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The SMILE study cohort: Baseline comparison of oral health and dental aesthetics among smokers in Italy, Poland, Moldova, and Indonesia

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ABSTRACT

Objectives: SMILE Study is an international multicenter randomized prospective controlled trial investigating oral health and dental aesthetic endpoints in smokers who switch to combustion-free nicotine alternatives (C-F NAs). This paper reports baseline characteristics of the smokers enrolled in the SMILE study cohort.
Methods: Conducted across Italy, Poland, Republic of Moldova, and Indonesia, the SMILE study recruited 460 adult daily smokers. Eligible participants were randomly assigned to continue smoking or switch to a C-F NA. Baseline assessments included Modified Gingival Index (MGI), quantitative light-induced fluorescence (QLF) imaging of plaque, digital spectrophotometry for tooth shade, extrinsic stain indices, and oral health–related quality of life. Standardized calibration, centralized training, and electronic case report forms ensured methodological consistency across sites.
Results: Significant cross-country differences were observed in age distribution, sex ratio, smoking intensity, Whitening Index for Dentistry (WID) values, and MGI gingival inflammation scores. Italians reported the highest WID values, while Indonesians showed the lowest MGI and plaque coverage, possibly reflecting cultural, behavioral, and product-specific factors. Multivariable regression identified age, sex, ethnicity, and cumulative smoking exposure as significant predictors of oral health outcomes.
Conclusions: Characterization of participants in the SMILE study cohort reveals marked inter-country variability in oral health and dental aesthetics among smokers, reflecting demographic, behavioral, and unique cultural factors.
Clinical Significance: This large multicenter study used standardized, objective technologies to assess smokers, providing essential benchmarks for evaluating smoking cessation and harm reduction strategies in future longitudinal analyses and supporting clinicians in tailoring personalized interventions.

1. Introduction

Dental plaque biofilm-induced periodontal diseases, including gingivitis and periodontitis, arise from the accumulation of an oral microbial biofilm above and below the gingival margin, leading to inflammation and downstream consequences to the periodontium [1]. Gingivitis is characterized by redness, swelling, and bleeding on periodontal probing, while periodontitis is additionally characterized by progressive loss of the connective tissue attachment to the root surface, radiographic alveolar bone loss, and increased pocket probing depth [2,3]. Cigarette smoking is a significant independent risk factor for periodontitis, increasing risk by 5- to 20-fold compared to non-smokers [4–8]. Smokers are prone to more severe periodontal tissue destruction even after adjusting for confounding factors such as age, education, diabetes history, alcohol consumption, and oral hygiene levels [9]. The European Organization for Caries Research (ORCA) has also identified smoking as a risk factor for caries [10–13]. In addition to periodontal disease, smoking is also a risk factor for oral cancer [14–16].

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Smoking also causes visible dental issues, such as tooth discoloration and tobacco staining, which depend upon the duration and frequency of smoking [17,18]. A large cross-sectional study in the United Kingdom [19] found that smokers were more likely to report dental discoloration and dissatisfaction with their tooth color compared to non-smokers. Even after at-home dental bleaching procedures, smoking keeps negatively affecting color stability [20]. This dissatisfaction with the appearance of their teeth due to enamel discoloration and tobacco stains is frequently perceived as a significant social problem for smokers [19]. Since oral health and dental aesthetics are common concerns among smokers, they may provide a stronger and more immediate motivation to quit than the distant risk of lung cancer or cardiopulmonary disease.

The 2022 WHO declaration highlights the importance of dental care and oral health for healthy longevity, advocating for stronger policies to address the determinants of oral diseases and other non-communicable diseases, and to reduce inequalities through universal healthcare access [21]. Recently, the WHO recommended integrating brief tobacco interventions into oral health programs in primary care, particularly for low- and middle-income countries, as part of its global action plan on preventing and controlling non-communicable diseases [22].

The SMILE Study began in October 2021 as part of a large international initiative coordinated by the Center of Excellence for the acceleration of HArm Reduction (CoEHAR) at the University of Catania to raise awareness about oral health and dental aesthetics among cigarette smokers [23]. The study is set to investigate whether better gingival/gum conditions, improved tooth color, and reduced dental plaque accumulation can occur in smokers who stop smoking after switching to combustion-free nicotine alternatives (C-F NA). The data gathered from this research will offer important insights into C-F NA's potential for lowering the risk of periodontal disease and, by extension, cardiovascular issues. Furthermore, the findings could play a crucial role in addressing the global smoking epidemic, particularly among smokers who are particularly concerned about issues like bad breath or unsatisfactory dental aesthetics. For these individuals, focusing on current oral issues (such as achieving a healthier, brighter smile) might be a more persuasive incentive to quit smoking than the more distant threat of lung cancer or cardiopulmonary diseases.

Conducted across four countries (Italy, Indonesia, Moldova Republic, and Poland), this multicenter, prospective, randomized controlled trial is among the largest in oral health and includes three parallel arms: smokers continuing with combustible cigarettes (control), smokers switching to C-F NAs (intervention), and never smokers (reference group). High smoking rates and diverse socioeconomic, healthcare, and cultural contexts enhance the study's relevance and generalizability. The trial captures how distinct healthcare systems, from well-established (Italy, Poland) to less developed healthcare systems (Moldova, Indonesia), may influence smoking behavior and C-F NA adoption. The SMILE Study introduces advanced digital technologies to objectively and accurately quantify tooth discoloration, using a spectrophotometer to measure tooth shade and a specialized scanner employing quantitative light-induced fluorescence to assess dental plaque and early caries lesions.

This article outlines the design of the SMILE study and provides an initial descriptive analysis of the enrolled smoking population, comparing baseline oral health outcomes and dental aesthetics measures across dental clinics in Italy, Poland, Moldova, and Indonesia. The specific objective is to assess similarities and differences among the smoking populations in these countries. The present analysis does not include comparisons between study arms, which will be reported in future publications.

2. Methods

2.1. SMILE study objectives

The primary objective of the SMILE study is to measure changes in inflammation of gingival tissues using the Modified Gingival Index (MGI), between baseline and study end among participants who continue smoking and those who switch to C-F NA. Secondary objectives include evaluating within- and between-group variations in MGI, plaque imaging, early caries lesions, tooth shade, tooth stain scores, and oral health–related quality of life at each study time point.

This paper presents a cross-sectional descriptive analysis of smokers enrolled in the SMILE study cohort at baseline, with the specific aim of comparing oral health status and dental aesthetics across four countries (Italy, Poland, Moldova, and Indonesia). The analysis focuses exclusively on variations between sites, without addressing differences between study arms based on smoking behavior. Comparative analyses involving the never-smoker cohort and the longitudinal assessment of randomized participants over the 18-month follow-up period will be reported in forthcoming publications.

2.2. Study design

The SMILE study is an 18-month prospective, multicenter, open-label cohort study with three parallel arms, using randomization and controls to assess oral health and dental aesthetics [23]. The protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. Eligible smokers were randomly assigned to the control group (Arm A), receiving standard care with cessation counseling, or the intervention group (Arm B), receiving a C-F NA of their choice plus cessation counseling. Willingness to either quit smoking or participate in the locally available smoking cessation programs is one of the exclusion criteria, so that only persistent smokers being the potential target of the harm reduction treatment strategy were included in the study. The reference group (Arm C) included never-smokers, which served as comparators for background changes in the study endpoints. Participants undergo regular oral health assessments across seven clinic visits: screening, enrollment and randomization, and follow-ups at weeks 12, 24, 52, and 76.

Eligible participants attended a screening visit (visit 0), during which sociodemographic data, medical and oral health history, nicotine and tobacco use, and interest in C-F NA were recorded. Participants with severe irreversible periodontitis were excluded.

At the enrollment visit (visit 0), inclusion/exclusion criteria were reverified, and smokers were reminded of the risks associated with smoking. Participants underwent baseline measurements, including MGI assessment, plaque/dental imaging, dental shade quantitation, and oral health questionnaires, followed by PMPR (Professional Mechanical Plaque Removal). Only smokers not intending to quit were randomly assigned to either the control (arm A) or intervention (arm B) group (1:4 allocation ratio). Arm A participants received standard care, including cessation counseling (very brief advice - VBA), while Arm B participants could choose a preferred C-F NA (ECs or HTPs) and also received VBA. Smokers in arm B received training, counseling, and a supply of their chosen C-F NA (either e-cigarettes or heated tobacco products). Products were resupplied at each visit according to the study schedule. The trial is unblinded due to the nature of the interventions, but data analyses were conducted blind to the study arms.

Participants attended five additional clinical visits for repeated measurements. Cigarette consumption, C-F NA use, and oral hygiene routines were monitored via a tracker app and personal diaries. The app also tracked protocol violations, collected adverse events, and sent reminders. Before each visit, participants had to avoid certain oral hygiene practices and smoking for specified periods to ensure consistent data collection. Any modifications in oral hygiene routines were recorded using the tracker app. A detailed description of trial visit measurements is provided in **Appendix 1**.

