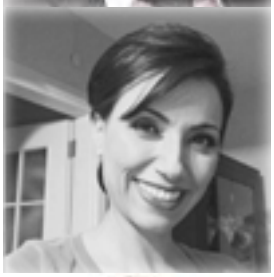
IS THERE ANY RELATION BETWEEN TYPE 1 HALITOSIS AND ORAL CANDIDA COLONISATION

Murat Aydın *DDS, PhD Microbiology
Reşatbey mah Gazipaşa Bulv, Emre apt, n:6, k:2, d:5 Adana-Türkiye, Phone:+0903224536262, E-mail: draydinmurat@gmail.com http://drmurataydin.com

Mustafa Çağrı Derici, MD, otolaryngelogist,
Adana Numune hastahnesi, Kulak Burun Boğaz servisi Adana-Türkiye drcagriderici@gmail.com

Yener Ünal PhD, istatisyen
Cumhuriyet Universitesi Fen fakültesi Sivas, Türkiye



Defne Yeler DDS, PhD Oral and Maxillofacial Radiolog
Cumhuriyet Universitesi diş hekimliği fakültesi Sivas, Türkiye
dyeler@gmail.com

Yusuf islam Demir, öğrenci
Atatürk Üniv. Diş hekimliği fakültesi, Erzurum Türkiye

* Corresponding

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ABSTRACT

Pathologic halitosis has been classified into 5 types: oral, airway, gastroesophageal, blood-borne and subjective, respectively. Type 1 (oral) halitosis mostly takes origin from anaerobic bacterial activities on oral surfaces. Despite some assertion appeared on candidial halitosis can be a hidden reason for oral malodor, *Candida*s' role has not been clearly investigated in halitosis patients. A total of 136 subjects were enrolled and divided into two groups. The study group comprised of 69 patients with halitosis who had over 0.7 ppm H_2S concentration in their oral cavity and the control group comprised of 67 healthly subjects. Self assesment scores for halitosis, *Candida* colony counts in saliva samples, oral NH_3, SO_2, H_2S, H_2 and volatile organic gas concentrations were measured and recorded. H_2S producing capacity of subjects was quantified by applying cysteine challenge test. In order to know what gases are emitted by *Candida* colonies, *Candida* samples were taken from patients' mouth with and without halitosis, and from *C. albicans* isolates were inoculated broth medium. After 3 days incubation at 37^oC , gas concentrations of headspace of flasks were read and compared by a portable multigas detector. The percentage of *Candida* positivity was 44.9% in the study group while it was 46.3% in the control group. There was no statistical significant difference between the groups according to the *Candida* growth (p=0.561). The oral gas concentrations were comparable in both groups ($p<0.05$). Oral H_2S concentration increased 9.65 fold with 20 mM cysteine rinse in patients with halitosis while it was 5.8 fold in controls. Self assesment for halitosis well correlated with clinic signs (p=0.001, r=0.8). Candida cultures apperantly showed increasing of H_2 and VOC concentrations. In this study, no association between *Candida* presence and oral halitosis was detected. No need antifungal therapy or diet to treat halitosis. On the other hand, cysteine challenge can be a useful diagnostic tool. Additonally, multigas detectors to quantify halitosis seems practical halitometers.

Keywords: *Candida, Halitosis, Hydrogen Sulfide, Breath Tests*

Makalenin Türkçe kopyası

INTRODUCTION

Halitosis is endogenously produced malodor emitted by oral, nasal, or alveolar ways. It is etiologically classified as type 0 (physiologic), type 1 (oral), type 2 (airway), type 3 (gastroesophageal), type 4 (blood-borne) and type 5 (subjective) halitosis.[1]

About 80–90% of halitosis cases fall in type 1 takes origin from the oral cavity and are due to oral bacteria producing volatiles by breaking down substrates such as amino acids.[2]

Primary substance associated with oral malodor in mouth air is composed by volatile sulfurs mostly consist of hydrogen sulfide (H2S), organic or nitrogen-based gases [3-5] To detect sulfurs in the mouth cavity is used for diagnostic purposes, especially H2S is thought enough representative for existing halitosis. [6,7]

The relationship between type 1 halitosis and anaerobic bacteria have been well documented. [8-10] However, any potential role of oral fungi has not been extensively investigated. Many websites and anecdotal articles have advocated antifungal therapy or diet to treat halitosis by asserting a potential relation between oral Candida and halitosis. Candida is the major fungus can inherently live in the mouth not only immune-compromised patients but also healthy people.

In the literature, Candida species and sulfurous gases were compared in subjects with or without halitosis. Neither hydrogen sulfide nor dimethyl sulfide was correlated with the amount of Candida except methyl mercaptan.[11] Other gases, such as organic or nitrogen-based volatiles have not been investigated in Candida positive patients with halitosis. However halitosis does not consist of 3 sulfur gases. 700 gases are emitted in the oral air, 3481 gases in the breath have been reported.[1]

Due to existance due of visiual similarity between candidal plaque and tongue coating, many web sites and anectodal papers assert Candida may take a role in halitosis mechanism or halitosis can be a subclinical candidasis of the oral cavity, by suspect from a hidden relation between candidiasis and type 1 halitosis. They offer carbohydrate restricted diets, or even predict anti fungal therapy for halitosis cases should be stopped if there is not such a relationship between Candida and halitosis. For this reason any potential connection between Candida and halitosis gains importance.

In this study, oral Candida isolation frequency in patients with halitosis was compared with healthy individuals to estimate potential role of Candida species on generation of type 1 halitosis. Oral gases (including organic, nitrogen based, sulfurous or hydrogen) when exist Candida and/or halitosis are compared. Additionally, self assesment score of participants and cysteine challenge test were examined as diagnostic tools for examination of halitosis.

MATERIAL METHOD

Patient selection

A total of 136 subjects were enrolled and divided into two groups in this study. The study group comprised of 69 patients (19-60 yo; mean age 34; 51 women) with halitosis and the control group comprised of 67 (22-62 yo; mean age 33; 44 women) healthy subjects.) Prior to the onset of the study, data were registered for each participant individually, including age, sex, medical history, tobacco use, and a list of current medications. The exclusion criteria included subjects with systemic diseases, those who had taken antibiotics or other antimicrobial therapy within 2 weeks prior to the examination, pregnancy, menstruation, current smokers, history of nasal and pharyngeal infection, sinonasal disorders (nasal polyps, chronic rhinosinusitis, allergic rhinitis, septum deviation), respiratory tract infections (asthma, malignancy), as well as those with taste and smell disorders, neurologic and psychiatric disorders (epilepsy, schizophrenia, depression, psychotic disorders, social phobia, obsessive or

delusional disorders), metabolic and endocrine disorders (diabetes mellitus, liver or kidney diseases), gastritis, using any dental prosthesis or alcohol.

All measurements were recorded in the morning between 8:30 and 11:30 (before lunch) and at least 2 h after eating or drinking. All patients received instructions before the examinations. Permission for this study was obtained from the Ethics Committee of Cumhuriyet University (2016-06-22)

Criteria Malodor assessment:

Self-report or social environment's complaint is most valuable diagnostic criteria for halitosis.[12] Patient or his social environment complain from halitosis, initial H2S reading higher than 0.7 ppm are major criteria for Type 1 halitosis.[12] In this study all patients in the study group were having these conditions, while subjects or his social environment who do not complain from halitosis and whose oral initial H2S level ≤ 0.7 ppm were served as control group. On the other hand, Type 5 halitosis cases are detected and excluded by using a previously published questionaire.[13] Eventually, Type 2,3,4,5 halitosis cases were eliminated. Only Type 1 (oral halitosis) patients remained in the halitosis group and Type 0 (no halitosis) subjects were placed in the control group.

Participants were requested to score their own halitosis on a 5 point scale with anchors of 0 no odor whatsoever and 5 extremely foul odor. A self-assessment was requested as recent halitosis "What is degree your oral halitosis ?" The answer for each participant was noted as halitosis level (HL).

Examination protocol:

The protocol was consisted of three steps. First, initial oral gas measurement was performed to each subject of both group. Second, microbiological examination to detect Candida presence in the mouth was made. Third, H_2S producing capacity measurement of each subject, by applying cysteine challenge test (Kleinberg I, 2002). Gas concentrations were measured after cysteine rinse as explained in the below paragraph, then, readings were compared with its initial value of the same subject. H_2S producing capacity is a ratio of maximum H_2S concentration in the mouth cavity after cysteine rinse to initial H_2S concentration of the person. (Aydin M. Özen ME. Kirbiyik U, 2016)

1) Gas measurement

Each participant's baseline VOC, NH_3, SO_2, H_2S, H_2 levels in oral air were used as individualized control data. Artificial malodor composed H2S was produced in the mouth by cysteine challenge test applied as first described by Kleinberg (2002), then, oral gas levels were measured and recorded for these 5 gases using a portable multi-gas detector (IBRID MX6 C526R311, IndSci). While breathing through the nose, the subject places his left index finger between the upper and lower left molars, and gently bites leaving a space between the anterior teeth. This degree of mouth opening is more representative of physiologic mouth position in the social environment. The aspirating tube which was connected to the gas detector had been previously validated for clinical studies. (Aydin M. Özen ME. Kirbiyik U, 2016) Subjects were instructed not to occlude the tube aperture with the tongue.

2) Microbiological examination

After initial halitosis measurement, a 3 ml of sterile serum physiologic was introduced the mouth for 3 minutes. Asked to patient gargle mouth. Then, serum physiological was aspirated from the mouth with sterile injector without needle, vortexed, serially diluted and inoculated to 10 BBL CHROMagar Candida medium (Becton Dickinson, Sparks, MD, USA) and aerobically cultured for 5 days at 37 ^{o}C. This medium was selected since each colony develops by showing a specific colorization that renders it readily identifiable. According to manufacturer, *Candida albicans* (light green), *C. tropicalis* (blue to purple), *C. krusei* (purple fuzzy), *C. membranefaciens, C.*

lisitaniae (Blue-right violet), *C, glabrata, C. parapsilosis* (Cream to white) were identified based on the colors of the cultured colonies. Further identification was applied to suspicious colonies when/if needed.

3) Cysteine challenge test protocol and H2S production capacity of individual

A modified protocol as previously described by Aydin M (2016) was carried out as follow: 20 mMol (2.43 g/L) of aqueous solution L-Cysteine (#1.02839.0100, Merck) was used to generate H2S with cysteine challenge. Five ml cysteine solution was introduced the mouth, held in contact with the dorsal tongue for 30 seconds. Gas detection was then carried out 3 minutes later as explained above. Oral bacteria act on cysteine substrate and releasing H2S in the oral cavity. An artificial halitosis raised in the mouth after cysteine gargle. The ratio of oral H2S concentrations before and after cysteine challenge represented H2S production capacity (HPC) of the patient.

In vitro experiment

In order to compare halitosis specific odor and Candida-related odor, it is necessary to know what gases are naturally emitted by Candida albicans in the culture medium. Additional experiments were achieved by 3 ml of clinically isolated and purified Candida cell suspension (105 CFU) was inoculated in 100 ml of broth media. Three ml of serum physiologic mouth washing samples were obtained from randomly chosen 12 subjects of Candida positive halitosis patients and 12 of Candida positive individuals from the control group. These samples were inoculated to the same kind of growth media in different flasks. (Fig. 1) Flasks were plugged that each plug was contained within a hole that can be tightly closed to serving to insert an aspirating tube into the headspace area of the flask. (Fig. 1) During gas measurement, aspirating tube that was loose-fitted to the holes was inserted into the flask through the hole. After initial gas measurement, flasks were incubated for 3 days at 37 ^{o}C. Gas concentrations of headspaces of flasks were re-measured and recorded. Eventually, this in-vitro culture experiment was achieved 36 times by using different inoculum.

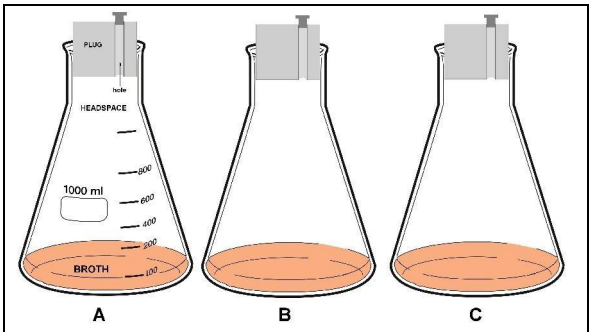

Fig.1 In-vitro experiment setup. Each flask consisted of 100 ml Sabouraud %2 Dextrose Broth (Sigma-Aldrich, S3306, USA) medium that contains vancomycin 7.5 mg/L, kanamycin 100 mg/L. 3 ml of mouth washing solution taken from subjects from Candida positive halitosis patients (A), from Candida positive control individuals (B), Candida albicans suspension (10^5 CFU) (C) were inoculated to the flasks. Gas concentrations were measured from headspaces of the flasks before and after incubation.

Statistical analysis

Effect of Candida growth on Halitosis was analyzed with the X2 test. Distribution or change of gas concentrations before/after the application was analyzed with Wilcoxon Signed Ranks Test. Mann-Whitney U test was used to detect the effect of Candida growth on gas profile in the culture medium.

The sample size for the study was calculated 130. The power of the test was set at 80 % with two-sided 95 % significance level.

RESULTS

Average HL was calculated as 3.26 (n=69, SD=1.27) for halitosis patients and 0.89 (n=67, SD=0.76) for control group individuals. The difference between HL values of the groups were statistically significant (p=0.001).

Of the 69 patients, 31 were Candida positive (positive rate: 46.3%). Of the 31 positive cases, 21 cases were less than 100 colonies (Data not shown). Candida albicans was detected in 75% of Candida positive cases alone or combined with other Candida species, and it was more predominant than other Candida species

The isolated Candiada species were *C. albicans* (90.2%), *C. tropicallis* (5.1%),*C. glabrata + C. parapsilosis* (3.6%),*C. krusei* (0.4%),*C. membranefaciens + C. lisitaniae* (0.04%) and others (0.4%) in both groups. There was no statistically signifiant difference between the groups according to the species of Candida ($p>0.05$).

Sixty two (44.9%) subjects of all (n=136) were found positive for Candida and half of them (31, 50%) were in the study group.

There was no statistical significant difference between the groups according to the Candida growth ($p=0.561$) (Table 1). Candida species isolated from all individuals are summarised in the Table 2. In halitosis group, initial oral gas concentrations did not show significant differentiation when *Candida* present or not present. (Table 3)

Table 1 Distrubiton of *Candida* growth in both group

		Candida		Total	P
		positive	negative		
Halitosis Group	n	31	38	69	0.561
	%	44.9%	55.1%	100.0%	
Control Group	n	31	36	67	
	%	46.3%	55.2%	100.0%	
Total	n	62	74	136	
	%	45.6%	54.4%	100.0%	

Tablo 2 Distribution of *Candida* species

İzole edilen türler	Colony count	
	Candida group(n=69)	Control (n=67)
C. albicans (90.2%)	1942	2125
C. tropicallis (5.1%)	107	126
C. glabrata + C. parapsilosis (3.6%)	99	66
C. krusei (0.4%)	6	13
C. membranefaciens + C. lisitaniae (0.04%)	2	0
Others (0.4%)	0	20
Total	2156	2350

Table 3 Initial gas concentration (ppm) (SD) of Halitosis patients (n=69)

	Candida positive	*Candida* negative	P value
VOC	1.481 (±1.347)	2.316 (±1.584)	0.223
NH3	3.903 (±2.211)	3.132 (±1.545)	0.093
SO2	0.000 (±0)	0.005 (v0.032)	0.370
H2S	1.245 (±1.440)	1.432 (±1.563)	0.611
H2	11.097 (±15.186)	10.158 (±16.621)	0.809

In both groups, cysteine challenge caused higher H_2S, while lower other gases (VOC, NH_3, and H_2) in the mouth cavity ($p=0.001$), however, no significant change was seen with SO_2 concentration (Table 4). With cysteine challenge, initial H_2S concentration increased from 1.348 to 13.019 ppm in patients with halitosis (HPC=9.65), while, from 0.587 to 3.457 ppm in control subjects (HPC = 5.8). Average increasing of H_2S with cysteine challenge was calculated as 11.6710 (n=69; SD= 11.167) and 2.8701 (n=67; SD= 1.270) ppm in halitosis and control

group, respectively (p=0.001), but the other gases were not.
Candida presence in the mouth did not alter initial gas concentrations in halitosis patients.

Table 4. average changes of gas concentrations (ppm) with cysteine challenge

Gases	halitosis group		control group	
	Before	After	Before	After
VOC*	1.941	1.548	1.248	0.845
NH_3*	3.478	2.551	0.851	0.627
SO_2	0.003	0.0	0.0	0.0
H_2S*	1.348	13.01	0.587	3.457
H_2*	10.58	6.029	15.43	8.746
*, statistically significant				

In vitro results
VOC and H2 gases were significantly higher (p<0.016) in all *Candida* cultures (n=36), but other gases were not (Table 5).

After *Candida* incubated to the growth media, organic gas concentration increased 1.98 fold (from 0.45 to 0.892 ppm) (p=0.000), H2 increased 23.3 fold (from 0.75 to 17.5 ppm) (p=0.000). There was not a significant difference between gas profiles of headspaces in culture media of Candida positive /negative people or control culture. (Table 5)

Table 5 In vitro gas changes in the culture media before/after inoculation of samples and *Candida* cels

Gases	*Candida* positive Halitosis patients (n=12)	*Candida* positive Control individuals (n=12)	*Candida* albicans cells (n=12)
VOC	0.283 → 1.017*	0.486 → 1.100*	0.450 → 0.892*
NH_3	0.333 → 0.167	0.000 → 0.000	0.167 → 0.583
SO_2	0.000 → 0.000	0.000 → 0.000	0.000 → 0.000
H_2S	0.217 → 0.100	0.000 → 0.000	0.000 → 0.000
H_2	0.833 → 19.667*	1.286 → 23.857*	0.750 → 17.500*
* Statistically significant			

DISCUSSION

Candida species isolated

Candida is the most frequently isolated commensal and opportunistic yeast fungus. *Candida* albicans is one of medically important *Candida* species that is a member of the normal oral microbiota[16,17] and is the predominant species isolated from the oral cavity with ratio *C. albicans* (66.70%), *C. glabrata* (11.71%), *C. parapsilopsis* (10.74%), *C. tropicalis* (9.19%) and *C. krusei* (1.15%).[18] Similar to literature findings, in this series, *C. albicans* (90.2%) was found the predominant species.

In this study tongue swab was not used due to it is difficult to standardize cell count can be transferred by swab.

Candida-halitosis relationship

Only 44.9 % of *Candida* positive samples fall into halitosis group. On the other hand, 46.3 % of control was found Candida positive. Distribution of Candida isolation frequency between both groups was not statistically different. No clear correlation between Candida isolation and halitosis was detected.

