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Prevalence estimation of halitosis and its association with oral health-related parameters in an adult population of a city in North Italy.

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Halitosis and risk indicators

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Abstract

Aim: No epidemiological data on halitosis are available from Italy. Thus, the aim of this population-based cross-sectional study was to estimate the prevalence of halitosis in an urban adult population from North Italy and to explore related oral risk indicators.

Materials and Methods: The survey used a two-stage probability sampling method to collect a representative sample of inhabitants, aged between 20 and 75 years, of the city of Turin. Seven hundred and forty-four adults were clinically examined (47% of sampled subjects) for oral malodor and periodontal conditions. Using a standardized questionnaire social, health and halitosis-related parameters were collected. Logistic models with interaction terms between tongue coating scores (TCS) and periodontal status were used to explore halitosis risk indicators.

Results: The prevalence estimate of halitosis according to the organoleptic assessment was 53.51% (95% CI: 48.55-58.50). A statistically significant correlation was found between organoleptic and gas chromatography measurements ($p < 0.0001$). Stronger associations between halitosis and periodontitis were observed in people having higher TCS: adjusted odds ratio considering low and high TCS in individuals with severe periodontitis were 2.95 and 20.77 ($p \leq 0.003$).

Conclusions: Due to the high prevalence of halitosis in the Turin population, its diagnosis and management should be incorporated in comprehensive dental care.

Clinical relevance

Scientific rationale for the study: No epidemiological study on the prevalence and risk indicators of halitosis has been carried out in Italy.

Principal findings: The prevalence of oral malodor among the inhabitants of an urban center in North Italy was high. Periodontal status, amount of tongue coating and inadequate oral hygiene practices were closely related to clinical halitosis. A statistically significant interaction between degree of tongue coating and periodontitis was observed.

Practical implications: Tongue coating level and severity of periodontal involvement would seem to exert a synergistic contribution to oral malodor.

Introduction

Halitosis is the common term used to define an unpleasant odor in expired air, which originates from oral or non-oral sources (Murata et al. 2002). In most cases (80–90%) it results from the release in the oral cavity of volatile sulfur compounds (VSCs) through the degradation of organic substrates by prevalently Gram-negative anaerobic bacteria (Delanghe et al. 1997, Nakano et al. 2002, Scully & Greenman 2012).

Halitosis has negative impact on many aspects of daily life and affects interpersonal social communication. Personal discomfort and social embarrassment are the main reasons people seek treatment by professionals (Quirynen et al. 2009). In spite of the health and social implications, few epidemiological and etiological studies have been conducted to assess the prevalence of halitosis and to identify related factors (Scully & Greenman 2012). The available epidemiological data are based mainly on convenience samples and on self-perception of oral malodor that is limited in accuracy and sensitivity (Rosenberg et al. 1991, Oho et al. 2001, Vandekerckhove et al. 2009).

The worldwide prevalence of halitosis is largely variable. Bad breath affects approximately 2.4-57.9% of the sampled groups when assessed by organoleptic or gas chromatographic measurements and 22-44.6% when considering self-reporting findings (Miyazaki et al. 1995, Söder et al. 2000, Iwanicka-Grzegorek et al. 2005, Knaan et al. 2005, Liu et al. 2006, Nadavnosky et al. 2007, Yokoyama et al. 2010). A recent cross-sectional study from Switzerland estimated that 32% and 11.5% of the population of the city of Bern had halitosis based on self-reported and objective criteria, respectively (Bornstein et al. 2009). A similar study from Sweden reported a prevalence of 2.4% of severe halitosis in the city of Stockholm (Söder et al. 2000).

No epidemiological study on the prevalence of halitosis has been carried out in Italy. Since data on the prevalence of bad breath seem to be highly variable depending on the population studied, additional information on specific risk factors in a given population may be helpful. Thus, the aim of the present study was two-fold: to estimate the prevalence of halitosis in an adult urban population from North Italy and to explore related oral risk indicators.

Material and Methods

Study design and sampling procedures

The study is part of a population-based cross-sectional survey conducted between December 2009 and July 2010 by the Section of Periodontology, Department of Surgical Sciences, C.I.R. Dental School, University of Turin (Italy) to estimate the prevalence of severe periodontitis. It was conducted in accordance with the World Medical Association Declaration of Helsinki and was approved by the Research Ethics Committee of the University of Turin (Italy). Informed written consent was provided by each participant.

The survey used a two-stage probability sampling method to collect a representative sample of the inhabitants, aged between 20 and 75 years, of the city of Turin located in North Italy. The sample size of 800 subjects allowed to obtain an estimate of severe periodontitis prevalence with a 95% confidence interval (95% CI) with a precision of 2.5% hypothesizing a disease prevalence of 15% (Petersen & Ogawa 2005). Further details on sample size calculation and study design were reported in Appendix.

