Salivary biomarkers in childhood-onset obsessive-compulsive disorder: Preliminary analyses of pro-inflammatory cytokines

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Work in the BCCH Provincial OCD Program is supported by grants from CIHR and MSFHR.
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Saliva as a non-invasive tool for evaluating mucosal immunity and inflammation in obsessive compulsive disorder (OCD)

**Evidence suggesting immune dysregulation in OCD**
- Increased prevalence of immune-related disorders in individuals with OCD compared to the general population
- Increased risk for development of childhood-onset OCD (CO-OCD) after throat infection (longitudinal register studies)
- Elevated peripheral pro-inflammatory cytokines and increased markers of microglial activation in some OCD cohorts; peripheral monocyte function may be modified by treatment with serotonin reuptake inhibitors
- Low plasma immunoglobulin A (IgA) in OCD and Tourette’s Syndrome compared to controls
- Increased levels of the salivary anti-microbial peptide alpha-amylase in adults with OCD compared to controls

**Non-OCD-specific factors affecting immune phenotypes**
- Early life adversity
- Acute and chronic stress
- Physical and psychiatric comorbidities

**Neuroimmune interactions**
- Sympathetic outflow from brain
- Hypothalamic-pituitary-adrenal axis
- Blood brain barrier permeability
- Enteric nervous system; gut microbiota
- Microglial activation and metabolism
- Myeloid cell differentiation/trafficking

**Systemic inflammation**
- Oral health
- Hygiene behaviours
- Dental appliances (e.g. braces, retainers)
- Physical comorbidities including infection
- Diet and activity levels

**Oral inflammation**
- Pelvic floor overactivity
- Altered spinal reflexes
- Autonomic imbalance
- Nervous system immaturity
- Behavioural factors

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Objectives of pilot study

1. To compare levels of immune-related components in saliva from participants with OCD and healthy controls (with a focus here on pro-inflammatory cytokines)

2. To evaluate the relationship between salivary pro-inflammatory cytokines and OCD phenotype
**Methods**

### Study Design
Cross-sectional; participants recruited from BCCH Provincial OCD Program (OCD group) and outreach events (healthy controls)

### Inclusion criteria
- 7-24 years old; clinician-diagnosed OCD or control with no self-reported psychiatric disorder

### Exclusion criteria
- Recent infection, immune-related disorder, immune-modulating medications, recent dentist visit

#### MEASURES

**Medical and demographic information**
- Medical questionnaire, OCD history

**Oral health questionnaire**
- Recent oral intake and oral hygiene measures

**Structured clinical interview**
- Diagnosis of OCD and comorbidities (DSM-IV)

**OCD severity**
- Children’s Yale-Brown Obsessive Compulsive Scale (CY-BOCS)

**Multiplex immunoassay \((n=40)\)**
- CRP, IL-1b, TNF-a, IL-6, IL-8

**Future analyses (full sample)**
- IgA, lysozyme, α-amylase, proteomics

**Morning passive drool collection**
- Determination of flow rate
- Stored at -80°C until analysis

#### Statistics
Differences between groups were compared using Mann-Whitney U-tests (continuous variables) or Pearson’s chi-squared test (categorical variables). Associations were evaluated with Spearman correlations. Multiple linear regression was used to evaluate potential predictors of cytokine levels.
Table 1. Summary of participant oral health characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control n=20</th>
<th>OCD n=20</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current age, mean ± SD (range)</td>
<td>15.6 ± 0.8 (14-17)</td>
<td>15.1 ± 2.1 (10-18)</td>
<td>0.300</td>
</tr>
<tr>
<td>CY-BOCS score, mean ± SD (range)</td>
<td>NA</td>
<td>25.4 ± 5.3 (15-36)</td>
<td>NA</td>
</tr>
<tr>
<td>Gender, % female of total</td>
<td>60</td>
<td>40</td>
<td>0.343</td>
</tr>
<tr>
<td><strong>Oral health</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any flossing, % of total</td>
<td>90</td>
<td>41</td>
<td>0.005*</td>
</tr>
<tr>
<td>Any cavities, % of total</td>
<td>50</td>
<td>66</td>
<td>0.330</td>
</tr>
<tr>
<td>Self-reported good oral health, % of total</td>
<td>90</td>
<td>88</td>
<td>0.863</td>
</tr>
<tr>
<td>Bleeding when brushing, % of total</td>
<td>7</td>
<td>13</td>
<td>0.452</td>
</tr>
<tr>
<td>Braces or retainer, % of total</td>
<td>43</td>
<td>38</td>
<td>0.549</td>
</tr>
</tbody>
</table>

