

# Turbidity of mouthrinsed water as a screening index for oral malodor

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**Objectives.** The objectives of this research were to examine the relationship between turbidity of mouthrinsed water and oral malodor, and to evaluate whether the turbidity could be used to screen oral malodor.

**Study design.** The subjects were 165 oral malodor patients. Gas chromatography and organoleptic test (OT) were used for oral malodor measurement. Oral examination along with collection of saliva and quantification of bacteria was conducted. Turbidity of mouthrinsed water was measured with turbidimeter. Logistic regression with oral malodor status by OT as the dependent variable and receiver operating characteristic (ROC) analysis were performed.

**Results.** Turbidity had a significant association with oral malodor status. In addition, ROC analysis showed that the turbidity had an ability to screen for presence or absence of oral malodor.

**Conclusion.** Turbidity could reflect or represent other influential variables of oral malodor and may be useful as a screening method for oral malodor. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:203-209)

Oral malodor, also known as halitosis, is a common complaint among the Japanese population.<sup>1</sup> Oral malodor can arise from a variety of sources including the sinuses, gastrointestinal tract, lungs, and most frequently, the oral cavity. Production of oral malodorous substances is commonly associated with by-products of bacterial metabolic degradation, which occurs on oral soft tissue surfaces, in periodontal pockets and on dorsal tongue surface. These products result from microbial fermentation of proteins, peptides, and mucin found in saliva, blood, gingival crevicular fluid, lysed neutrophils, desquamated epithelial cells, and residual food retained in the oral cavity.<sup>2</sup> The most conspicuous malodorous substances are volatile sulfur compounds (VSCs), with hydrogen sulfide (H<sub>2</sub>S), methyl mercaptan (CH<sub>3</sub>SH), and dimethyl sulfide ((CH<sub>3</sub>)<sub>2</sub>S) accounting for approximately 90% of VSCs.<sup>3</sup>

Many oral bacteria, especially gram-negative anaerobic species, produce a diverse array of malodorous compounds such as short-chain organic acids including valeric acid, butyric acid, putrescine, and skatole, besides VSCs.<sup>2</sup> Species that produce such malodorous

compounds include *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *Porphyromonas endodontalis*, and *Eubacterium species*.<sup>4,5</sup>

Turbidity is defined as an expression of optical property that causes light to be scattered and absorbed, rather than transmitted in straight lines, through the sample. The turbidity expresses the murkiness or dirt level of the sample. The turbidity of mouthrinsed water, which is measured visually or using a turbidimeter, has been reported to be a promising method for screening oral health status.<sup>6</sup> In a previous study, Hakuta et al. estimated oral hygiene status of elderly study subjects by visually evaluating the dirt levels of mouthrinsed water.<sup>7</sup> Our former study suggested that turbidity reflected oral health conditions such as salivary flow and bacterial level.<sup>8</sup>

Therefore, turbidity of mouthrinsed water can be an index to show the hygiene level of the oral cavity, which is mainly derived from bacteria accumulation and food debris on the teeth, tongue, oral mucosa, and in saliva. Because these elements are causes of oral malodor, measurement of turbidity could be useful to screen oral malodor. There are no studies investigating the association between turbidity and oral malodor. The purposes of this research were to examine the

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## Statement of Clinical Relevance

The turbidity of mouthrinsed water appears to be a simple, rapid, and objective screening measure for the presence or absence of oral malodor.

relationship between turbidity of mouthrinsed water and oral malodor, and to evaluate whether the turbidity could be used to screen oral malodor.

## MATERIALS AND METHODS

### Subjects

The subjects were oral malodor patients, who visited the Fresh Breath Clinic, Dental Hospital, Tokyo Medical and Dental University from April, 2009 to January, 2010. After excluding subjects who were edentulous and had missing data on study variables, a total of 165 patients (47 males and 118 females; mean age = 49.3, standard deviation (SD) = 14.1), who agreed to join the study and signed the informed consent form, were used for the analysis. The study protocol was approved by the Ethics Committee for Human Research, Tokyo Medical and Dental University (Approval No. 132).

### Oral malodor measurement

Two types of methods, gas chromatography (GC) and organoleptic test (OT), were used for oral malodor measurement and clinical diagnosis of oral malodor. To reproduce genuine oral malodor, patients were advised not to have food or drink and to refrain from their usual oral hygiene practice on the morning of the appointment. They were also instructed to stop eating strong smelling foods for at least 48 h, using strong scented perfumes for 24 h, and smoking or drinking alcohol for 12 h before the day of malodor assessment to exclude confounding smells. Only patients who adhered to the above protocol underwent the assessment. Oral malodor measurements were conducted between 9 and 11 o'clock in the morning because morning breath odor has been used as a model to investigate offensive mouth breath.<sup>9</sup> Patients were instructed to close their mouth for 3 min prior to each malodor measurement and breathe only through their nose during that time.

### Gas chromatography

A GC-8A (Shimadzu, Kyoto, Japan) equipped with a flame photometric detector was used for the GC analysis. It has an auto-injection system with a 10 mL Teflon (Du Pont, Tokyo, Japan) sample loop and a column packed with 25% 1,2,3-tris (2-cyanoethoxy) propane on an 80/100 mesh Shimalite AW-DMCS-ST support system at 60 °C. The Teflon tube was directly inserted into the oral cavity of a patient through the lips and teeth for the malodor measurement, and 20 mL of mouth air was aspirated with a syringe connected to the outlet of the auto-injection. Following the aspiration, a 10 mL sample of air was transferred to the column and chromatographed by a sulfur chemiluminescence

detector that specifically responded to sulfur. The concentrations of VSC gases (ng/10 mL), H<sub>2</sub>S, CH<sub>3</sub>SH, and (CH<sub>3</sub>)<sub>2</sub>S, were determined by their characteristic retention times, and quantities were calculated by comparing their peak areas with those of dilutions of standard gases of H<sub>2</sub>S, CH<sub>3</sub>SH, and (CH<sub>3</sub>)<sub>2</sub>S prepared with a PD-1B permeator (Gastec Company, Kanagawa, Japan).<sup>10</sup>

### Organoleptic test

The OT was performed by trained dentists. The standardization of examiners was carried out with the T&T Olfactometer (Daiichi Yakuhin Sangyo Co., Tokyo, Japan), an odor solution kit for examining the olfactory sense, to calibrate the consistency of judgment before the measurements.<sup>11</sup> Judges rated the malodor on a 0-5 scale, referring to previous criteria<sup>12,13</sup> where a score of 0 represented absence of odor, 1 barely appreciable odor, 2 slight malodor, 3 moderate malodor, 4 strong malodor, and 5 severe malodor. Patients with scores of 0 and 1 were classed as normal, whereas those with scores of 2 and higher were classed as having malodor. Examiners were blind to the concentrations of VSCs by GC, to avoid possible judgment biases.

### Oral examination

All subjects underwent a standard oral examination following the oral malodor measurement. The numbers of teeth present and decayed teeth were recorded. Periodontal pocket depths were assessed at 6 sites on each tooth with a periodontal probe (PCP UNC 15, Hu-Friedy Mfg. Co., Inc., IL, USA). The deepest pocket was recorded for each tooth. Gingival bleeding was recorded if the bleeding was observed following the pocket depth measurement. Oral hygiene status was evaluated by the plaque index of Silness and Loe criteria.<sup>14</sup> The thickness of tongue coating was evaluated by modified Oho criteria.<sup>15</sup> The scores of tongue coating were as follows: 0 – none, 1 – thin, 2 – moderate, and 3 – thick.

### Collection of saliva

Unstimulated whole saliva was obtained by requesting the subjects to spit saliva into a disposable paper cup for 5 min. Flow rate of saliva (mL/min) was calculated by weighing the volume of saliva.

### Turbidity measurement

The turbidity was measured by absorbance at 660 nm, using the turbidimeter (WA1; Nippon Denshoku, Japan) (Figure 1). Subjects were asked to swish 20 mL distilled water for 10 strokes in their mouths and then spit into the paper cups. The samples were transferred

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