Research Article

Oral Bacteria Species Detection In Breath-Malodor Children

Dr. Sarworini B. Budiardjo¹, Ariadna Djais, PhD²

¹Dept. of Pediatric Dentistry, Fac. of Dentistry, Univ. of Indonesia, Jakarta, Indonesia
²Dept. of Oral Biology, Fac. of Dentistry, Univ. of Indonesia, Jakarta, Indonesia

Aim: To investigate oral bacteria species in breath-malodor children. Design: Cross-sectional, descriptive analytic of microbiological and clinical Volatile Sulfur Compounds (VSC’s). Method: Nine children, mean ages 7 years-9 month, DMF-T less than 2, and mild chronic-gingivitis. Assessment of breath-malodor using group of 2:3 organoleptic-scoring-scale (oss). Oral bacteria samples were swept from unstimulated-saliva, by absorbent paper-tape-steryle from sublingual-salivary-glands, and dental-plaque from mesial-surface of upper-second-primary-molars using individual steryle tip instrument. Identification of bacteria species was accomplished using standard methods. All material sample diluted in 0.9% NaCl to 10 ml, then cultured into 20 Brucella blood agar plates, stored in an anaerob atmosphere gas pack system, at room temperature of 37°C Celsius for 5 days, respectively. Results and discussion: The BPB gram negative anaerob in breath-malodor-children can be identified from saliva and dental plaque, and synchronized between the number of BPB and organoleptic-scoring-scale (oss). The number of BPB Saliva is less than BPB Dental Plaques, BPB level 2 oss less than BPB level 3 oss. BPB Saliva level 2 oss is 282±229 cfu/ml, and level 3 oss is 2316±38.58 cfu/ml; significant different (p<0.05); and BPB Dental Plaque level 2 oss is 585.4±188.5, and level 3 oss is 1538.5±502.68; significant different (p<0.05). BPB Saliva less than BPB Dental Plaque because the energy bond BPB Dental Plaque more powerful. The number of BPB gram negative anaerob in breath-malodor-children accordance to increasingly high organoleptic-scoring-scale due to Volatile Sulfur Compound (VCS’s) production.

Keywords: breath-malodor children, VSC’s, Black-Pigmented-Bacteria (BPB) gram negative-anaerob, saliva, dental-plaque, organoleptic scoring scale (oss).
INTRODUCTION:
Research about breath malodor had started since 60 years ago, the prevalence reach half of human population but in children has not been reported. (1), (2), (3) Halitosis, oral malodor, breath malodor, fetor ex ore or bromopnea is a word and a term in dentistry used to describe the bad breath odor. (1), (4) This issue already declared human disturbing since thousands of years ago by the Jews, Greece and Roman authors. The problem of breath-malodor has plenty to attract the attention not only of the particular health, dental health, also from the public. (4) The breath-malodor is not a disease but showed a systemic symptom as well as psychologically experienced person and a local such as of oral conditions. (5) Etiology halitosis is multifactorial and approved that 80-90% originates from oral cavity. (7) The main role creating bad breath is Volatile Sulfur Compounds (VSC’s). (2,4) Therefore halitosis is defined as exhaled air containing more than 75 parts per billion of odor-producing VSCs. (8) In the oral cavity, VSC’s is produced by anaerobic bacteria which mainly located on the surface of tongue dorsum, gingival sulcus, abscess due to non-vital teeth, rampant caries and systemic diseases that cause xerostomia. Beside that, the systemic conditions and various extra-oral factors lead to production of volatile compounds eliminated from the inhaled air so it can caused bad breath. (4) Some authors state that the majority of humans, have oral malodor experience at once a day especially when waking up sleep or after eating certain types of food, 50% of the population experiencing oral malodor, where 25% of the population experienced severe oral malodor. (9,10).

