenthesis).
[c] Microbes that are not normally considered as member of the oral or oropharyngeal flora.
[d] Significantly higher prevalence of isolation in Healthy vs other groups ($P < 0.05$, χ^2 test).
[e] Significant difference quantity of bacterial species (% proportion) between: Healthy vs Shallow Pocket; Healthy vs Deep Pocket groups ($P < 0.05$, Fisher's PLSD).
[f] Significant higher prevalence of isolation in Pseudo-Pocket vs other groups ($P < 0.05$, χ^2 test).
[g] Significant difference quantity of bacterial species (% proportion) between Pseudo-Pocket vs Deep Pocket groups ($P < 0.05$, Fisher's PLSD).
[h] Significant lower prevalence of fusiform species isolated in Pseudo-Pocket vs other groups ($P < 0.05$, χ^2 test).
[i] Data including species with frequency of isolation ≤ 15%.

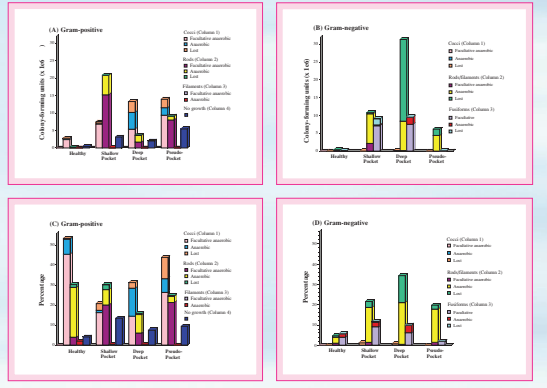

Fig 2. **Quantity (colony-forming units/paper point) and relative mean proportion of predominant cultivable bacterial types from subgingival plaque samples of renal transplant recipients. No growth refers to bacterial isolates that failed to survive after primary culture. Panel A: quantity of gram-positive species and no growth. Panel B: quantity of gram-negative species. Panel C: proportion (% of total) gram-positive species and no growth. Panel D: proportion (% of total) gram-negative species. Note the larger quantity of colony-forming units from Shallow Pocket and Deep Pocket samples. Substantial amount of gram-negative rods were lost upon subculturing in Deep Pocket group. A multiple comparison (Fisher's PLSD) was performed on different bacterial types expressed as proportion of the total bacteria recovered. Significant different ($P < 0.05$, Fisher's PLSD) of data values were observed among several bacterial types (I to X) between the clinical groups: I) total gram-positive species: Healthy vs Shallow pocket and Deep Pocket groups, Deep Pocket vs Pseudo-Pocket groups; II) total obligatory anaerobic gram-positive species: Healthy vs Shallow Pocket groups; III) total facultative anaerobic gram-positive species: Deep Pocket vs Healthy and Pseudo-Pocket groups; IV) total gram-positive cocci: Shallow Pocket vs Healthy, Shallow Pocket vs Pseudo-Pocket groups, V) facultative anaerobic gram-positive cocci: Healthy vs Shallow Pocket, Healthy vs Deep Pocket groups; VI) obligatory anaerobic gram-positive cocci: Shallow Pocket vs Deep Pocket groups; VII) facultative anaerobic gram-positive rods: Shallow Pocket vs Deep Pocket groups; VIII) obligatory anaerobic gram-positive filaments: Healthy vs Deep Pocket, Healthy vs Pseudo-Pocket groups; IX) total gram-negative species: Deep Pocket vs Healthy and Pseudo-Pocket groups; X) total gram-negative rods: Healthy vs Deep Pocket groups.**

CONCLUSIONS

1. **Subgingival microflora of renal transplant recipients with inflammatory periodontal conditions are mainly comprised of gram-negative rods and spirochetes;**
2. **Non-oral microbes were highly prevalent in the subgingival plaque of the renal transplant recipients;**
3. **A substantial amount of the renal transplant subgingival flora are not recoverable by anaerobic culture (spirochetes and various lost species);**
4. **Based on the above, we postulate that the subgingival biofilm of renal transplant recipients would be an unique microbial entity regardless the various periodontal conditions.**

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