Fewer participants with OCD reported flossing compared to controls, which may relate to avoidance behaviours associated with OCD symptoms or more generally to functional impairment or disruption of routines.
Table 2. Bivariate correlations among pro-inflammatory markers

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CRP</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$p$</td>
<td>$r_s$</td>
<td>$p$</td>
<td>$r_s$</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.496</td>
<td>0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.249</td>
<td>0.121</td>
<td>0.748</td>
<td>&lt;0.0005*</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.245</td>
<td>0.127</td>
<td>0.712</td>
<td>&lt;0.0005*</td>
<td>0.691</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.345</td>
<td>0.029*</td>
<td>0.706</td>
<td>&lt;0.0005*</td>
<td>0.695</td>
</tr>
<tr>
<td>Age</td>
<td>-0.282</td>
<td>0.078</td>
<td>-0.232</td>
<td>0.150</td>
<td>-0.128</td>
</tr>
</tbody>
</table>

- There were significant bivariate correlations among all cytokines.
- Cytokine levels tended to be higher in younger versus older participants.
- All analytes except CRP were higher in females compared to males, most notably IL-1β (336 ± 252 versus 198 ± 183 pg/ml, $p=0.027$) and IL-6 (12.9 ± 11.9 versus 6.5 ± 6.5 pg/ml, $p=0.051$).
Figure 1. Salivary CRP and cytokine concentrations

The only overall difference between OCD and control groups (without adjusting for other variables) was lower total protein (associated with less flossing) in the OCD group.
Table 3. Linear regression of participant factors associated with salivary pro-inflammatory cytokine levels

Linear regression models including age, gender, oral health measures, and presence of severe OCD explained a large proportion of the variance in IL-6 (shown below) as well as IL-1β (46%, \( p=0.059 \)) and TNF-α (37%, \( p=0.015 \)). Use of braces or a retainer and presence of severe OCD were common significant predictors for levels of all three salivary cytokines.

<table>
<thead>
<tr>
<th>Predictor for IL-6</th>
<th>Standardized β</th>
<th>( p )</th>
<th>Overall model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R-square</td>
</tr>
<tr>
<td>Gender</td>
<td>0.25</td>
<td>0.108</td>
<td>0.627</td>
</tr>
<tr>
<td>Age</td>
<td>-0.484</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td>Severe OCD</td>
<td>0.516</td>
<td>0.002*</td>
<td></td>
</tr>
<tr>
<td>Cavities</td>
<td>0.363</td>
<td>0.016*</td>
<td></td>
</tr>
<tr>
<td>Flossing</td>
<td>0.144</td>
<td>0.315</td>
<td></td>
</tr>
<tr>
<td>Braces/retainer</td>
<td>0.348</td>
<td>0.034*</td>
<td></td>
</tr>
<tr>
<td>Bleeding with brushing</td>
<td>-0.187</td>
<td>0.209</td>
<td></td>
</tr>
<tr>
<td>Good oral health</td>
<td>-0.258</td>
<td>0.081</td>
<td></td>
</tr>
</tbody>
</table>

63% of the total variance in IL-6 was explained by a linear regression model in which younger age, presence of severe OCD, cavities, and use of braces or a retainer were significant individual predictors.
Summary

- Saliva can be easily collected from children and youth with OCD; measurement of pro-inflammatory cytokines in this readily-accessible fluid is feasible.
- Demographic factors including age and gender were associated with salivary pro-inflammatory cytokine levels.
- Participants with OCD were less likely to floss than participants in the control group. This finding points to the importance of characterizing oral hygiene when measuring salivary inflammation and understanding the impact of psychiatric symptoms on oral health.
- The presence of severe OCD and use of braces or a retainer were common factors associated with salivary IL-1β, IL-6, and TNF-α in linear regression models adjusted for other potential confounding variables.
Conclusions and Future Directions

- Saliva may provide a minimally-invasive tool for assessing mucosal immunity and neuroendocrine-immune interactions in psychiatric disorders. Because its composition reflects both systemic and local mucosal factors, evaluation of oral health in any assessment of potential salivary biomarkers is essential.

- Preliminary data suggest that the presence of severe OCD in addition to factors related to oral health affect salivary cytokine levels. Recruitment and further analyses are ongoing.

- Disease- or stress-associated salivary changes may ultimately aid in identifying subgroups for prognostic or treatment purposes.