Volatile Sulfur Compounds (VSC’s) Bad oral hygiene provide a conducive atmosphere for anaerobic bacteria in putrefaction of food debris supporting VSC’s production. Thus can be assumed that bad oral hygiene supports VSC’s production, the main cause of halitosis in children. VSC’s is a sulfur-containing gas, foul smelling and easily vaporized. (4) The main components of the causes of oral malodor is VSC’s namely Hydrogen Sulfide (H2S), Methyl Mercaptan (CH3SH), Dimetilsulfida [(CH3)2S], Propionic acid, Butyric acid, Putrescine, and Cadaverine. This agent is the proteolytic degradation of the remaining food, saliva, blood, and epithelial cells containing substrate sulfur by bacteria anaerobe gram-negative. VSC’s-forming substrate is a sulfur-containing amino acids like cysteine, cystine and methionine can be found in the saliva and gingival fluid. Dorsum of the tongue and gingiva crevice full of microbes gram-negative anaerobic bacterium which produce VSC’s such as Porphyromonas gingivalis, Fusobacterium nucleatum, Prevotella intermedia, and Treponema denticola. (5) Several predisposing factors of halitosis in children were decrease of saliva flow, predominant anaerobic gram-negative bacteria in the oral cavity, high protein diet, and low-carbohydrate diet can cause pH saliva more alkalis and increases the epithelial cell necrosis in the oral cavity. Some medicines can also cause systemic oral malodor, such as Alcohol, Dimethyl Sulfoksida (DMSO), Nitrites, Amyl, Disulphiram Isosorbid Dinitrat and Cytotoxic Drugs. The malodor formed within 72 hours after eating due to saliva excretion from the composition food. According to research that cause halitosis VSC’s in the oral cavity unlike the VSC’s found on extrinsic factors of halitosis because Methyl Mercaptan and Hydrogen Sulfide is not found on the VSC’s extrinsic factors. (3)

Microorganism in malodor Some microorganisms that enhance the malodor, found in periodontitis, gingivitis lesions and the dorsum of the tongue like Veillonella spp. and Prevotella SP. (11,12) The anaerobic gram-negative bacteria is normally found in the dorsal tongue or onethird of posterior tongue. It is a part of tongue that often not swept clean in a mechanical interaction by the teeth and palate durum, the toothbrush and gargle with mouthwash or antibacterial. (13) Research conducted by Paryavi found bacteria that dominates the oral cavity with a high frequency of sugar consumption is a gram-negative cocci bacteria species Veillonella SP. (VL) and P. oralis. VL. (14) Denepitiya and Kleinberg, 1982; Singer and Kleinberg, 1983, salivary sediment is metabolically and microbiologically similar to pooled dental plaque. Sulser, 1939, Berg and Fosdick, 1946; Berg 1947, clarifying that the odor was a result of the bacteria acting on salivary proteins and peptides to yield compounds producing the odor. Drs. Kleinberg and Codipilly, declare that Gram-negative bacteria is significant role in oral malodor formation such as fusobacteria, the black pigmented anaerobes, haemophilus, and veillonella as well as amino acids other than VSC’s are participants in malodor production, gram-positive bacteria are not involved. (8) In 1970 Tonzetch at all, stated that halitosis associated with the Volatile Sulphur Compounds (VSC’s) especially hydrogen sulphide and metilmecaptan and if concentration of
VSC’s ascend on breathing it can be called halitosis.\(^3,15\)