In the literature, oral Candida isolation rate of patients with halitosis was found 28% [11] 25.2% (Koga C, 2014).[19] Furthermore, no differences in total sulfur gas levels were found between Candida negative and positive group. Although, methyl mercaptan (MM) concentration was found higher in both the C. albicans positive patients and the patients with some particular Candida species. [19] In the current study, no specific attention was paid to check MM in this case series, but, sensor configuration of the MX6 gas detector covers hundreds of organic, sulfur, or nitrogen-based gases including MM. MM gas, if any, would be detected by PID sensor as a VOC by the MX6.

Cysteine challenge test and HPC:

Cysteine is a simple amino acid, easier to use it as an energy source by oral bacteria present in the mouth cavity than

complex proteins. Cysteine transformation to H2S in the oral cavity is a general phenomenon independent of age, sex or presence of periodontal disease, but dependent on pH, and a dose of cysteine. [6]

For this reason, using cysteine challenge in halitosis studies is a widely used and well accepted way of concocting such a study. [7,12,15,20,21]

Cysteine is used as the substrate for H2S formation, orally H2S sharply increased after cysteine rinse, in both group, while other oral gases decreased, due to oral bacteria began to use cysteine as an energy source instead of carbohydrates or complex proteins those are responsible from producing other gases rather than H2S. Depends on individual's oral ecology or microbiota, the H2S peak level after a cysteine rinse reflects the individual's oral halitosis capacity or represents particular H2S producing the capacity of that individual independently momentarily alternations of oral gas concentration. [12] In this study, HPC of patients with halitosis (9.65) was higher than those of controls (5.88) which were not affected by Candida presence in the mouth ($p<0.05$). This specificity can be used as a diagnostic tool for clinic examination of halitosis in future studies.

HL

Different assessments and halitometric threshold for pathologic halitosis were discussed in the literature.[22] Therefore, precise estimates of diagnosis of halitosis by using exact arithmetical cut-off value are not possible to obtain[23-25] or there are no accepted clinical protocols for the diagnosis of this problem.[1,12]

The most descriptive question of the anamnesis and the first and most convincing tool to diagnose a halitosis is self or other people's assessment that covers all types of halitosis despite some of the patients with halitosis may not be aware of this situation.[26] The fact that the human nose is capable of smelling and defining not only sulfurous but also other odorous gases.

In this study, HL was found a significant indicator of halitosis ($p=0.001$). Self-estimation of halitosis seems a useful tool for halitosis diagnosis. It seems possible to use HL as a simple diagnostic tool for halitosis

In-vitro experiment

Candida uses carbohydrates as energy source by producing alcohol and organic gases from carbohydrates.[27] Approximately 250 VOCs have been identified from fungi where they occur as mixtures of simple or aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols and their derivatives.[28,29] This clearly explains why the all Candida positive culture media emitted VOC and H2 in the in vitro experiment. In vitro experiment led to know what gases are inherently emitted by *Candida* cells. Also, this gave opportunity to compare gas concentrations released from diferent originated *Candida* cells; 1-clinically isolated, 2-halitosis patients and 3-healthly control subjects. Parallel to literatur findings, every *Candida* positive saliva samples taken from either halitosis or control group showed significant higher levels of VOC and H2 (Table 5). This can be interpretted as Candidas may be responsible from some hydrogen or volatile organic content in the mouth but not directly halitosis.

However, by a limitation of this study, it was difficult to specify which particular organic gases were released could not be distinguished from culture media or mouth.

Halitosis consists of not only sulfurous, but also organic gases. If *Candida* had been responsible from oral malodor due to organic gases, Candida positive control subjects would also have high VOC concentrations in their mouth cavities, and/or Candida negative patients in the study group would not complain from halitosis.

Conclusion

1. No clear association was found between oral Candida colonization and oral halitosis.

2- No need antifungal therapy or diet to treat halitosis.

3- Self halitosis assesment is well correlated with clinic findings

4- Multigas detectors like used in this study, are better due to the capacity of detection wider variety of gas groups. While, popular halitometers (such as Halimeter or Oral Chroma) detect only one or few sulfurous compounds on a restricted scale.

5- Cysteine challenge test well reflects individual's halitosis production capacity.

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