An invitation letter and a questionnaire were sent to all the sampled subjects. The letter explained the study purpose and reported the instruction for the halitosis measurement. Subjects were asked not to eat garlic, onion or spicy food 48 h prior to their appointment and to abstain from smoking, chewing gum, using any oral rinse and freshener and drinking alcohol or coffee at least 12 h before the visit. On the morning of the appointment, they were asked not to use scenting personal products and to refrain from brushing their teeth.

Questionnaire

A structured questionnaire was completed by each subject and reported at the time of the clinical examination. It inquires about socio-demographic and lifestyle factors (education level and smoking habit), medical history, presence and intensity of bad breath, and oral hygiene practices (frequency of tooth-brushing, tongue scraping, interdental devices use, professional oral hygiene sessions).

Organoleptic assessment

Organoleptic testing (OLT) of mouth malodor was performed on standardized way by a trained and experienced clinician who was masked to any other data recorded during the study. All measurements were recorded between 8:30 and 11:30 h in the general practitioners' (GP) medical offices. In order to avoid smell fatigue the second patient was evaluated at least 15 min. after the previous one (Tsai et al. 2008). The subjects were asked to close their lips tightly for 3 min. in upright position and then to exhale gently and briefly from the mouth through a plastic tube. They were at a distance of about 10 cm from the odour judge. The degree of bad breath was determined using the 0-5 Rosenberg point scale where 0 represented absence of odour; 1, questionable odour; 2, slight odour; 3, moderate odour; 4, strong odour; 5, extremely severe odour (Rosenberg & McCulloch 1992). The OLT was used to diagnose clinical oral malodor in this study and subjects were diagnosed as having oral malodor when their OLT score ≥ 2 (Murata et al. 2002).

Reproducibility of OLT and measurement of VSC levels

The intra-examiner reproducibility of OLT was assessed prior to and during this investigation on the mouth odour of patients attending the GPs' medical offices, but not involved in the survey. The Cohen's Kappa values were 0.97 and 0.94, respectively.

Furthermore, the reliability of the odor judge's ratings was assessed against the senior member of the Section of Periodontology who served as "reference evaluator" on the first 250 consecutive study participants. The Cohen's Kappa statistics ranged from 0.86 and 0.92 indicating a strong degree of agreement.

These first 250 consecutive study subjects were also examined for VSC concentration following the organoleptic assessment. The VSC level was quantified with a portable gas chromatograph (OralChroma^{TM} Abilit Corp., Osaka, Japan) which measures the concentration of hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulphide [$(CH_3)_2S$]. A disposable plastic 1-ml syringe was inserted deep into the patient's oral cavity and held between lips for 3 min. Then the plunger was pulled slowly, pushed again and pulled for a second time before removal from the mouth. A dedicated needle was attached and 0.5 ml of mouth air was injected into the measurement device. After 8 min., the process was completed and the concentrations of the three gases were displayed in parts per billion (p.p.b.). The VSC threshold levels according to the manufacturer's instructions were as follows: H_2S >112 p.p.b. or CH_3SH >26 p.p.b. or $(CH_3)_2S$ > 8 p.p.b.

Oral and periodontal examination

All 744 subjects underwent oral and periodontal examination by an experienced clinician who was not involved in the halitosis assessment. Probing depth (PD), recession of the gingival margin and clinical attachment level were measured using a periodontal probe (PCP UNC-15; Hu-Friedy, Chicago, IL, USA) at six sites per tooth, excluding third molars. The percentages of total surfaces demonstrating plaque or bleeding on probing were expressed as full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS). The periodontal status was assessed according to the Centers for Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) case definition for moderate and severe periodontitis in population-based epidemiological survey (Page & Eke 2007). Subjects diagnosed with moderate or severe periodontitis were further stratified by extent and characterized as "localized" (≤30% of sites involved) and "generalized" (>30% of sites involved) according to Armitage (1999).

The tongue-coating score (TCS) was calculated by multiplying the thickness score by the area score (Oho et al. 2001). The area was reported as a score of 0–3 as follows: 0, no coating; 1,

tongue coating covering less than 1⁄3 of tongue dorsum; 2, tongue coating covering 1⁄3 – 2⁄3 of tongue dorsum; 3, tongue coating covering greater than 2⁄3 of tongue dorsum. Thickness was reported as a score of 0–3 (0, no tongue coating; 1, thin tongue coating with papillae visible; 2, moderate tongue coating with papillae invisible; 3, thick tongue coating). The maximum value was 9. The presence of pathology of the oral mucous membranes was also recorded.

Statistical analysis

In order to produce estimates of prevalence of halitosis as assessed by OLT, each age and sex stratum was weighted for the inverse of the probability to be selected using as reference the population in Turin at 01/01/2010 (data from the National Institute of Statistics). The effect of cluster sampling by GPs was considered so that each regression model was adjusted for autocorrelation within GPs.

The distributions of subjects' characteristics were summarized using percentages and frequencies. The chi-square test was performed to examine the relationship of clinical oral malodor and socio-demographic, behaviour and oral health variables.

Agreement between the self-reported bad breath assessment and OLT grading was tested through a Cohen's Kappa test. The Pearson's correlation coefficient was calculated to determine the association between VSC concentrations and OLT scores.

In order to estimate crude and adjusted odds ratio (ORs) of halitosis risk indicators logistic regression models were performed. In the multivariate model the combined effect of CDC/AAP periodontitis diagnosis and TCS (categorized in 0-1, 2-4, ≥ 5) on clinical halitosis was investigated after adjusting for age strata, gender, level of education (categorized in three levels: low or primary and secondary school level; intermediate or high school level; and high or university education or more) and smoking habit (current smoker *versus* non- and past-smoker). As explorative analysis we detected also the association between clinical halitosis and some oral health-related variables such as FMBS% (in quartiles), tooth-brushing

frequency (≥ 2/day *versus* <2/day), interdental cleaning (yes *versus* no), and professional scaling frequency in the last year (≥ 1 session *versus* none) adjusted for socio-demographic variables.

Statistical analysis was conducted using the Statistical Package STATA/SE 13 (Stata Statistical Software: Release 13; StataCorp LP, College Station, TX, USA).

Results

As reported in Fig. 1, among 1600 individuals invited to participate in the study, 802 subjects returned the questionnaire (response rate 50.12%). Because 58 refused the halitosis examination, 744 subjects were included in the analysis (47% of the initially contacted 1600). No difference was detected in socio-demographic and lifestyle characteristics between individuals with and without halitosis examination as reported in the Table A1.

The organoleptic assessment of halitosis revealed that 412 (55.38%) of participants had oral malodor (score 2-5), among them 289 subjects (38.87% of the whole sample) were diagnosed to have a light/moderate malodor (score 2-3) and 123 individuals (16.53% of the whole sample) a strong bad breath (score 4-5).

In the target population the estimated prevalence of halitosis of any grade, of grade 2-3 and of grade 4-5 was 53.51% (95% CI: 48.55-58.50), 36.71% (95% CI: 33.17-40.25) and 16.80% (95% CI: 13.77-19.83), respectively. The observed prevalence of oral malodor increased with age as reported in Table 1. In young subjects (age < 30 years) the halitosis prevalence was 32.50% (95% CI: 22.24-42.76) and it increased to 65.28% (95% CI: 58.57-72.00) in the 50-59-year-old age group and then levelled off.

The agreement between self-reported halitosis and organoleptic assessment was low (Cohen's *K* index = 0.152, 95% CI: 0.096-0.208). Amongst 179 subjects complaining halitosis (24.06%), 123 (68.72%) reported having a light malodor, 40 (22.34%) a moderate and 16 (8.94%) a strong bad breath. As reported in Table 2, the analysis of the correlation between VSC levels and OLT grading in the first 250 study participants revealed a strong correlation

between them ($p < 0.001$). The mean values for the VSC measurements are presented in Table A2.

The socio-demographic, health behavioural characteristics and oral health status of all study subjects according to the presence of clinical halitosis are shown in Tables 3 and 4. There were statistically significant differences in the halitosis distribution with regard to the socio-demographic variables, especially we observed an higher prevalence of halitosis in men (61.29% in males *versus* 51.15% in females), subjects older than 50 years ($p < 0.001$), with lower level of education (65.18% in low educated *versus* 43.54% in high educated) and current smokers (66.29% in smokers *versus* 52.02% in non-smokers). As expected, the prevalence of halitosis increased in subjects with higher percentage of sites displaying microbial plaque or inflammation ($p < 0.001$), severe periodontitis (80.29% in severe *versus* 45.33% in moderate periodontitis subjects) and heavy tongue coating (82.67% in TCS ≥ 5 *versus* 38.33% in TCS 0-1).

With regard to oral hygiene, halitosis was negatively associated with daily use of toothbrush and interdental devices and frequency of scaling sessions.

Oral health characteristics of study subjects by periodontal and halitosis diagnosis are summarized in Table 5. In the severe periodontitis group, 19.71% subjects had no clinically detected halitosis. Among them, 89.09% presented with a localized form of periodontitis, 78.18% had FMBS values <50%, and 70.91% did not have tongue coating. In contrast, 38.84% of individuals with severe periodontitis and clinical halitosis were affected by the generalized form of disease, 61.61% presented FMBS values > 50%, and 72.32% a TCS ≥ 2.

Crude and adjusted ORs for putative risk indicators for clinical halitosis are reported in Table 6. Adjusting for oral health-related variables, the effect of socio-demographic variables on the halitosis probability became null, only education level effect remained. Both univariate and multivariate analysis underlined the association between oral health-related variables and the halitosis probability. In particular, the probability of halitosis increased by increasing the

tongue coating levels. This relationship was significantly different according to the severity of periodontitis (p-value for interaction ≤0.003). The association between the other oral health-related variables (FMBS, tooth brushing frequency, inter-dental cleaning and scaling frequency) and halitosis underlined in univariate analysis was confirmed even adjusting for socio-demographic characteristics (Table A3).

Discussion

In our study, based on a sample of the general population of Turin, the estimated prevalence of clinical oral malodor in the Turin population was 53.51% (95% CI: 48.55-58.50) comprising 36.71% (95% CI: 33.17-40.25) and 16.80% (95% CI: 13.77-19.83) of the individuals suffering from moderate (OLT score 2-3) and strong breath odor (OLT score 4-5). There is limited research on the prevalence of halitosis in population-wide or community-based samples. Based on these epidemiological data the worldwide prevalence of halitosis is largely variable with a range from 10% to 50% (Frexinos et al. 1998, Söder et al. 2000, Al-Ansari et al. 2006, Liu et al. 2006, Nadanovsky et al. 2007, Bornstein et al. 2009). Among few studies conducted in Europe, a Swiss epidemiologic survey (Bornstein et al. 2009) reported a halitosis prevalence of 11.5% based on the organoleptic evaluation of 419 inhabitants of the city of Bern (21% of the initially contacted 2000). This low response rate might have compromised the reliability of the data. In a study by Söder et al. (2000) on a representative sample of the Stockholm population the prevalence of severe halitosis (score 5) was approximately 2.4%.

The current data were considerably higher than the percentages previously reported. Differences in the methods and criteria employed to measure and define oral malodor may partly explain such discrepancies. Most of these investigations used subjective criteria from questionnaire to estimate the percentage of affected people in a population (Frexinos et al. 1998, Al-Ansari et al. 2006, Nadanovsky et al. 2007). These data have to be considered with some caution, because self-estimation of oral malodor has been demonstrated to be largely

unreliable (Pham et al. 2012). The perception of malodor is different in culturally diverse populations (Rayman & Almas 2008), and objective assessment does not correlate well with patient's perception of the own bad breath (Rosenberg et al. 1995). In the present investigation only in 55.24% of the participants there was a correspondence between self- and odour judge assessment.

It is noteworthy that, in contrast with data from the literature, the actual prevalence of halitosis detected by OLT measures was higher than the percentage of self-reported halitosis (Rosenberg et al. 1995, Iwanicka-Grzegorek et al. 2005, Pham et al. 2012). It is important to point out that the tendency to overestimate the own bad breath level was mainly demonstrated in convenience samples attending specialized halitosis clinics (Quirynen et al. 2009).

We employed an organoleptic examination for measuring the severity of oral malodor. Nowadays, the OLT method is still suggested as the primary indicator of halitosis and is regarded as a kind of reference standard for the diagnosis of oral malodor (Greenman & Rosenberg 2005, Bollen & Beikler 2012). This method is easy to perform and reflects the every-day situation when halitosis is detected. Its most important disadvantage is a certain degree of subjectivity. However, Greenman et al. (2004) suggested that organoleptic examination is applicable even in large-scale survey if appropriate calibration of the odour judge is carried out. In agreement with previous reports, only one calibrated and masked examiner who demonstrated reliability in smelling halitosis assessed the organoleptic measurements (Liu et al. 2006, Bornstein et al. 2009, Pham et al. 2012). Ideally, two or more odour judges should assess the intensity of the breath malodor to minimize intra-observer variability (Wozniak 2005). However, oral malodor measurements by a panel of judges may introduce problems of reproducibility (Rosenberg & McCulloch 1992).

Additional instrumental analysis of breath air was performed using a sulphur monitor portable. Due to the operative difficulties in performing VSC measurements in the GPs' medical offices, the VSC levels were measured only in a subgroup of the study population.

All measurements were conducted by the same examiner, and there was good agreement between organoleptic test and H_2S, CH_3SH and $(CH_3)_2S$ concentrations. The VSC values of subjects without clinical oral malodor were below the threshold levels for oral malodor, whereas those with an organoleptic score of 2 or more were higher than the threshold levels proposed by the manufacturer to diagnose halitosis.

Periodontal pockets and tongue coating are well-known risk factors for halitosis as provide an ideal environment for growth of bacterial species that produce VSCs and other odoriferous molecules (Haraszthy et al. 2007, Apaztidou et al. 2013). Several studies observed a positive correlation between VSCs in mouth air and OLT scores, and the severity of periodontal disease (Morita & Wang 2001, Takeuchi et al. 2010, Romano et al. 2010, Pham et al. 2012). However, not all patient suffering from gingivitis or periodontitis have oral malodor (Bosy et al. 1994, Stamou et al. 2005, Calil et al. 2009).

Tongue dorsum is a rich source of VSCs. It has been suggested that approximately 60% of VSCs originate from the tongue surface in patients with periodontitis (Yaegaki & Sanada 1992) and that the tongue coating volume tends to increase in case of periodontal involvement (Quirynen et al. 1998, Van der Sleen et al. 2010). However, no study assessed the importance of interaction between TCS and periodontitis in the pathogenesis of halitosis.

The findings from the present investigation suggest that periodontal conditions and tongue coating level may exert a synergistic contribution to oral malodor. Among individuals without a diagnosis of periodontal disease a TCS ≥ 5 increased of more than 10-times the probability of having halitosis, confirming its pivotal role in causing bad breath.

In the periodontitis patients the strength of the associations between periodontal disease and clinical halitosis was different both by the level of tongue coating and the severity of periodontal involvement. This was more evident in the severe periodontitis group.

The significance of tongue coating in moderate and severe periodontitis is unclear. In agreement with Amou et al. (2013) the present results would seem to suggest that VSCs

production in subjects with moderate periodontitis originate mainly from periodontal pockets. Pham et al. (2011) reported that periodontal treatment played an important role in controlling oral malodor in periodontitis patients while tongue cleaning contributed to a lesser extent.

In contrast to moderate periodontitis, in the severe form of disease heavy tongue coating would seem to play a pivotal role in the pathogenesis of halitosis. It has been suggested that the microbial composition of tongue biofilm in addition to its thickness and extension has an impact on oral malodor production (Rosenberg et al. 1991). Presence and proportion of specific periodontopathogenic bacteria in tongue coating are closely associated with oral malodor and severity of periodontal conditions (Apatzidou et al. 2013).

However, it is important to highlight that halitosis is a complex issue in which different oral health-related parameters take part. Due to their collinearity, it was not possible to insert them in one logistic regression model and to test their own contribution to oral malodor. In contrast with previous investigations that considered as indicative of periodontal disease the number of sites $\geq$ 5 mm (Tsai et al. 2008, Calil et al. 2009, Pham et al. 2012) or the mean PD (Stamou et al. 2005), the diagnosis of moderate and severe periodontitis was made according to the CDC/AAP suggested case definition for population-based surveillance of periodontitis (Page & Eke 2007, Costa et al. 2009). Based on this classification more then 60% of subjects with severe periodontitis and halitosis presented with high values of FMBS and about 40% had heavy tongue coating. On the contrary, among severe periodontitis subjects without clinical halitosis 78% of the subjects exhibited FMBS <50%, 70% had no tongue coating, and 90% presented with a localized form of periodontitis. These findings emphasize that oral malodor is caused by a combination of factors, including periodontal status and tongue coating that provide a synergistic contribution.

Limitations of the present study are the sample size calculation based on the expected prevalence of severe periodontitis, the moderate response rate (50.12%), and the wide interval confidence of some of the associations detected. Thus, it is not possible to rule out that

patients suffering from halitosis were overestimated in the examined population. However, it is important to point out that for the most part studies did not perform the sample size calculation (Liu et al. 2006, Al-Ansari et al. 2006, Bornstein et al. 2009, Yokoyama et al. 2010) or based it on inconsistent prevalence estimates due to diverse halitosis assessments and cut-off points among different studies (Nadanovsky et al. 2007).

Although national surveys are needed to confirm whether the present findings may be generalized more broadly, the present study suggested that there was a high prevalence of clinical halitosis in the Italian population we studied. Periodontal status and amount of tongue coating were closely related to halitosis. The combined effect of severe periodontitis and amount of tongue coating would seem to have a significant impact on oral malodor. This association illustrates the complex nature of halitosis and may warrant further investigations into the interplay of factors associated with the etiology of oral malodor.

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Table 1. Observed prevalence of halitosis by age and gender

	Overall		Females		Males	
	Prev	**95% CI**	**Prev**	**95% CI**	**Prev**	**95% CI**
Age (years)						
20-29	32.50	(22.24, 42.76)	36.96	(23.01, 50.91)	26.47	(11.64, 41.30)
30-39	44.83	(35.78, 53.88)	41.56	(30.55, 52.57)	51.28	(35.59, 66.97)
40-49	49.69	(41.91, 57.46)	46.46	(36.64, 56.29)	55.00	(42.41, 67.59)
50-59	65.28	(58.57, 72.00)	60.87	(51.95, 69.79)	71.79	(61.81, 81.78)
60-75	65.82	(59.18, 72.46)	58.76	(48.97, 68.56)	72.73	(63.95, 81.50)

Table 2. Analysis of correlation between GC measurements and OLT scores in the first 250 study subjects

GC measurement	R*	P-value
Total VSC	0.75	<0.001
H_2S	0.76	<0.001
CH_3SH	0.72	<0.001
$(CH_3)_2S$	0.61	<0.001

*Pearson's correlation coefficients (R) and significance levels for comparisons between specific GC measurements and organoleptic ratings; GC, gas chromatography; OLT, organoleptic testing; VSC, volatile sulphur compounds.

Table 3. Socio-demographic and lifestyle characteristics of subjects according to the halitosis diagnosis

	No Halitosis *N* = 332		Halitosis *N* = 412		Total *N* = 744		p-value
	N	%	N	%	N	%	
Gender							0.006
Female	212	48.85	222	51.15	434	58.33	
Male	120	38.71	190	61.29	310	41.67	
Age (years)							<0.001
20-29	54	67.50	26	32.50	80	10.75	
30-39	64	55.17	52	44.83	116	15.59	
40-49	80	50.31	79	49.69	159	21.37	
50-59	67	34.72	126	65.28	193	25.94	
60-75	67	34.18	129	65.82	196	26.35	
Education level							<0.001
Low	109	34.82	204	65.18	313	42.07	
Middle	140	49.30	144	50.70	284	38.17	
High	83	56.46	64	43.54	147	19.76	
Smoking							0.001
Non- and past-smoker	273	47.98	296	52.02	569	76.48	
Current smoker	59	33.71	116	66.29	175	23.52	
Self-perceived halitosis							<0.001
No	282	49.91	283	50.09	565	75.94	
Yes	50	27.93	129	72.07	179	24.06	

Table 4. Oral health-related variables according to the halitosis diagnosis

	No halitosis (N = 329)		Halitosis (N = 407)		Total[a] (N=736)		*p*-value
	N	**%**	***N***	**%**	***N***	**%**	
Periodontitis (CDC/AAP case definition)							<0.001
No/mild	116	69.05	52	30.95	168	22.83	
Moderate	158	54.67	131	45.33	289	39.26	
Severe	55	19.71	224	80.29	279	37.91	
FMPS (%)							<0.001
0-25	87	91.58	8	8.42	95	12.91	
25-50	157	67.38	76	32.62	233	31.65	
50-75	59	34.10	114	65.90	173	23.51	
75-100	26	11.06	209	88.94	235	31.93	
FMBS (%)							<0.001
0-25	180	77.25	53	22.75	233	31.66	
25-50	117	45.53	140	54.47	257	34.92	
50-75	27	19.15	114	80.85	141	19.16	
75-100	5	4.76	100	95.24	105	14.26	
TCS							<0.001
0-1	222	61.67	138	38.33	360	48.91	
2-4	81	35.84	145	64.16	226	30.71	
≥ 5	26	17.33	124	82.67	150	20.38	
Toothbrushing frequency							<0.001
< once/day	0	0.00	10	100.00	10	1.36	
once/day	18	25.71	52	74.29	70	9.51	
≥ twice/day	311	47.41	345	52.59	656	89.13	
Interdental cleaning							<0.001
No	154	34.68	290	65.32	444	60.33	
Yes	175	59.93	117	40.07	292	39.67	
Scaling frequency							0.002
< once /year	141	38.84	222	61.16	363	49.32	
≥ once/year	188	50.40	185	49.60	373	50.68	
Total	**329**	**44.70**	**407**	**55.30**	**736**	**100**	

[a]Numbers did not add up to the total number (744) due to eight edentulous subjects.

FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; TCS, tongue coating score.

Table 5. Oral health characteristics of subjects by periodontal and halitosis diagnosis

	No/mild periodontitis				Moderate periodontitis				Severe periodontitis			
	No halitosis (*N*=116)		Halitosis (*N*=52)		No halitosis (*N*=158)		Halitosis (*N*=131)		No halitosis (*N*=55)		Halitosis (*N*=224)	
	N	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**
FMPS (%)												
<50	97	83.62	22	42.31	113	71.52	35	26.71	34	61.82	27	12.05
≥ 50	19	16.38	30	57.69	45	28.48	96	73.29	21	38.18	197	87.95
FMBS (%)												
<50	111	95.69	36	69.24	143	90.51	71	54.20	43	78.18	86	38.39
≥ 50	5	4.31	16	30.76	15	9.49	60	45.80	12	21.82	138	61.61
TCS												
0-1	84	72.41	16	30.77	99	62.66	50	38.17	39	70.91	62	27.68
2-4	26	22.41	22	42.31	41	25.95	49	37.40	14	25.45	74	33.04
≥5	6	5.18	14	26.92	18	11.39	32	24.43	2	3.64	88	39.28
Periodontitis												
generalized					2	1.27	7	5.34	6	10.91	87	38.84
localized					156	98.73	124	94.66	49	89.09	137	61.16

Localized periodontitis: <30% of sites involved; generalized periodontitis: ≥ 30% of sited involved.

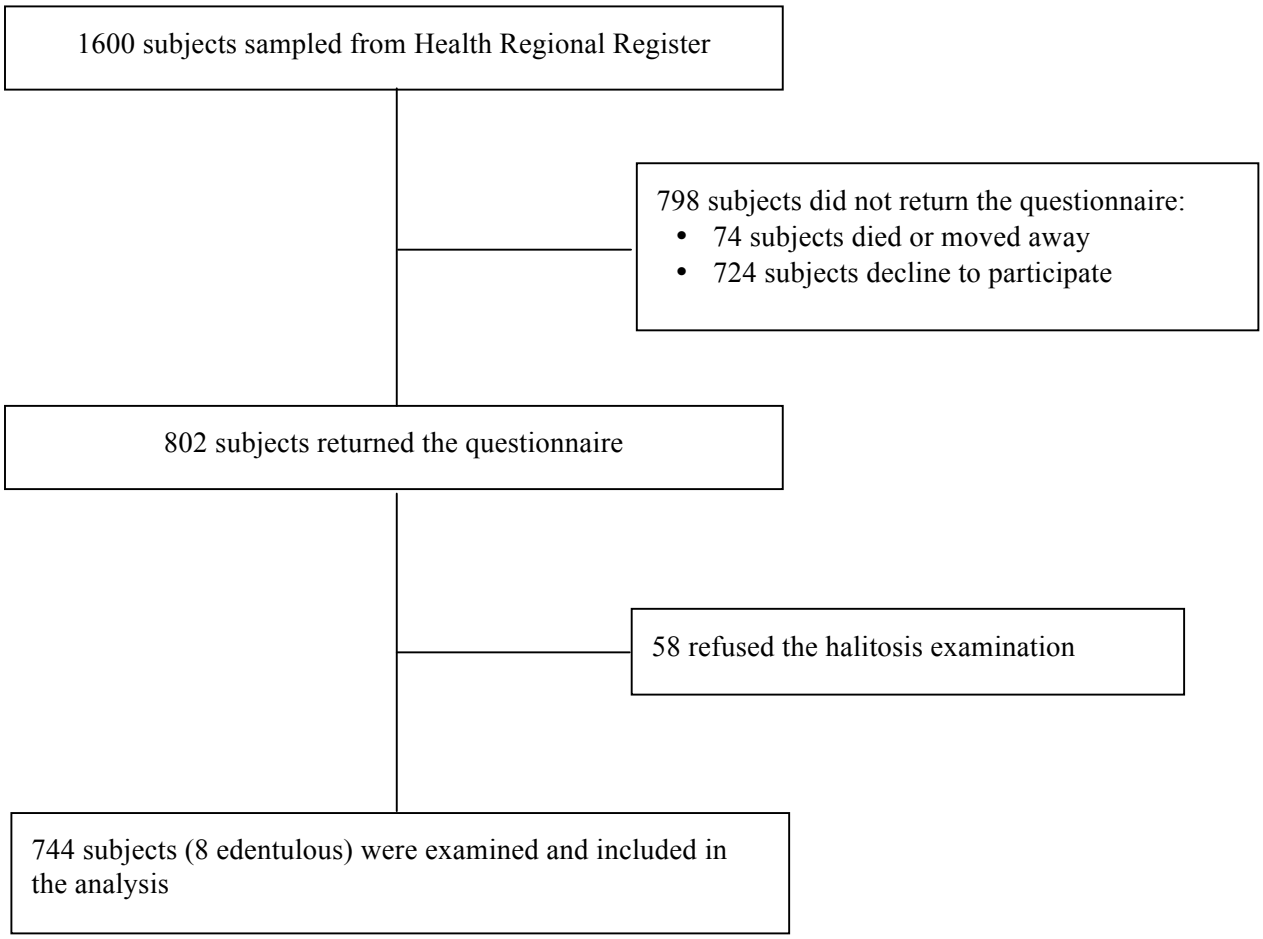
FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; TCS, tongue coating score.

Table 6. Crude and adjusted effects on clinical halitosis

	Crude effects			Adjusted effect		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-Value**
Gender						
Female	1			1		
Male	1.51	(1.12, 2.03)	0.006	1.22	(0.85, 1.73)	0.278
Age (years)						
20-29	1			1		
30-39	1.69	(0.93, 3.06)	0.084	1.18	(0.60, 2.32)	0.634
40-49	2.05	(1.17, 3.60)	0.012	0.92	(0.48, 1.77)	0.800
50-59	3.91	(2.24, 6.80)	<0.001	1.46	(0.76, 2.81)	0.261
60-75	1.51	(1.12, 2.03)	0.006	1.31	(0.67, 2.57)	0.431
Education level						
Low	1			1		
Middle	0.55	(0.40, 0.76)	<0.001	0.69	(0.46, 1.03)	0.071
High	0.41	(0.28, 0.61)	<0.001	0.56	(0.34, 0.90)	0.017
Smoking status						
No	1			1		
Yes	1.81	(1.27, 2.58)	0.001	1.21	(0.78, 1.88)	0.385
FMPS (%)						
0-25	1					
25-50	4.06	(2.74, 6.02)	<0.001			
50-75	14.34	(8.53, 24.11)	<0.001			
75-100	67.92	(6.30, 175.45)	<0.001			
Tooth brushing frequency						
< twice/day	1					
≥ twice/day	0.32	(0.19, 0.56)	<0.001			
Inter-dental cleaning						
No	1					
Yes	0.36	(0.26, 0.48)	<0.001			
Scaling frequency						
< once /year	1					
≥ once/year	0.63	(0.47, 0.84)	0.002			
Periodontitis						
No or mild	1					
Moderate	1.85	(1.24, 2.76)	0.003			
Severe	9.09	(5.85, 14.11)	<0.001			
TCS						
0-1	1					
2-4	2.87	(2.04,4.04)	<0.001			
≥ 5	7.69	(4.80,12.33)	<0.001			
TCS in patients with no/mild periodontitis						
0-1				1		
2-4				4.40	(2.00, 9.69)	<0.001
≥ 5				10.52	(3.42, 32.34)	<0.001
TCS in patients with moderate periodontitis						
0-1				1		
2-4				2.31	(1.34, 3.98)	0.003
≥ 5				3.10	(1.56, 6.15)	<0.001
TCS in patients with severe periodontitis						
0-1				1		
2-4				2.95	(1.46, 5.96)	0.003
≥ 5				20.77	(4.80, 89.93)	<0.001

FMPS, full-mouth plaque score; TCS, tongue coating score

Fig. 1 Flow chart of the study


1600 subjects sampled from Health Regional Register
798 subjects did not return the questionnaire:
• 74 subjects died or moved away
• 724 subjects decline to participate
802 subjects returned the questionnaire
58 refused the halitosis examination
744 subjects (8 edentulous) were examined and included in the analysis

Appendix. Study design and sampling procedures

A population-based cross-sectional representative epidemiological survey was conducted by the Section of Periodontology, Department of Surgical Sciences, C.I.R. Dental School, University of Turin (Italy) between December 2009 and July 2010. The target population comprised adults, aged between 20 and 75 years, living in Turin (Italy). Turin is one of the biggest industrial and business cities located in North Italy. It was inhabited by 910,504 persons at the time of sampling procedures.

To obtain an estimate of severe periodontitis prevalence with a 95% confidence interval (95% CI) with a precision of 2.5% we needed to examine 800 individuals hypothesizing a disease prevalence of 15% as reported in literature (Petersen & Ogawa 2005). Considering a response rate of 50%, 1600 individuals were randomly selected from the Health Regional Register of Pidemont using a stratified two-stage sampling design. The Health Regional Register collects demographic information of the entire population resident in Turin grouped according to the state-provided general practitioners (GPs) to whom they are assigned. In Italy all residents are covered by the National Health System, assigned a public GP and enrolled in the Regional Health Registries.

The first stage units were GPs stratified by the four districts of Turin to ensure a geographic and socioeconomic coverage over the whole of Turin. The probability to be selected was proportional to the number of subjects attending to each GP.

The second stage units were the subjects cared by each GP, who were sampled using a random sampling technique. Overall 20 GPs were sampled, and 1600 patients were selected and invited to participate in the study through an invitation letter, explaining the purpose of the study and including a through description of the clinical (periodontal and halitosis) examination. The invitation letter was accompanied by a structured questionnaire about socio-demographic, lifestyle factors and medical history. The questionnaire was completed by each subject and collected at the time of the halitosis examination.

Table A1. Characteristics of subjects according to the organoleptic testing (OLT)

	Subjects without OLT (*N*=58)		Subjects with OLT (*N*=744)		Total (*N*=802)		*p*-Value (χ^2 test)
	N	**%**	***N***	**%**	***N***	**%**	
Gender							0.578
Female	36	62.07	434	58.33	470	58.60	
Male	22	37.93	310	41.67	332	41.40	
Age (years)							0.619
20-29	9	15.52	80	10.75	89	11.10	
30-39	11	18.97	116	15.59	127	15.84	
40-49	13	22.41	159	21.37	172	21.45	
50-59	11	18.96	193	25.94	204	25.44	
60-75	14	24.14	196	26.34	210	26.17	
Education level							0.154
Primary and secondary school	42	72.41	597	80.24	639	79.68	
University education	16	27.59	147	19.76	163	20.32	
Smoking habit							0.484
Non- or past-smoker	42	72.41	569	76.48	611	76.18	
Current smoker	16	27.59	175	23.52	191	23.82	

Table A2. Volatile sulphur compounds concentration according to the degree of oral malodor in the first 250 consecutive study participants

	Absence of odour or questionable odour (N= 126)	Slight malodor (N= 47)	Moderate malodor (N=38)	Strong or severe malodor (N=39)
H_2S (p.p.b,)	49.28 ± 42.45	174.57 ± 87.01	455.66 ± 246.47	1101.26 ± 466.84
CH_3SH (p.p.b.)	14.47 ± 11.49	62.17 ± 52.18	203.74 ± 123.69	680.82 ± 331.83
$(CH_3)_2S$ (p.p.b.)	4.87 ± 4.15	28.46 ± 24.96	49.92 ± 27.23	120.25 ± 90.75

Values represent mean ± standard deviation. The degree of oral malodor was classified by the results of the organoleptic test: 0: absence of odour; 1: questionable odour, 2: slight odour; 3: moderate odour; 4: strong odour, 5: extremely severe odour.

Table A3. Adjusted effects of oral health-related variables on clinical halitosis

	Adjusted effect for gender, age, smoking habit and level of education		
	OR	**95% CI**	***p*-Value**
FMBS (%)			
0-25	1		
25-50	3.77	(2.50, 5.69)	<0.001
50-75	16.36	(9.43, 28.38)	<0.001
75-100	64.34	(4.47, 169.15)	<0.001
Toothbrushing frequency			
< twice/day	1		
≥ twice/day	0.40	(0.23, 0.71)	0.002
Interdental cleaning			
No	1		
Yes	0.40	(0.29, 0.55)	<0.001
Scaling frequency			
< once /year	1		
≥ once/year	0.64	(0.46, 0.87)	0.005

FMBS, full-mouth bleeding score.