**Examination and diagnosis**

Halitosis diagnosis is confirmed by a search of medical history, clinical examination and measurement breath malodor. Medical history is necessary to determine a chronic systemic disease and the consumption of drugs that cause malodor. Such as diabetic patient breath smelling acetone. Clinical examination could determine the head and neck structure especially intra oral to evaluate periodontal condition, xerostomia, seccesion of saliva flow, caries, disstorse restoration, orthodontic appliance and oral ulceration. VSC’s measurement technique is complicated because the gasses are complex, it is difficult to sample, the samples change with exposure to air or with time, there is no standard to measure against, and the subject population is limited. Halitosis or breath malodor examination consist of 2(two) method, (1) Sensoric or organoleptic method and (2) Instrument method using Halimeter. The sensoric method is the most reliable method for enforced by the examiner's perception of the patient's airway. In this technique, the examiner is sitting face to face to the patient within a distance 10-15 cm to smell the breath of patients from the patient's mouth. Another technique is to wipe the dorsal part of the tongue using a plastic spoon and evaluate existing odor on a spoon. Halitosis measured with 5 level scales or organoleptic scoring scale as follow, 0=absence of odor, 1=questionable odor, 2=slight malodor, 3=moderate malodor, 4=strong malodor, and 5=severe malodor. Patients were asked not to drink, eat or use the oral cavity cleaning products for 2 hours before the examination. In addition, patients were also asked to not eat onions or spicy food for 48 hours prior to the examination procedure. The instrument method is done by using a tools Halimeter portable sulfide monitor. The Halimeter is an instrument with a special gas sensor that detects a range of compounds: hydrogen sulfide, methyl mercaptan, other thiols, and dimethyl sulfide. Patients were asked to hold their breath in advance with a flexible straw is inserted into the mouth in a state of half-open or into the nostrils. Then the patient is asked to exhale his breath. The Halimeter is connected to computer, and with the Haligram software, it can easily monitor on the screen what the gas sensor detects. The Halimeter enables measurements of bad breath to be quantified in parts per billion (ppb). As a standard, 100-200 ppb is quite acceptable and is regarded as normal, the ranges from 50 ppb to 600 ppb, very bad malodor 600 ppb and 50 ppb did not smell bad at all. However, many different variables can affect the result.\(^16\)

**Material and method**

Research conducted in Pediatric Dental Clinics and Oral Biology Laboratorium, Faculty of Dentistry, University of Indonesia. Respondent is 9 children, mean aged 7 years 9 month, DMF-T less than 2, and mild chronic gingivitis. Breath malodor diagnostic measuring by level of organoleptic scoring scale (oss). The examiner facing to the participant in a distance of 10-15 cm, and smells the mouth breath and grouping to level 2 oss or level 3 oss. The unstimulation saliva is collecting from sublingual-salivary-glands by absorbent paper-tape-sterile, and the dental plaque from the mesial surfaces of upper second primary molar using individual sterile tips instrument. The specimen stored in the anaerobic atmosphere using gas pack system, respectively. Laboratorium procedures is follows, the saliva and dental plaque diluted in NaCl 0.9% solution to 10 ml. A total of 0.5 ml of saliva and dental plaque sample, respectively is poured evenly on the surface of 20 Brucella blood agar plates. Culture the Brucella blood agar in the anaerobic atmosphere using gas pack system, under room temperature 37 degrees Celsius for 5 days.

**RESULTS AND DISCUSSION**

Halitosis or breath malodor refers to organoleptic scoring scale (oss), level 2 is slight malodor and level 3 is moderate malodor. From 9 participants, 5 children included in level 2 and 4 children at level 3. The material sample are saliva and dental plaque, and types of bacteria grouped in two, namely Black Pigmented Bacteria (BPB) Saliva and Black Pigmented (BPB) Dental Plaque. Bacterial count by Elisa method in colony forming unite (cfu) per millimeter or cfu/ml.

<table>
<thead>
<tr>
<th>No.</th>
<th>TYPE of BACTERIA</th>
<th>Level 2 oss (cfu/ml)</th>
<th>Level 3 oss (cfu/ml)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BPB Saliva</td>
<td>282±229</td>
<td>2316±3858</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2</td>
<td>BPB Dental Plaque</td>
<td>5854±188.5</td>
<td>15385±502.68</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The Table 1 shows number of BPB Saliva (cfu/ml) and BPB Dental Plaque (cfu/ml) in level 2 oss is less than level 3 oss. The BPB Saliva level 2 oss is 282±229 cfu/ml, and level 3 oss is 2316±3858 cfu/ml; p<0.05, and the BPB Dental Plaque level 2 oss is 5854±188.5 cfu/ml, and level 3 oss is...
CONCLUSION
Breath malodor in children caused by Volatile Sulfur Compound (VCS’s) which produced by gram negative-anaerob Black Pigmented Bacteria (BPB). Number of BPB Saliva and BPB Dental Plaque in level 2 oss less than a number of BPB Saliva and BPB Dental Plaque in level 3 oss.

REFERENCES:
