DOCTORAL DISSERTATION IN ODONTOLOGY

SEIDA EROVIC-ADEMOVSKI

TREATMENT OF INTRA-ORAL HALITOSIS

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I. Erovic Ademovski S, Lingström P, Winkel E, Tangerman A, Persson G. R, Renvert S. Comparison of different treatment modalities for oral halitosis. Acta Odontologica Scandinavica 2012; 70: 224-233.

II. Ademovski S.E, Persson G.R, Winkel E, Tangerman A, Lingström P, Renvert S. The short-term treatment effects on the microbiota at the dorsum of the tongue in intra-oral halitosis patients- a randomized clinical trial. Clinical Oral Investigations 2013; 17: 463-473.

III. Erovic Ademovski S, Mårtensson C, Persson G.R, Renvert S. The effect of periodontal therapy on intra-oral halitosis: a case series. Journal of Clinical Periodontology 2016; 43: 445-452.

IV. Erovic Ademovski S, Mårtensson C, Persson G.R, Renvert S. The long-term effect of a zinc acetate and chlorhexidine diacetate containing mouth rinse on intra-oral halitosis- a randomized clinical trial. (In review) Journal of Clinical Periodontology.

ABSTRACT

Intra-oral halitosis (bad breath) is reported to affect 15-83 % of the adult population. Having intra-oral halitosis is a social and psychological handicap, and may cause people in the person's social circle to increase the physical distance or to turn their faces in another direction to avoid the unpleasant smell from the exhaled air. Such behaviours may affect the individual's self-confidence resulting in insecurity in social and intimate relations. The oral health-related quality of life status has also been reported to be lower in individuals with halitosis. Approximately 90% of what is considered as bad breath is the result of the degradation of organic substrates (proteins) by anaerobic bacteria of the oral cavity. Intra-oral halitosis can be assessed using both subjective and objective methods to evaluate the subject's exhaled air. The most common one and the one often referred to as the "gold standard", is the organoleptic scoring system (OLS). OLS is a subjective method evaluating the strength of halitosis in exhaled air using a scale from 0-5. One objective method to assess the presence of volatile sulphur compounds in exhaled air is to use a sulphide monitor measuring the total sum of the volatile sulphur compounds (T-VSC) in exhaled air. The three gases (hydrogen sulphide (H_2S), methyl mercaptan (MM) and dimethyl sulphide (DMS)) in exhaled air related to intra-oral halitosis can be assessed separately using a simplified gas chromatograph. Different treatment models such as periodontal treatment, tongue scraping and rinsing with Zn ion containing products have been used to reduce intra-oral halitosis. The present thesis has evaluated the efficacy of different treatment models in the treatment of intra-oral halitosis.

Paper I

In Paper I, the effects on intra-oral halitosis using a mouth rinse containing zinc acetate (0.3%) and chlorhexidine diacetate (0.025%) (ZN/CHX) with or without the adjunct use of a tongue scraper were assessed. Twenty-one subjects without a diagnosis of periodontitis were randomized in a cross-over clinical trial. Organoleptic scores (OLS) total volatile sulphur compounds (T-VSC) and the presence of hydrogen sulphide (H_2S), and methyl mercaptan (MM) were assessed. Evaluations were made before rinsing, immediately after rinsing, 30 minutes after rinsing, and 14 days after rinsing. OLS scores were significantly lower following active rinse combined with tongue scraping ($p< 0.001$) at all time points. After 30 min, and at day 14, the T-VSC values, H_2S and MM were lower in the active rinse sequence than in the placebo rinse sequence. The placebo rinse sequence with tongue scraping reduced T-VSC at 30 min but not at 14 days. Similar reductions of T-VSC, H_2S and MM values following the active rinse sequence with or without tongue scraping were found. In conclusion, the use of a tongue scraper did not provide additional benefits to the active mouth rinse, but reduced OLS scores, and tongue coating.

Paper II

In Paper II the effects on the microbiota at the dorsum of the tongue were studied following rinsing with a Zn/CHX containing mouth rinse with or without adjunct tongue scraping on volatile sulphur compounds (VSCs) in exhaled air. Bacterial samples from the dorsum of the tongue were assessed by checkerboard DNA–DNA hybridization. At day 14, 48% of the individuals rinsing with the active rinse and using the tongue scraper, and 14% of the individuals in the placebo and tongue scraping sequence were considered effectively treated for their intra-oral halitosis. At day 14 in the active rinse sequence, significantly lower bacterial counts were identified in samples from the dorsum of the tongue ($p<0.001$) for 15/78 species including *Fusobacterium sp*., *Porphyromonas gingivalis, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Tannerella forsythia*. In successfully treated subjects a decrease in bacterial counts from baseline to day 14 were found for 9/74 species. Data from study II showed that VSC values were not associ-

ated with bacterial counts in samples taken from the dorsum of the tongue. The active rinse alone had effects on intra-oral halitosis and reduced bacterial counts of species associated with intra-oral halitosis. Tongue scraping had no additional effects on the microbiota studied.

Paper III

In Paper III the effects of non-surgical periodontal therapy on intra-oral halitosis was evaluated. Non-surgical periodontal therapy was performed in sixty-eight adults with confirmed intra-oral halitosis. Three months after therapy OLS scores ($p<0.01$), T-VSC ($p<0.01$), and MM ($p<0.05$) values were significantly lower. The non-surgical therapy resulted in a significant reduction of probing pockets (PPD), bleeding on probing (BOP) and plaque indices (PI). Successful periodontal therapy was defined as a BOP<20% and a ≥50% reduction of total PPD (total PPD was calculated by adding all pockets ≥ 4 mm from the entire dentition). The 34 individuals with successful periodontal treatment demonstrated reductions in OLS ($p<0.01$) scores, and T-VSC ($p<0.01$) values. Effective treatment for intra-oral halitosis (T-VSC value <160 ppb, H_2S value <112 ppb and MM value <26 ppb) was identified in 11/68 individuals. Thus, non-surgical periodontal therapy resulted in few individuals who were considered as effectively treated for intra-oral halitosis.

Paper IV

In Paper IV the long-term effects of a zinc acetate and chlorhexidine diacetate (Zn/CHX) mouth rinse on intra-oral halitosis were evaluated 3, and six months after treatment. Forty-six adults with intra-oral halitosis were randomized into a 6-month, double-blinded, placebo-controlled clinical study. Using assessments of organoleptic scores (OLS), total volatile sulphur compounds (T-VSC), hydrogen sulphide (H_2S), and methyl mercaptan (MM) concentrations in exhaled air the presence of intra-oral halitosis was evaluated. At three and six months, individuals rinsing with the Zn/CHX rinse presented with reductions of the OLS, T-VSC, H_2S, and MM in exhaled air. At six months 68.2% of individuals using the Zn/CHX rinse experienced a 1 or 2 category improvement in

OLS compared with 19.1% of placebo-treated subjects. In the group rinsing with the Zn/CHX mouth rinse 91% of subjects were categorized as being effectively treated for their intra-oral halitosis (i.e. H_2S < 112 ppb). Compared to 43% in the placebo group. In conclusion, a Zn/CHX mouth rinse provides effective long-term efficacy against intra-oral halitosis, assessed both objectively and subjectively.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Att leva med dålig andedräkt (Intra-oral halitosis) kan vara ett socialt och psykologiskt handikapp. Det kan resultera i att personer i omgivningen vänder bort ansiktet för att undvika den obehagliga lukten. Ett sådant beteende kan påverka individens självförtroende och leda till osäkerhet i sociala och privata relationer. Personer med dålig andedräkt har sämre livskvalité. Att ha dålig andedräkt är vanligt och mellan 15 och 83 % har rapporterats ha dålig andedräkt. Den huvudsakliga anledningen till dålig andedräkt är att bakterier i munhålan bryter ner proteiner och bildar svavelinnehållande illaluktande gaser. Dålig munhygien, tungbeläggningar och ett inflammerat tandkött förklarar 80-90 % av all dålig andedräkt. En persons andedräkt kan undersökas med både subjektiva och objektiva metoder. Den vanligaste metoden som ofta anges som "gold standard", är att lukta på patientens utandningsluft. Undersökaren bedömer sedan styrkan av dålig andedräkt, så kallad organoleptisk scoring (OLS) enligt en skala från 0 till 5 där 0 står för ingen dålig lukt och 5 står för extremt dålig andedräkt. En objektiv metod för att mäta den sammanlagda förekomsten av flyktiga svavelföreningar (T-VSC) i utandningsluften, är att använda en sulfidmätare. De tre gaser i utandningsluften som kopplas till dålig andedräkt är väte sulfid (H_2S), metylmerkaptan (MM) och dimetylsulfid (DMS). Dessa kan mätas separat i en portabel gaskromatograf. Olika behandlingsmodeller har använts för att reducera dålig andedräkt. Denna avhandling har utvärderat effekten av behandling av inflammerat tandkött, skrapning av tungan och sköljning med Zink-jon innehållande munsköljmedel på dålig andedräkt.

Studie I

I studie I, utvärderades effekten på dålig andedräkt av ett munsköljmedel innehållande zinkacetat (0,3%) och klorhexidindiacetat (0,025%) (Zn/CHX) eller ett placebo sköljmedel med eller utan tillägg av en tungskrapa. Tjugoen försökspersoner utan tandlossning randomiserades i en cross-over studie. Försökspersonernas andedräkt kontrollerades före sköljning, direkt efter sköljning, 30 minuter efter sköljning, och 14 dagar efter sköljning. OLS värden var signifikant lägre efter aktiv sköljning i kombination med tungskrapa vid alla tidpunkter. Efter 30 minuter, och vid dag 14 var värdena för T-VSC, H_2S och MM lägre efter sköljning med Zn/CHX jämfört med sköljning med placebo. Efter sköljning med placebo kombinerat med användning av tungskrapa minskade värdet på T-VSC vid 30 minuters mätning, men inte vid dag 14. Efter användning av sköljvätskan som innehöll Zn/CHX med eller utan användning av tungskrapa minskade värdena för T-VSC, H_2S och MM. Tilläggsbehandling med tungskrapa gav ingen ytterligare reduktion av värdena jämfört med bara sköljning med den aktiva sköljsekvensen. Användning av tungskarapa minskade däremot värdena på OLS och mängden diagnosticerad tungbeläggning

Studie II

I studie II, utvärderades effekten av ett Zn/CHX innehållande munsköljmedel eller placebo med eller utan komplement av tungskrapa på mikroorganismer på tungan och på flyktiga svavelföreningar (VSCs) i utandningsluften. Bakterieprover från tungan bedömdes med en checker-board DNA-DNA-hybridiserings metod. VSC värdena som registrerades vid dag 0 var korrelerade till förekomsten av flera bakteriearter. Baserat på VSC värden vid 14 dagar hade 57% av de som sköljt med den aktiva substansen ingen dålig andedräkt. Efter användning av den aktiva substansen kombinerat med tungskrapa hade 48% ingen dålig andedräkt Sköljning med placebolösning resulterade i att 5% inte hade dålig andedräkt efter 14 dagars sköljning. Om placebosköljning kombinerades med användning av en tungskrapa var 14% klassade som effektivt behandlade. I prover tagna från tungryggen vid dag 14 identifierades signifikant färre bakterier efter användning av den aktiva sköljsekvensen. Femton av sjuttioåtta bakteriearter inklusive *Fusobacteri-*

um sp., Porphyromonas gingivalis, Pseudomonas aeruginosa, Staphylococcus aureus, och Tannerella forsythia var signifikant lägre i prover från tungryggen ($p<0,001$), efter användning av det aktiva sköljmedlet. Hos individer som bedömdes effektivt behandlade för sin dålig andedräkt vid dag 14 var 9/74 bakteriearter reducerade i prover från tungryggen. Flyktiga svavelföreningar var inte förknippade med bakterietal i prover tagna från tungan. Sköljning med ett Zn/CHX innehållande munsköljmedel hade effekt på dålig andedräkt och minskade förekomsten av bakteriearter som är förknippade med dålig andedräkt. Tungskrapning hade inga ytterligare effekter på de mikroorganismer som studerades.

Studie III

I studie III utvärderades effekten av icke-kirurgisk parodontal behandling (tandhygienistbehandling innefattande munhygieninstruktion, borttagning av tandsten samt polering av tänderna) på 68 vuxna individer med konstaterad dålig andedräkt. Tre månader efter behandling konstaterades minskade värden för OLS ($p<0,01$), T-VSC ($p<0,01$), och MM ($p<0,05$). Den icke-kirurgiska behandlingen resulterade i en signifikant reducering av parodontala fickor, blödning vid sondering och plackindex (bakteriebeläggningar på tänderna). Individernas totala fickdjup beräknades genom att addera alla fickor ≥ 4 mm i hela bettet vid studiens början och slut. Framgångsrik parodontal behandling definierades om blödning vid sondering var <20% och om det sammanlagda värdet av de fickor som var över 4 mm hade reducerats med ≥50%. Hos de 34 individer som på detta sätt bedömdes vara parodontalt effektivt behandlade minskade OLS, och T-VSC värdena signifikant ($p<0,01$). Elva av 68 individer klassades som effektivt behandlade för sin dåliga andedräkt dvs hade T-VSC <160 ppb, H_2S <112 ppb och ett värde på MM <26 ppb). Icke-kirurgisk parodontal behandling resulterade i få individer som ansågs effektivt behandlade för sin dåliga andedräkt.

Studie IV

I studie IV utvärderades effekten av (Zn/CHX) munsköljmedel på dålig andedräkt efter sex månader. Fyrtiosex vuxna med dålig andedräkt randomiserades i en 6-månaders, dubbel-blind, kliniskt kontrollerad studie. Sköljning med ett Zn/CHX innehållande munsköljmedel resulterade i en signifikant reduktion av OLS, T-VSC, H_2S och MM i utandningsluften vid både tre och sex månaders uppföljning. Efter 6 månaders sköljning med den aktiva substansen uppvisade 68,2% en förbättring i OLS (ett eller två stegs förbättring på den femgradiga skalan) jämfört med 19,1% av de individer som sköljt med en placebolösning. Hos gruppen som sköljde med ett Zn/CHX innehållande munsköljmedel var 91 % av individerna effektivt behandlade för sin dåliga andedräkt (H_2S <112 ppb), jämfört med 43% i placebogruppen. Användning av ett Zn/CHX innehållande munsköljmedel reducerar effektivt och har en långtidseffekt mot dålig andedräkt utvärderat med såväl subjektiva som objektiva metoder.

ABBREVIATIONS

BOP	Bleeding on probing
CAL	Clinical attachment level
CHX	Chlorhexidine
DMS	Dimethyl sulphide
DT	Decayed teeth
GI	Gingival index
H_2S	Hydrogen sulphide
MM	Methyl mercaptan
NS	No significant difference
OLS	Organoleptic scores
PD	Pocket depth
PI	Plaque index
ppb	Parts per billion
PPD	Probing pocket depth
TC	Tongue coating
T-VSC	Total sum of volatile sulphur compounds
VSC	Volatile sulphur compounds
WTCI	Winkel tongue coating index
Zn/CHX	Zinc acetate/Chlorhexidine diacetate

INTRODUCTION

The olfactory system odour and scent

The olfactory system is the part of the sensory system used for smelling. Through the nasal cavity, scent molecules reach the receptor cells located in the olfactory epithelium (Ward et al. 2003). There are between 6 and 10 million receptor cells located in the olfactory epithelium (Ward et al. 2003). The human nose can recognize 10 000 different scents. The olfactory system cannot be turned off and prompts immediate emotional responses (Bradford et al. 2009). The olfactory receptors send information both to the neocortex for conscious processing and to the limbic brain system for emotional processing, allowing us to associate a particular smell with distinct memories (Grammer et al. 2005).

Body odour derives from different body fluids. The glandular system of the skin is the primary source for human body odours. Pheromones are chemical messengers (odour signals) produced by the human body. Such pheromones may activate specific psychological and behavioural responses (Grammer et al. 2005). The scents are related to kin recognition, mate selection, menstrual cycle synchronicity and emotional contagion (Bader et al. 2002, Weisfeld et al. 2003, Pause 2012, Semin et al. 2013). Heterosexual males considered the odour of a female ovulatory period to be more pleasant and sexy than during the luteal period (Trouton et al. 2012). Human odours can be related to the person's emotional state such as stress, sadness, and fear (Bradford et al. 2009).

Human preferences and behaviours are influenced by scents. Industry is using different scents in marketing their products. It is well known that scents influence consumers into react in certain ways. Warm ambient scents may influence the consumers into increased purchasing of premium products (Madzharov et al. 2015). Scents have also been used in dental offices to reduce anxiety among the patients (Lehrner et al. 2000).

Halitosis

The term halitosis derives from the Latin word 'halitus' (breath), and the Greek word 'nosos' (disease). The term halitosis has been used since 1930s (Ramdurg & Mendigeri 2014). Halitosis is a general term describing an unpleasant odour from oral and systemic sources (Tonzetich 1977). Terms such as bad breath, breath malodour, oral malodour, intra-oral halitosis, fetor ex ore and fetor oris have also been used to describe an unpleasant odour originating from the oral cavity (Tangerman 2002, Van der Sleen et al. 2010). There are documents dating back to the Greek and Roman times describing halitosis. Also the Islamic theology describes the importance to prevent bad breath (Mandel 1988, Fischman 1997). In the Talmud (a central text of Rabbinic Judaism) bad breath was considered a major problem, so a woman was entitled to seek divorce if her husband had bad breath. According to the Talmud, priests with bad breath were not allowed to carry out holy duties in the temple. In 1898, Joseph Howe published the monograph “The breath, and the diseases which give it a fetid odour”.

In the Mediterranean countries, a resin from the Pistacia lentiscus tree (labdanum) has been used to refresh the breath for thousands of years. Other folk cures include the use of parsley (Italy), cloves (Iraq), guava peels (Thailand), and eggshells (China) (Mandel 1988, Rosenberg 1996, Fischman 1997, Huwez et al. 1998, Shifman 2002).

Halitosis often classifies as Genuine halitosis, Pseudo halitosis or Halitophobia. In individuals with pseudo-halitosis others do not perceive an obvious malodor, although the patient complains of its existence. The condition can be improved by counseling (using lit-

erature support, education and explanation of objective examination results) and simple oral hygiene measures (Yaegaki & Coil 2000, Amstrong et al. 2010, Seemann et al. 2014).

If after treatment for genuine or pseudo–halitosis, the patient is still convinced that he/she suffers from halitosis although no physical or social evidence exists to suggest that halitosis is present, the condition is referred to as halitophobia. Most halitophobic patients interpret other people's behavior, such as covering the nose, averting the face or stepping back, as an indication of their own bad breath (Yaegaki & Coil 2000, Amstrong et al. 2010, Seemann et al. 2014)

In 2014 Seemann et al. recommended to use the word halitosis and to distinguish between intra- and extra-oral halitosis. Intra-oral halitosis originates from the mouth and extra-oral originates from other sources. Extra-oral halitosis can be subdivided in to blood-borne and non-blood-borne halitosis. Tangerman and Winkel (2007) aimed to explain the origin and cause of intra-oral and extra-oral halitosis. The degree of intra-oral halitosis determined by organoleptic scoring of mouth breath and the concentration of H_2S and MM in mouth breath is strongly correlated (Tangermann 2002, Tangerman & Winkel 2007). MM is considered the main contributor to intra-oral halitosis and DMS the main contributor to extra-oral or blood-borne halitosis whereas MM and H_2S cannot be found in blood-borne halitosis (Tangerman 2002, Tangerman & Winkel 2007).

Intra-oral halitosis is accordingly used to describe bad breath from the oral cavity. Bacterial degradation of proteins in the oral cavity is the primary source of intra-oral halitosis (Rosenberg & McCulloch 1992, Tangerman 2002, Quirynen et al. 2009). Individuals suffering from intra-oral halitosis present with higher concentrations of volatile sulphur compounds (VSCs), including hydrogen sulphide (H_2S), and methyl mercaptan (MM) (Tonzetich 1977). Data have indicated that H_2S and MM are the main VSCs in individuals with intra-oral halitosis. H_2S has a rotten egg aroma,

MM has a cooked cabbage smell, and DMS has cabbage, cauliflower, and garlic aromas (Swiegers & Pretorius. 2007).

The oral cavity is considered as the primary source of intra-oral halitosis (Tangerman & Winkel 2007, Quirynen et al. 2009). Halitosis can also be related to diseases such as chronic sinusitis, pneumonia, liver diseases and cancer in the respiratory system (Tangerman & Winkel 2010, Seemann et al. 2014). Extra-oral blood borne halitosis originates from systemic diseases i.e.; liver cirrhosis, uremia, diabetic ketoacidosis, metabolic disorders, and medications i.e.; disulfiram, dimethyl sulfoxide and cysteamine (Tangerman & Winkel 2010 Seemann et al. 2014). Extra-oral non-blood borne halitosis also originates from pathological conditions such as throat infection; tonsillitis, nasal infection; sinusitis and postnasal drip, infection of respiratory system; lung infection, lung disease; lung cancer, and tuberculosis (Tangerman & Winkel 2010, Seemann et al. 2014). The most common source of extra- oral halitosis is; tonsillitis (71%) followed by sinusitis (9.5%), foreign body in the nose (9.5%) and diabetes mellitus (9.5%) (Seemann et al. 2006). Actions taken to treat intra-oral halitosis will not help those suffering from extra-oral halitosis. Such individuals should be referred for medical examination and treatments.

Social interaction and intra-oral halitosis

Intra-oral halitosis is considered as an unattractive aspect of social interaction. In a Dutch survey, 14.5% out of 1000 individuals answered that they on a daily basis were exposed to individuals with halitosis. Sweat malodour was considered as the strongest 'downer' when meeting a person for the first time (de Jongh et al. 2014). To have halitosis is a social and psychological handicap and may cause people in the person's social circle to increase the physical distance, or to turn their faces in another direction to avoid the unpleasant smell from their breath when communicating (Scully et al. 1997, de Jongh et al. 2016).

Decreased self-confidence and insecurity in social and intimate relations were major reasons for individuals seeking treatment at specialized breath odour clinic (McKeown 2003). If a person per-

ceives a constant bad breath problem, he/she may avoid social relations affecting the person's well-being (McKeown 2003, Lu et al. 2016). Kursun et al. (2014) reported 62% to have anxiety caused by halitosis. Individuals with halitosis and with a strong social anxiety disorder were reported to have difficulties overcoming their halitosis anxiety (Zaitsu et al. 2011). The anxiety of having halitosis is reinforced when others cover their noses or avert their faces in social contacts as such behaviours are interpreted as an indication of halitosis (Yaegaki & Coil 1999).

In a Swedish population, the 49-item Oral Health Impact Profile (OHIP) was used to assess oral health-related quality of life in 1309 subjects. Bad breath was the most prevalent condition, and especially reported by young individuals (Oghli et al. 2017). In another study, the authors identified that individuals with intra-oral halitosis were reported to have poorer oral health related quality of life status as compared to those without halitosis (Lu et al. 2016).

Prevalence of intra-oral halitosis

The prevalence rates of intra-oral halitosis vary greatly between studies (from 1.5% to 100 %) and may be explained how halitosis has been assessed or defined. In some studies prevalence rates of intra-oral halitosis are based on self-perceived assessments. Self-percieved reports of halitosis should be interpreted with caution as the perceptions of halitosis vary between individuals. Self-assessment of intra-oral halitosis is difficult.

Many individuals with halitosis are, not aware of the fact that their breath may be obnoxious (Rosenberg 1996). In a study based on dental students, the perception of halitosis did not always reflect the actual situation (Rani et al. 2015). Cultural differences may, in part explain differences in reported prevalence rates of intra oral halitosis based on self-perceived assessments (Mumghamba et al. 2006, Seemann et al. 2006, Hammad et al. 2014, Lu et al. 2014, Villa et al. 2014, Kim et al. 2015, and Rani et al. 2015).

Age and gender may also be factors influencing the perception of intra-oral halitosis. Among male Swiss army recruits 1.5 % per-

ceived that they had halitosis frequently (Bornstein et al. 2009a) whereas 14 % of young mothers in Tanzania reported that they had bad breath, and that this was associated with gingival bleeding on tooth brushing, and periodontal pockets ≥ 6 mm (Mumghamba et al. 2006). Sixty-two percent of elderly individuals living in a nursing home in Sweden were convinced of having halitosis (Zellmer et al. 2016). There are, however, conflicting results regarding the impact of age on intra-oral halitosis (Nadanovsky et al. 2007, Samnieng et al. 2012).

Socioeconomic factors have been reported to be related to self-reported halitosis (Lopes et al. 2016). Inadequate oral hygiene practices have also been strongly associated with self-reported halitosis (Al-Ansari et al. 2006). Other self-perceived factors reported to be related to halitosis are poor health status, overweight or obese, stress, lower economic levels, high intake of fast food, instant noodles and the low intake of fruits and vegetables (Kim et al. 2015). Among undergraduate dental students the prevalence of self-perceived halitosis was found to significant correlate with smoking and dryness of mouth (Setia et al. 2014).

Depending on methods used to assess intra-oral halitosis figures ranging from 15 % (Nadanovsky et al. 2007) to 83 % (Zürcher et al. 2012) have been reported (Table 1).

Intra-oral halitosis varies during the day, and higher levels have been reported in morning breath (Miyazaki et al. 1995). If 75 ppb of H_2S in the exhaled air was considered as the socially acceptable level, 23% in the late morning and 6% in the early afternoon had halitosis (Miyazaki et al. 1995). Another study, however, failed to show significant variation during the day (Samnieng et al. 2012).

Table 1. Prevalence of intra oral halitosis.

Author	Location	Age	Sample size (N)	Intra-oral Halitosis (%)
Aimetti et al. 2015	Italy	20-75	744	55.38
Bornstein et al. 2009a	Switzerland	18-25	580	OLS 2 = 25, T-VSC > 75 ppb = 42.6
Bornstein et al. 2009b	Switzerland	18-94	419	OLS ≥ 2 = 31.5 > 75 ppb = 72.1
Chen et al. 2016	China	22-70	720	> 110 ppb = 33.2
Liu et al. 2006	China	15-64	2000	27.5
Lu et al. 2014	China	18-82	911	OLS ≥ 2 = 77.3
Miyazaki et al. 1995	Japan	18-64	2672	6-23
Hammad et al. 2014	Jordan	18-68	205	78
Nalçaci et al. 2008a	Turkey	7-11	628	14.5
Nalçaci et al. 2008b	Turkey	7-15	30	VSC > 110 ppb= 26.7
Nadanovsky et al. 2007	Brazil	1-87	344	15
Villa et al. 2014	Italy	6-16	101	OLS 2= 15, > 100 ppb=37.6

Author	Location	Age	Sample size (N)	
Samnieng et al. 2012	Thailand	>60	428	H_2S 60.5
				MM 62.9
				DMS 80.7
Seemann et al. 2006	Germany	6-76	407	66.8
Quirynen et al. 2009	Belgium	2-90	2000	75.8
Tangerman & Winkel 2007	Netherland		58	81
Takeuchi et al. 2010	Japan	Mean 46	823	61.3
Oho et al. 2001	Japan	Mean 47	155	45
Zürcher et al. 2012	Switzerland	6–83	465	82.7
Zellmer et al. 2016	Sweden	66-99	124	54
Yokoyama et al. 2010	Japan	16	474	39.6

Factors associated with intra-oral halitosis

Several factors have been associated with intra-oral halitosis. In a majority of studies examining factors linked to intra-oral halitosis tongue coating and periodontitis have been identified as predominant factors (see Table 2).

Table 2. Factors associated with intra-oral halitosis.

		Correlation to VSC or OLS									
Author	Sample size (N)	TC	BOP	PI	DT	CAL	PD	GI	Smo king	Perio-dontitis	Caries
Aimetti et al. 2015	744	x								x	
Bornstein et al. 2009 a	580	x							x		
Bornstein et al. 2009 b	419	x							x		
Calil et al. 2009	72	x									
Figueiredo et al. 2002	PD> 3 mm n=21							x			
	PD≤ 3 mm n= 20						x				
Hammad et al. 2014	205	x							x		
Lee et al 2003	40	x	x				x	x			
Miyazaki et al. 1995	2672	x		x	x				x		x

Correlation to VSC or OLS											
Author	Sample size (N)	TC	BOP	PI	DT	CAL	PD	GI	Smo king	Perio-dontitis	Caries
Morita et al. 2001	82	x	x				x		x		
Nalçaci et al. 2008 a	628										x
Liu et al. 2006	2000	x		x			x				
Lu et al. 2014	911	x							x		
Takeuchi et al. 2010	823	x	x	x			x	x			
Tsai et al. 2008	72	x	x								
Pham et al. 2012	Gingivitis n=80	x	x	x							
	Periodontitis n=137	x	x	x	x	x	x				
Quirynen et al. 2009	2000	x					x				
Yokoyama et al. 2010	474	x		x				x			

TC: Tongue coating, BOP: Bleeding on probing, PI: Plaque index, DT: Decayed teeth, CAL: Clinical attachment level, PD: Pocket depth, GI: Gingival index

Tongue coating

Normally, the tongue has a pink colour. If dead cells and bacteria remain on the surface of the tongue a white or pale yellow layer is formed on the surface, and referred to as tongue coating. Individuals with intra-oral halitosis appear to have more tongue coating than individuals without intra-oral halitosis (Oho et al. 2001). The thickness of tongue coating has been associated with levels of H_2S (Samnieng et al. 2012). Tongue coating has been reported as a major reason for intra-oral halitosis (Yaegaki & Sanada 1992a, Quirynen et al. 2009). Miyazaki et al. (1995) concluded that in the younger generation intra-oral halitosis was caused primarily by tongue coating and in the older individuals by periodontal diseases in combination with tongue coating. Tongue coating is associated with a more pronounced bacterial diversity and to the prevalence of *Leptotrichia wadei* and *Peptostreptococcus stomatitis* was reported to be higher in tongue coating samples from children with intra-oral halitosis than in children without intra-oral halitosis (Ren et al. 2016). Tongue coating has been closely associated with clinically confirmed intra-oral halitosis whereas high PI scores are associated with self-perceived intra-oral halitosis (Rani et al. 2015).

Microbiota

Intra-oral halitosis is the result of bacterial degradation of proteins in the oral cavity (Rosenberg & McCulloch, 1992, Tangerman & Winkel 2007, Quirynen et al. 2009). The degradation of sulphur-containing amino acids (i.e. cysteine and methionine), by the microbiota produce volatile sulphur compounds (VCS´s) such as hydrogen sulphide (H_2S), methyl mercaptan (MM) (CH3SH) and dimethyl sulphide (DMS) [(CH3)2S] (Kleinberg & Westbay 1990, Yageaki & Sanada 1992a, De Boever et al. 1994 & 1995, Hughes & McNab 2008). Examples of microorganisms involved in this process are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tanerella forsythia*, *Fusobacterium nucleatum* and *Treponema denticola*.

Periodontal conditions and intra-oral halitosis

Studies have demonstrated that intra-oral halitosis is associated with both gingivitis, and periodontitis (Quirynen et al. 2009, Pham

et al. 2012, Zellmer et al. 2016). Quirynen et al (2009) reported gingivitis to be the cause in 4 % of cases and periodontitis in 7 % of cases. Gingival bleeding and periodontal pockets have been associated with high levels of MM (Samnieng et al. 2012). High levels of MM and H_2S were also reported in periodontitis patients by Yaegaki & Sanada (1992 a,b). In addition, elevated VSC levels have been linked to progression of periodontitis (Makino et al. 2012).

Other factors linked to intra-oral halitosis

Data suggest, that hormonal cause of intra-oral halitosis may occur but this is not common (Quirynen et al. 2009). It appears that individuals who drink coffee have lower OLS and VSC´s scores (Lu et al. 2014). In contrast, cigarette smokers have significantly higher VSC scores. Intra-oral halitosis is also associated with mouth breathing (Motta et al. 2011), hypo salivation, fixed prosthodontics and dementia (Zellmer et al. 2016).

Methods used for the assessment of intra-oral halitosis

Intra-oral halitosis can be assessed using both subjective and objective assessments of exhaled air. The organoleptic scouring method (OLS) is often referred to as the "gold standard" for the assessment of intra-oral halitosis (Greenman & Rosenberg 2005, Vandekerckhove et al. 2009). This method is a subjective classification of an individual's intra-oral halitosis. The sense of smell can be trained and verified using an identification test (Sensonics Inc., Haddon Heights, NJ, USA) (Laleman et al. 2014, Seemann et al. 2014). One or several individuals trained to evaluate the exhaled air grade the exhaled air using an organoleptic scoring index. Different types of OLS indexes have been proposed (Rosenberg et al. 1991a, b, Yaegaki & Coil 2000, Murata et al. 2002, Bornstein et al. 2009b). Rosenberg et al. in 1991 proposed an OLS score using a 0-5 scale. To perform the test the patient is asked to hold his/her breath for approximately one minute and then slowly exhale the air from the mouth. The investigator judging the smell of the exhaled air should be positioned close to the patient (approximately at a distance of 10 centimetres). The investigator then grades the smell of the exhaled from 0 to 5: 0 = no odour, 1 = barely noticeable odour,

2 = slight but clearly noticeable odour, 3 = moderate odour, 4 = strong odour, 5 = extremely strong odour close to saturation (Rosenberg et al. 1991a,b). The patient could also exhale the air into a tube that is inserted through a privacy screen. In this way the examiner does not see the patient. The examiner then judges the odour on the other side of the screen at the other end of the tube/straw (Murata et al. 2002).

Different objective measurements for the detection of VSC can also be used. A portable gas chromatograph (OralChroma™), and two portable sulphur monitors (Halimeter® and Breathtron®) are currently available (Laleman et al. 2014). The Halimeter® gives an information of the combined amount of VSC, H_2S, MM and DMS (Rosenberg et al. 1991a,b, Tangerman & Winkel 2008, Vandekerckhove et al. 2009). The instrument draws air from a plastic straw placed inside the patient's mouth. The total amount of VSC´s is determined in parts per billion (ppb). The Halimeter® is more sensitive for H_2S, whereas the detection sensitivity for MM and DMS is lower (Furne et al. 2002, Vandekerckhove et al. 2009). This instrument is primarily used for the analysis of intra-oral halitosis (Tangerman & Winkel 2008). Different threshold limits have been used to indicate halitosis (Nalçaci et al. 2008a, Villa et al. 2014, Bornstein et al. 2009 a,b, Lu et al. 2014,). A VSC level $\leq$ 75 ppb has been proposed as a socially acceptable cut-off level (Yaegaki & Sanada 1992c). VSC values obtained by the Breathtron® device are correlated to organoleptic registrations and VSCs measured by gas chromatography (Ueno et al. 2008).

The OralChroma™ measures VSC`s (H_2S, MM and DMS) separately and at low concentrations. The results can be graphically shown on a computer screen (Laleman et al. 2014). The sample of air is collected via a 1 ml syringe, which is injected in the OralChroma™, after 8 minutes the concentration of the three gases is displayed in ppb or ng/10 ml (Murata et al. 2006, Tangerman & Winkel 2008). Thus, Yaegaki et al. (2012) reported that the detection limit for each gas was 0.54 ng/10 ml. According to the manufacturer of the OralChroma™ device the following cut-off values for intra-oral halitosis have been proposed; $H_2S \geq 122$ ppb, MM $\geq$ 26

ppb and for DMS ≥ 8 ppb (Vandekerckhove et al. 2009, Laleman et al. 2014).

Several studies have reported a correlation between organoleptic scores and Halimeter® readings (Rosenberg et al. 1991b, Tangerman & Winkel 2007, Bornstein et al. 2009b Quirynen et al. 2009, Vandekerckhov et al. 2009, Dadamio et al. 2013a). Correlations between organoleptic scores and OralChroma™ readings have been reported by Tsai et al. (2008) and Vandekerckhove et al. (2009). With regard to the organoleptic score to detect patients with or without intra-oral halitosis, the sensitivity and specificity for the Halimeter® was 63% and 98%, respectively, and for the OralChroma™ 69% and 100% by using the threshold suggested by the manufacturer. By lowering the values, sensitivity could be improved without a significant decrease in specificity for both devices (Vandekerckhove et al. 2009).

Treatment of intra-oral halitosis

Individuals suffering from intra-oral halitosis may try to mask their bad breath. Mint lozenges and, chewing gums are commonly used in attempts to reduce intra-oral halitosis. It has, however, been shown that the effect is of short duration (Reingewirtz et al. 1999). It has also been demonstrated that the levels of MM increase after the use of a sugarless chewing gum and that mint does not reduce the amount of MM (Yaegaki et al. 2002). In a recent review, data supporting the use of hydrogen peroxide, baking soda, essential oils and flavours in the management of oral malodour are inconclusive (Dadamio et al. 2013b). Other treatment strategies that may have long lasting effects on intra-oral halitosis should therefore be evaluated.

Periodontal treatment

Periodontitis has been documented as one of the causes of intra-oral halitosis (Quirynen et al. 2009). It therefore seems logical that a reduction of the periodontal inflammation would reduce the levels of VSCs in exhaled air. Tooth brushing alone is, however, not efficient in reducing intra-oral halitosis (Aung et al. 2015). In contrast, treatment of gingival inflammation in children (Kara et al.

2006), and non-surgical periodontal treatment in adults are effective methods reducing the extent of intra-oral halitosis (Quirynen et al. 1998, Tsai et al. 2008). Periodontal treatment combined with tongue scraping/brushing has also been shown to reduce VSC levels in the oral cavity (Quirynen et al. 2005, Takeuchi et al. 2010).

Tongue cleaning

There are several studies suggesting that tongue cleaning/scraping can reduce the levels of VSCs in exhaled air (Lee et al. 2003, Casemiro et al. 2008, Tsai et al. 2008, Pham et al. 2011, Lu et al. 2014). One published review of the literature reported that the efficacy of tongue cleaning/scraping is conflicting (Van der Sleen et al. 2010) whereas another systematic review demonstrated that there was a minor superiority of tongue scrapers as compared to brushing in reducing halitosis (Outhouse et al. 2006).

Mouth rinsing

Different mouth rinse solutions have also been used in the treatment of intra-oral halitosis (Fedorowicz et al. 2008, Blom et al. 2012). Mouth rinses containing metal salts, essential oils, chlorhexidine, chlorine dioxide and cetylpyridinium chloride in different combinations have been shown to reduce VSCs in exhaled air (Pitts et al. 1983, Rosenberg et al. 1992, Kozlovsky et al. 1996, Frascella et al. 2000, Silwood et al. 2001, Young et al. 2001, Borden et al. 2002, Winkel et al. 2003, Peruzzo et al. 2007, Shinada et al. 2010). Metal salts, essential oils, chlorhexidine, chlorine dioxide and cetylpyridinium chloride are also known to have an antibacterial effects (Young et al. 2001 & 2003, Roldan et al. 2003 & 2004, Sreenivasan et al. 2005 & 2013, Shinada et al. 2010, Gu et al. 2012). Data have shown that zinc (Dadamio et al. 2013b), clorindioxid (Silwod et al. 2001) and CHX (Quirynen et al. 2005) reduces VSC levels in exhaled air. CHX splits the disulphide bonds contained within H_2S and MM to release sulphur ions, which are successively bound by Zn ions to form insoluble and non-odorous zinc-sulphides (Young et al. 2003). In a recent systematic review, the authors concluded that due to very limited evidence, the potential effect of a specifically formulated dentifrice and mouth-washes,

or a tongue scraper for the treatment of intra-oral halitosis is unclear (Slot et al. 2015).

Lactobacillus salivarius WB21 containing tablets have been shown to reduce halitosis (Iwamoto et al. 2010, Suzuki et al. 2014). Two recent reviews have identified that probiotic therapy also can be used to reduce intra-oral halitosis (Anusha et al. 2015, Janczarek et al. 2016). Probiotic therapy following oral disinfection with CHX may reduce the severity of halitosis over longer periods in children (Jamali et al. 2016). Adjunctive use of probiotics in addition to local debridement also results in clinical benefit in terms of pocket depth reduction and reduced intra-oral halitosis (Penala et al. 2016). Lack of effectiveness has also been reported (Marchetti et al. 2015).

OBJECTIVES

The objectives were:

- In Paper I, to compare the efficacy of four intervention modalities to control for intra-oral halitosis in study individuals with a diagnosis of intra-oral halitosis but without a diagnosis of periodontitis.

- In Paper II, to investigate in study individuals with confirmed intra-oral halitosis but without evidence of periodontitis if (1) the microbiota at the dorsum of the tongue is related to VSC and (2) if any of four different treatment modalities employed over 14 days reduced the counts of individual bacteria at the dorsum of the tongue.

- In Paper III, to evaluate the effects of non-surgical periodontal therapy on intra-oral halitosis 3 months after therapy.

- In Paper IV, to evaluate the long-term (6 month) effect of a Zn/CHX containing mouth rinse in periodontally healthy individuals with genuine intra-oral halitosis, compared to a placebo mouth rinse.

MATERIALS AND METHODS

The four papers included in the present thesis are based on research performed at the Dental Hygiene Clinic, Kristianstad University under the umbrella of the Oral Health, General Health and Quality of Life Research Center, Kristianstad University. The microbiological analyses were performed at the University of Bern, Switzerland. The research activities were performed between 2008-2016.

In Papers I and II, a cross-over, double-blinded short term study design was used including individuals with confirmed intra-oral halitosis but with no evidence of periodontitis. In paper III, a case series including individuals with confirmed intra-oral halitosis and chronic periodontitis without surgical treatment needs were studied over three months. In paper IV, a randomized clinical trial approach was employed with a follow-up period of six months.

In all four papers intra-oral halitosis was evaluated using subjective assessments of the exhaled air using the OLS score as proposed by Rosenberg et al. (1991a,b). Additionally, objective registrations of exhaled air was performed using the Halimeter® (Interscan Corporation, Chatsworth, CA, USA) to assess T-VSC, the OralChroma™ (ABIMEDICAL Corporation, Kawasaki City, Japan), to assess H_2S, MM and DMS. The Winkel tongue coating index (WTCI) was used to assess the extent of tongue coating. All evaluations of intra-oral halitosis were performed by one and the same investigator (SEA).

All individuals included in the studies were carefully instructed to adhere to the following rules before returning to the clinic for assessments of their breath odour; (I) not to consume food containing onions, garlic, or hot spices within 48 hours before assessments, (II) not to drink alcoholic beverages within 12 hours before assessments, (III) not to eat or drink within 4 hours before assessments (subjects were allowed to drink water until 3 hours before assessments), (IV) not to perform oral hygiene measures, tongue cleaning or use any mouth rinse in the morning of the examination day, and (V) not to use scented cosmetics, perfume or after-shave lotions in the same morning as the study assessments were performed.

In Papers I, II, and IV the mouth rinse used contained water, glycerin, sorbitol, alcohol (1.8%), zinc acetate (0.3%), chlorhexidine diacetate (0.025%), sodium fluoride (0.05%), hydrogenated castro oil, citric acid, acesulfame potassium, menthol and mentha piperita. The mouth rinse was administered by rinsing 10 ml in the mouth for 1 minute, twice daily. Morning rinsing was performed after tooth brushing, post-breakfast, and evening rinsing was done before bed-time. The mouth rinse solutions were distributed in identical coded bottles. The study subjects, and the examiner (SEA) were unaware of sequence of assignment.

Advertisements in the local newspaper, on message boards, and on the web page at Kristianstad University, Sweden, were used to recruit subjects. Participants were also recruited from the Dental Hygiene Clinic at Kristianstad University, Sweden. The study individuals were screened for the presence of intra-oral halitosis.

In the present research series, all study individuals were diagnosed with intra-oral halitosis. All study participants enrolled in the research resulting in papers I and II did not have a diagnosis of periodontitis defined as not having any PPDs > 5 mm.

All individuals enrolled in the research resulting in paper III had a diagnosis of moderate periodontitis deemed not to require surgical intervention.

All individuals in paper IV had been effectively treated for periodontitis.

Paper I and II

Twenty-one adults fulfilling the criteria below were included in a cross-sectional study.

Inclusion criteria: (1) halitosis of intra-oral origin, (2) OLS ≥ 2 and (3) T-VSC ≥160 ppb, as determined with a Halimeter®.

Exclusion criteria: (1) untreated periodontitis defined as the presence of more than one periodontal pocket with a probing pocket depth ≥ 6 mm, (2) open caries lesions, (3) pregnancy, (4) systemic medications known to cause hypo-salivation, (5) systemic antibiotic therapy within the preceding 3 months before the study, (6) current smoker, or (7) a medical history with a disease known to be associated with extra-oral halitosis.

Subjects were randomly assigned to a protocol sequence order (Latin square). The following sequences were included: (I) the active test mouth rinse alone, (II) the active test mouth rinse with the use of a tongue scraper, (III) the inactive mouth rinse alone and (IV) the inactive mouth rinse with the use of a tongue scraper. The different test sequences were separated by a wash-out period of one week.

Study assessments were performed as follows: (I) Day 1: baseline values before intervention, (II) Day 1: immediately after intervention, (III) Day 1: 30 min after intervention and (IV) Day 14: 8–12 h after the last intervention the evening before.

Paper II

This paper was based on additional analyses of microbial samples collected from individuals included in paper I. Bacterial samples were taken with Catch all swabs (CatchAll™, Sample collection swab, Epicentre, Madison, WI, USA). The swab was moved across the dorsum of the tongue in several strokes back to forward as well as across the tongue. The samples were processed by checkerboard DNA–DNA hybridization. A software program (ImageQuant, Amersham Pharmacia, Piscataway, NJ, USA) was used to analyse the digitized information. Signals were compared against standard lanes of known bacterial amounts (105 cells). A total of 74 bacterial species were studied. The microbiological processing of the study material was performed at the Oral Microbiology Laboratory, University of Bern, Switzerland.

Paper III

A total of 300 individuals were screened for enrolment. The following inclusion criteria were used: (1) an OLS score ≥2 (Rosenberg et al. 1991a,b), (2) a level of T-VSC >110 ppb determined with a portable sulphur compound detector (Halimeter®), (3) ≥20 teeth, (4) the presence of bleeding on probing (BOP) ≥30%, (5) a minimum of four periodontal sites at different teeth with periodontal pockets ≥4 mm and (6) completion of the study. Seventy healthy adults (34 females) fulfilled the inclusion criteria and were included in the study.

Oral examinations were performed at baseline, and 3 months after treatment. Once the baseline registrations were performed, all study individuals received oral hygiene instructions, and non-surgical periodontal treatment were provided within a period of 2 weeks. At 4 and 8 weeks, PI scores were recorded and individualized re-enforcement of oral hygiene procedures were given. Two licensed dental hygienists performed non-surgical periodontal treatments adjusted to the participant's individual needs. Participants received supra- and sub-gingival debridement using ultrasonic (NSK, Varios2, Nakanishi Inc, Kanuma Tochigi, Japan), and hand instruments (LM-Instruments Oy, Parainen, Finland). Each treatment session was completed with professional tooth cleaning

using a prophylactic paste (Abrasion RDA 170, Clean Chemical Sweden CCS®, Borlänge, Sweden), and Air-Flow treatment (Air-Flow Master Piezon®, Electro Medical Systems S.A, Nyon, Switzerland). Following the registrations at 3 months, study participants were defined as being effectively treated for intra-oral halitosis if they met the following criteria: T-VSC values <160 ppb, H_2S value <112 ppb, and a MM value <26 ppb (Ademovski et al. 2013). In addition, and based on BOP values <20%, and a ≥50% reduction of the total sum of PPD measurements (calculated from the total sum of probing depths ≥4 mm for the entire dentition) at 3 months, the participants were categorized as having received periodontally successful treatment.

Paper IV

Forty-six periodontally healthy adults recruited among the 70 individuals from study III were included in the present study.

The subjects were required to have (1) ≥ 20 teeth, (2) bleeding on probing (BOP) ≤ 20%, (3) halitosis of intra-oral origin, (4) an OLS ≥ 2, and (5) a T-VSC concentration >160 parts per billion (ppb) to be included in the study.

The study comprised a 6-month treatment period, with study visits at baseline, month 3, and month 6. Before entering the study, one dental hygienist (SEA) performed non-surgical periodontal debridement according to individual needs. All participants received supra- and/or sub-gingival debridement by an ultrasonic device (NSK.Varios2, Nakanishi Inc, Kanuma Tochigi, Japan), and/or by hand instruments (LM-Instruments Oy, Parainen, Finland). The treatment session at baseline ended with Air-Flow treatment of the teeth (Air-Flow Master Piezon®, Electro Medical Systems S.A, Nyon, Switzerland) and a professional tooth cleaning with a prophylactic past (Abrasion RDA 170, Clean Chemical Sweden CCS®, Borlänge, Sweden). At baseline, the study individuals were randomized using a computer-based randomization software program SPSS 22.0 (IBM Corp., Armonk, NY, USA) into two groups, one rinsing with the active mouth rinse and the other group rinsing with a non-active placebo rinse. The clinical examiner, and the in-

dividuals participating in the study were blinded to group assignment.

Statistics

Paper I

The Kolmogorov-Smirnov test was used to identify that data for all variables failed to demonstrate a normally distribution pattern. The Kruskal-Wallis ANOVA and Univariate ANOVA with the Bonferroni post-hoc tests were used to compare baseline sequence conditions. Further data analysis between and within study sequences for the study group sequences were studied by Wilcoxon signed rank test, by Kruskal-Wallis ANOVA and by repeated Mann Whitney U-tests. Data were also assessed by Spearman rank correlation. Significance was declared at $p < 0.05$. IBM® SPSS® Statistics Standard 18.0 software package for PC, IBM Corp., Somers, NY, USA was used for the statistical analyses.

Paper II

Statistical analysis by Kolmogorov–Smirnov tests identified that the study data did not present with a normally distribution pattern. Statistical analysis was performed using nonparametric test including Mann–Whitney U tests and related samples Wilcoxon signed-rank test to assess differences in bacterial counts between and within study groups. Correlations between bacterial counts and VSC scores were assessed with Pearsons' and Spearman rank bivariate correlation. Due to multiple observations, significance was declared at $p<0.001$. The statistical analysis was performed with a statistical software package (IBM® SPSS® Statistics Standard 18.0 software package for PC, IBM Corp., Somers, NY, USA).

Paper III

The statistical package SPSS 22.0 (IBM®, Corporation Somers, NY, USA) for Windows was used for statistical analyses. The Kolmogorov Smirnov test identified that the data set did not have a normal distribution pattern and should be analysed with non-parametric statistical methods to determine whether significant differences existed within individuals for the variables tested before and after treatment (Wilcoxon sign rank test). To determine whether there was a significant correlation between tongue coating and intra-oral halitosis the Spearman Rank Correlation test was used. Reliability between assessment methods was performed by kappa α- analysis for intra-class correlation. Significance was declared at $p < 0.05$.

Paper IV

The intention to treat (ITT) population was defined as all subjects randomized and exposed to study treatment who hat at least one follow-up assessment for efficacy. The Kolmogorov-Smirnov test was used to test for normal distribution of the data. Wilcoxon Signed Rank Test was employed to determine whether significant differences existed within individuals for the variables tested at the different time points. Mann-Whitney U tests were used to determine difference between the active mouth rinse, and the control placebo rinse at baseline, three and six months after rinsing. Significance was declared at $p<0.05$. All statistical analyses were done using SPSS 22.0 (IBM Crop., Armonk, NY, USA).

Ethical approval

The Regional Ethics Committee at Lund, Sweden approved the studies (protocol IDs: 150/2007 & 2011/424).

RESULTS

Paper I

After two weeks significantly lower OLS scores were obtained following the sequence using active rinse alone ($p < 0.01$), or in combination with a tongue scraper ($p<0.001$). Lower OLS scores were also obtained following the sequence using the placebo rinse and tongue scraping ($p<0.01$). No differences in OLS values were found between baseline and 14 days for the placebo rinse sequence. The propotion of the different OLS scores asessed after 14 days are presented (Figure 1).

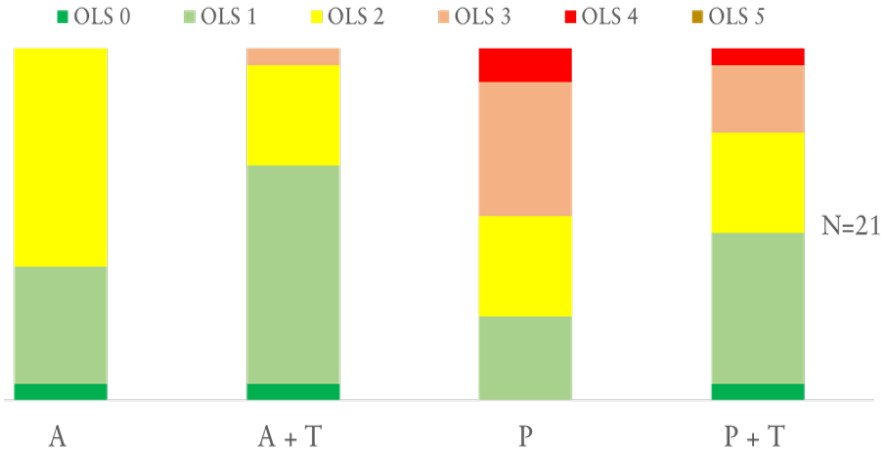

Figure 1. Organoleptic scores 0-5 at day 14.
A = Active rinse alone
A+T =Active rinse + tongue scraper
P= Placebo rinse alone
P+T = Placebo rinse + tongue scraper

T-VSC, H_2S and MM values were significantly lower following the active rinse sequense compared to the placebo rinse sequense at day 14. Statistical analysis failed to demonstrate differences for T-VSC, H_2S and MM values between the placebo rinse sequences. Statistical analysis also failed to demonstrate differences for T-VSC, H_2S and MM values between the the active rinse sequences. In figure 2 the mean percentage reduction at day 14 compared to baseline values for T-VSC, H_2S and MM by treatment sequenses are presented.

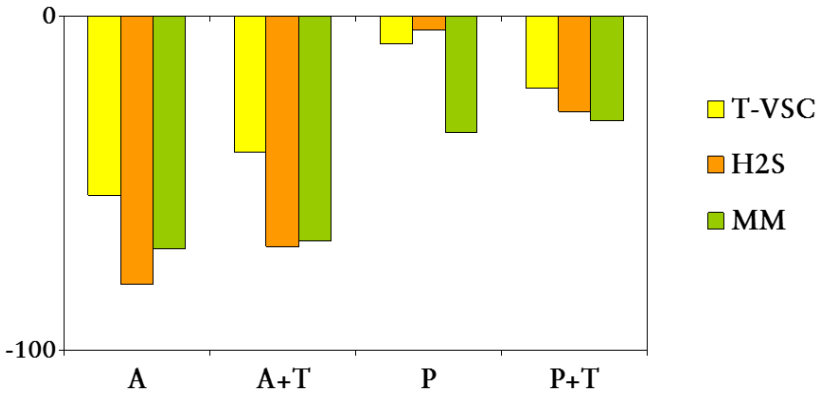

Figure 2. Mean reduction (%) of Total sum of volatile sulphur compounds (T-VSC), Hydrogen sulphide (H_2S) and Methyl mercaptan (MM).
A= active rinse alone
A+T = active rinse + tongue scraper
P= placebo rinse alone
P+T = placebo rinse + tongue scraper

Paper II

Statistically significant differences between baseline and day 14 were observed for the active rinse and active rinse and tongue scraping sequences ($p<0.01$). At baseline VSC levels were significantly correlated to the following bacterial species: *Actinomyces. israelii, Actinomyces neuii, Actinomyces odontolyticus, Aggregatibacter actinomycetemcomitans (serotype a), Atopobium parvulum, Prevotella bivia, Prevotella disiens, Prevotella nigrescens, Pseudomonas. aeruginosa, Staphylococcus epidermis, Staphylococcus constellatus, Streptococcus mitis, Tannerella forsythia, and Veillonella parvula.*

Two weeks following therapy the subjects were considered as successfully treated for intra-oral halitosis if the T-VSC values were < 160 ppb and H_2S values < 112 ppb, and MM values < 26 ppb. The number of individuals successfully treated are presented in table 3. The number of individuals having an OLS value ≤ 1 are also presented in table 3.

Table 3. Subjects considered as successfully treated for intra-oral halitosis at day 14.

	T-VSC < 160, H_2S < 112 & MM < 26 N (%)	OLS value ≤ 1 N (%)
A	12 (57.1)	8 (38.1)
A+T	10 (47.6)	14 (66.6)
P	1 (4.8)	5 (23.8)
P+ T	3 (14.3)	10 (47.6)

Statistical analysis failed to demonstrate differences in bacterial counts at day 14 for the placebo rinse with or without the use of a tongue scraper, and for the active rinse combined with the tongue scraper between those who were effectively treated for intra-oral halitosis in comparison to those who had intra-oral halitosis also at day 14. In the active rinse sequence, the bacterial counts at day 14 were lower for 15/78 bacterial species ($p < 0.001$) in individuals who were effectively treated for intra-oral halitosis as compared to those who were still identified as having intra-oral halitosis at day 14 (table 4). The species presented in table 4 decreased in counts at day 14 compared to baseline for subjects with a successful treatment outcome (active rinse sequence).

Table 4. Bacterial changes at day 14.

Active rinse sequence	
Lower bacterial counts in successfully treated cases compered to non-successfully treated cases at day 14	**Changes between baseline and day 14 in successfully treated cases**
Aerococcus christensenii	*Aggregatibacter actinomycetemcomitans (Y4)*
Actinomyces israelii	*Capnocytophaga gingivalis*
Actinomyces naeslundii	*Campylobacter rectus*
Campylobacter. Gingivalis	*Fusobacterium. nucleatum sp. Naviforme*
Eubacterium saburreum	*Parvimonas micra*
Fusobacterium .nucleatum sp.naviforme	*Porphyromonas gingivalis*
Fusobacterium . nucleatum sp.polymorphum	*Prevotella melaninogenica*
Mobiluncus mulieris	*Staphylococcus. aureus ATCC*
Peptostreptococcus anaerobius	*Ttreponema. Denticola*
Porphyromonas gingivalis	
Pseudomonas aeruginosa	
Staphylococcus aureus ATCC	
Staphylococcus. aureus yellow strain	
Staphylococcus. aureus white strain	
Tannerella forsythia	

Paper III

Three months after non-surgical parodontal therapy a significant reduction of BOP, PI and the number of PPD ≥4 mm, was observed. If the provided therapy resulted in a BOP value below 20% and a 50% reduction of the total probing pocket depth (calculated from the total sum of probing depths ≥4 mm for the entire dentition) the individuals were considered effectively treated. Using criteria to define successful treatment, 50% of the individuals were considered effectively treated. For the 34 periodontally successfully

considered effectively treated. For the 34 periodontally successfully treated individuals, reductions in OLS ($p<0.01$) and T-VSC ($p<0.01$) scores were observed three months after therapy. In contrast, the statistical analysis failed to show improvements for OLS, T-VSC, H_2S or MM scores for the 34 participants who were categorized not successfully treated from a periodontal perspective. Successfully treated for intra oral- halitosis using different cut-off values for halitosis definitions are presented in table 5.

Table 5. Distributions of individuals using different definitions of successful treatment of intra-oral halitosis in individuals who either had successful or non-successful periodontal treatment result.

	Three months after periodontal treatment N (%)		
Cut-off value	**Whole group**	**Periodontally successfully treated**	**Periodontally not successfully treated**
T-VSC< 110 & OLS<2	3 (4.4)	2 (5.9)	1 (2.9)
T-VSC< 160 & H_2S<112 & MM< 26	11 (16.2)	7 (20.6)	4 (11.8)
T-VSC< 160	17 (25)	7 (20.6)	10 (29.4)
H_2S< 112 & MM< 26	25 (36.8)	15 (44.1)	10 (29.4)
H_2S< 112	28 (41.1)	16 (47.1)	12 (35.3)
MM< 26	27 (39.7)	16 (47.1)	11 (32.4)

Paper IV

In this double blinded randomised clinical trial, the effects of an active mouth rinse (A) in comparison to a placebo mouth rinse (P) were evaluated over six months with an interim assessment at three months. Over time increases in PI and BOP scores, as well as the numbers of 4 mm and 5 mm probing depths were observed for both study groups (NS). Compared to baseline, data analyses at three and six months identified significant reductions of OLS ($p<0.01$), T-VSC ($p<0.01$), H_2S ($p<0.001$) and MM ($p<0.01$) values in the active treatment group (A). Six months after treatment, differences between treatment A and P were found for OLS ($p<0.05$), T-VSC ($p<0.05$), H_2S ($p<0.01$), MM ($p<0.01$) values, and with lower values in the test group. Changes from baseline to six months in OLS scores are presented in figure 3.

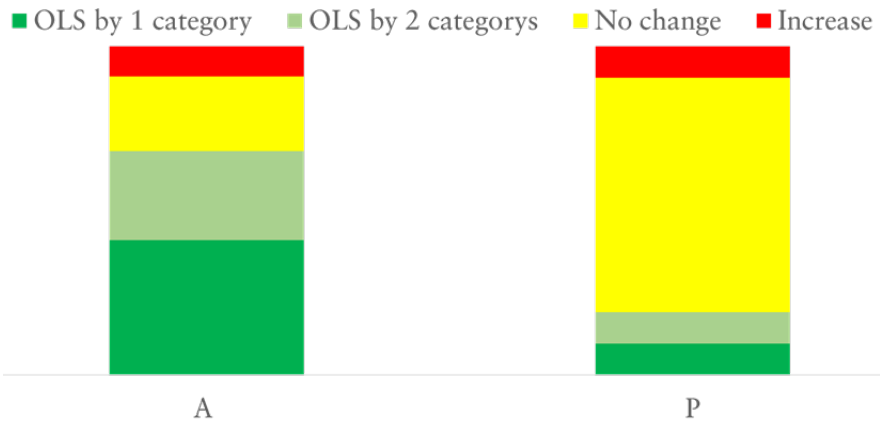

Figure 3. Changes from baseline to 6 months after treatment. Reduction of the organoleptic scores by 1 category, 2 categories increase and no change presented for the active (A) and placebo (P) treatments.

In table 6 the number of individuals successfully treated for intra-oral halitosis using different definitions are presented. In addition the number of individuals needed to treat to achieve one successfully treated individual for intra-oral halitosis after six months of treatment is also presented.

Table 6. Six months after successful treatment of intra-oral halitosis based on the following definitions.

Cut- off value	Placebo *n* = 21(%)	Active *n* = 22(%)	NNT in the active treatment group
T- VSC<160 & OLS<2	1 (5)	5 (23)	5.6
T- VSC<160 & H_2S<112 & MM< 26	3 (14)	9 (41)	3.8
OLS < 2	1(5)	5 (23)	5.6
T- VSC<107	2 (10)	6 (27)	5.6
T- VSC<160	3 (14)	11 (50)	2.8
H_2S< 112 & MM< 26	4 (19)	17 (77)	1.7
H_2S< 112	9 (43)	20 (91)	2.1
MM< 26	5 (24)	18 (82)	1.7

In figure 4 the mean values of T-VSC, H_2S and MM at baseline, 3 months after treatment and 6 months after treatment are presented for the active and placebo treatments.

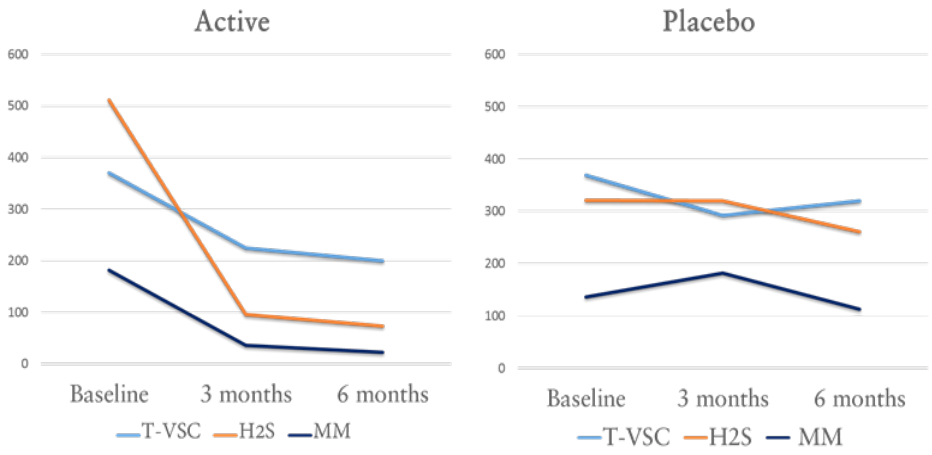

Figure 4. Presenting the mean T-VSC; Total sum of volatile sulphur compounds, H_2S; Hydrogen sulphide an MM; Methyl mercaptan.

DISCUSSION

The present thesis is primarily focused on treatment efficacy of intra-oral halitosis. In addition, contributing factors to the development of intra-oral halitosis are also studied.

The papers included in this thesis consist of three randomized clinical control studies of the effect of a Zn/CHX mouth rinse, both short term (Paper I, II) and long-term (Paper IV). One case series in which non-surgical periodontal treatment is evaluated after three months are also included (paper III). The data from the included papers were not normally distributed and non-parametric tests were performed used to evaluate efficacy. The data failed to demonstrate baseline differences at each sequence for the four treatment modalities in (Paper I and II) and no differences in any baseline measurements in (Paper IV). In Paper I, II and IV the active ingredients in the mouth rinse used were zinc acetate and chlorhexidine diacetate. Zinc and chlorhexidine have been reported to have antibacterial effects (Roldan et al. 2004, Cousido et al. 2010, Gu et al. 2012).

In Paper I the analysis demonstrated that rinsing alone with the active rinse solution (zinc-acetate and chlorhexidine-diacetate) was the most effective approach to reduce VSC in exhaled air. The adjunct use of a tongue scraper provided limited additional benefit on the reduction of intra-oral halitosis. Other studies have suggested that mechanical methods including tongue brushing or tongue scraping to clean the dorsum of the tongue reduced the levels of

VSC in exhaled air (Yaegaki & Sanada 1992 a, b, Danser et al. 2003, Tsai et al. 2008).

The data in (Paper III) demonstrated a correlation between pre-treatment degrees of tongue coating and intra-oral halitosis. Tongue coating has previously been reported by others to be correlated with intra-oral halitosis and that the tongue may be the primary location for bacteria producing VSCs (Miyazaki et al. 1995, Roldan et al. 2005, Tsai et al. 2008, Bornstein et al. 2009a,b, Calil et al. 2009, Van Tornout et al. 2013, Lu et al. 2014). Mechanical cleaning of the tongue may have a short time (less than 30 min) effect on intra-oral halitosis (Seemann et al. 2001). In Paper I, improved OLS scores were maintained over two weeks following tongue scraping. Tongue scraping in combination with active rinse provided limited additional effect on intra-oral halitosis in comparison to active rinse alone.

The use of the tongue scraper (Paper II) failed to alter the microbiota in samples from the dorsum of the tongue. These results are consistent with another study also demonstrating that tongue scraping does not reduce bacterial counts at the dorsum of the tongue suggesting that the microbiota at the tongue is resilient (Bordas et al. 2008). However, other studies have demonstrated that tongue scraping has an impact on the microbiota at the dorsum of the tongue (Roldan et al. 2005, Tsai et al. 2008). The differences in results obtained may be due to how the microbial samples were collected and analysed.

The amounts and diversity of bacteria identified in Paper II are consistent with what has been reported by Baumgartner et al. (2009) using the same sampling method. The use of a tongue scraper may not be sufficient in eliminating bacteria that are located in fissures and crypts of the tongue as well as the most posterior part of the tongue. Tongue scraping may for a short period of time remove tongue coating (Bordas et al. 2008, Chérel et al. 2008). In fact, data have shown that shortly after scraping the tongue the bacteria have re-grown enough to produce significant amounts of VSCs. This may explain why tongue scraping has been

reported to have limited and short-lived effect on oral halitosis (Outhouse et al. 2006). In a recent systematic review, the authors concluded that due to very limited evidence the potential effect of a specifically formulated dentifrice and mouth-wash, or a tongue scraper for the treatment of intra-oral halitosis is unclear (Slot et al. 2015).

Some investigators have found improvements of intra-oral halitosis following tongue cleaning in patients with periodontitis (Tsai et al. 2008, Takeuchi et al. 2010). It should, however, be pointed out that periodontal treatment has been reported to be more effective than tongue cleaning in reducing intra-oral halitosis in patients with periodontitis (Pham et al. 2011). In the treatment of intra-oral halitosis, another study failed to identify a benefit by adding tongue cleaning to supra-gingival plaque control (Silveira et al. 2012).

In Paper III, VSC values were reduced after non-surgical periodontal therapy including both supra- and sub-gingival debridement resulting in improvements of oral hygiene. Studies demonstrating that periodontitis is associated with intra-oral halitosis are consistent with our finding that improvement of periodontal conditions also results in the reduction of intra-oral halitosis (Miyazaki et al. 1995, Morita & Wang 2001, Liu et al. 2006, Samnieng et al. 2012). The results from Paper III are consistent with other studies demonstrating reductions of VSC levels shortly after non-surgical periodontal therapy (≤4 weeks) (Quirynen et al. 1998, 2005, Kara et al. 2008, Tsai et al. 2008, Takeuchi et al. 2010). The studies by Tsai et al. (2008), and Takeuchi et al. (2010) also used mouth rinse and performed a tongue cleaning procedures. In Paper III non-surgical periodontal therapy resulted in significant improvement of VSCs. Notwithstanding, few individuals were effectively treated (T-VSC <160 ppb, a H_2S value<112 ppb and a MM value <26 ppb) for intra-oral halitosis.

In Paper IV, study individuals with a BOP<20% demonstrated effective reduction in intra-oral halitosis after rinsing with a zinc and chlorhexidine containing mouth rinse. Between 82-91% of indi-

viduals using the Zn/CHX mouth rinse achieved a reduction in H_2S and MM concentrations to desired levels (i.e. 112 and 26 ppb, respectively) following continuous use for 6 months. At 6 months 68.2% of individuals using the Zn/CHX rinse experienced a 1 or 2 category improvement in OLS compared with 19.1% of placebo-treated subjects. These findings confirm both the sustained intra-oral halitosis protection effect by a Zn/CHX mouth rinse and highlight its long-term efficacy.

Intra-oral halitosis has traditionally been assessed by OLS and measurements of malodourous gases in exhaled air. Various Halimeter® cut-offs for clinical relevancy have been used (Yaegaki & Sanada 1992c, Tangerman & Winkel. 2007, Vandekerckhove et al. 2009). The issue of clinical relevancy is important for users who are not interested in ppb concentrations or category changes in OLS, but that the treatment reduces or eliminates intra-oral halitosis to a socially acceptable level.

If OLS values should be considered as the gold standard for evaluating intra-oral halitosis the cut of values for objectively defining intra-oral halitosis must be redefined. The manufacturer of the Halimeter® has suggested values between 80 and 140 ppb to be in the normal range, whereas values of 150 and above to indicate intra-oral halitosis). Vandekerckhove et al. (2009) used 160 ppb as a cut-off value and Yaegaki & Sanada (1992c) proposed 75 ppb of T-VSC in, mouth air to be socially acceptable. This difference in threshold values used for one of the parameters (T-VSC) highlights the complexity to make a clear definition of successful treatment of patients with intra-oral halitosis.

From clinic perspectives, it is important to define criteria for intra-oral halitosis that can be assessed objectively. Such criteria should be in agreement with clinical perception of intra-oral halitosis, and perceived as socially acceptable levels of malodour gases in exhaled air.
Based on the findings from the present thesis the following clinical recommendations for the treatment of intra-oral halitosis can be made:

- Improve oral hygiene, provide treatment of gingivitis and periodontitis, and recommend the use of a tongue scraper

- If treatment does not resolve the presence of intra-oral halitosis, additional treatment with Zn/CHX mouth rinse should be recommended

CONCLUSIONS

I. Rinsing with a zinc-acetate and chlorhexidine diacetate containing mouth rinse resulted in a clinically relevant reduction of intra-oral halitosis during a study period of 2 weeks. The use of a tongue scraper did not provide additional benefits to the active rinse. The removal of tongue coating debris with a tongue scraper does not seem to influence VSC levels in breath air in subjects who do not have periodontitis.

II. Rinsing with a mouth rinse, containing zinc and chlorhexidine, reduces VSC levels and bacterial counts of several species that may be associated with intra-oral halitosis in subjects without a diagnosis of periodontitis. The adjunct use of the tongue scraper had no adjunct effects on VSC release or in reducing bacterial counts in samples from the dorsum of the tongue in subjects without a diagnosis of periodontitis, but with pre-existing intra-oral halitosis.

III. Non-surgical periodontal therapy resulted in reductions of OLS, MM and T-VSC values 3 months after therapy. Few individuals were effectively treated for intra-oral halitosis.

IV. A mouth rinse containing Zn/CHX provides effective long-term efficacy against intra-oral halitosis, assessed both objectively, and subjectively sustainable over 6 months

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