2.3. Study population

Based on sample size calculations, 460 smokers were recruited

between Oct 2021 and Jun 2023, with 92 participants allocated to Arm A and 368 to Arm B. The detailed rationale and methodology for sample size determination are described in the study protocol paper [23].

Eligible participants were healthy adult smokers who self-reported daily smoking of >5 cigarettes for at least five consecutive years and showed no clinical signs of severe irreversible periodontitis. Recruitment was carried out through multiple strategies, including referrals from dentists and dental hygienists, targeted advertising among university students, searches through patient databases from dental clinics, hospital staff outreach, and word of mouth.

Participants were enrolled if they met all the following inclusion criteria:

- Adults aged 18–50 years
- Smoking ≥5 tobacco cigarettes per day for at least five consecutive years
- Verified smoking status with exhaled breath carbon monoxide (CO) levels ≥7 ppm
- Willingness to switch to a C-F NA if randomized
- Refusal to participate in smoking cessation programs
- Presence of at least 10 natural anterior teeth (cuspid to cuspid, upper and lower jaws)
- Healthy individuals not on regular medications for chronic conditions

Participants were excluded if they met any of the following criteria:

- Significant oral soft tissue pathology or gingival overgrowth (excluding plaque-induced gingivitis)
- Periodontitis, defined upon the 2018 international classification of Periodontal and Peri-Implant Diseases, but with interdental CAL set at ≥3 mm to define periodontitis, allowing “health” to include incipient/mild interdental periodontal attachment loss:

Interdental clinical attachment loss (CAL) ≥3 mm at ≥2 nonadjacent teeth or buccal/oral CAL ≥3 mm with pocketing ≥3 mm at ≥2 teeth.

- Presence of fixed or removable orthodontic appliances or dentures
- Recent use of medications that:
  - Interfere with the cyclooxygenase pathway (e.g., anti-inflammatory drugs) within 3 days before study visits
  - Exhibit antibacterial activity (e.g., antibiotics) within 7 days before study visits
- Regular use of nicotine or tobacco products other than cigarettes within 14 days of screening
- Pregnancy, breastfeeding, or intention to become pregnant during the study period

The SMILE study was conducted in four dental clinics across Italy, Poland, Moldova, and Indonesia, selected to align with CoEHAR’s mission to promote oral health and smoking abstinence in low- and middle-income regions. Factors such as low operational costs, strong interest in participation, and high smoking prevalence influenced their choice. Italian site led training and coordination. All four countries' high smoking prevalence allowed the study to explore diverse socioeconomic, healthcare, and cultural factors influencing smoking behavior. The variation in healthcare systems (from well-established in Italy and Poland to less developed in Moldova and Indonesia) strengthens the study’s global relevance. In addition, Indonesia’s widespread use of clove cigarettes ("kreteks") offers unique insights into cultural attitudes toward tobacco and nicotine use.

The SMILE study complies with the Declaration of Helsinki and Good Clinical Practice Guidelines and has been approved by the ethics committees of the four participating dental clinics. All participants provided written informed consent. The study is registered on ClinicalTrials.gov with the identifier NCT04649645 (https://clinicaltrials.gov/ct2/show/NCT04649645) and on the International Registered Report Identifier (IRRID) with the identifier DERR1–10.2196/53,222. Further information can be obtained on the website https://smilestudy.coehar.org

2.4. Study endpoints

2.4.1. Primary endpoints

2.4.1.1. Modified gingival index (MGI). Modified Gingival Index (MGI) was selected as the primary endpoint due to its reliability and sensitivity in detecting gingival inflammation, assessed at color change [24]. Given the strong association between smoking and periodontal disease, MGI provides a direct measure of smoking’s impact on oral health. Widely used in clinical studies [24–26], MGI is non-invasive, easy to administer, and validated, making it ideal for longitudinal research studies like SMILE. Higher MGI values reflect poorer gingival health, as they indicate increased levels of inflammation. A mean change of 0.08 in MGI is considered clinically meaningful [27,28], offering a clear and quantifiable metric for assessing the impact of smoking cessation and tobacco harm reduction strategies on oral health.

The MGI scoring system evaluates gingival inflammation by visually inspecting both papillary and marginal units of each tooth, using a five-point ordinal scale. A score of 0 indicates normal, healthy gingiva; 1 corresponds to mild inflammation with slight color change and edema but no bleeding; 2 reflects mild inflammation affecting the entire gingival unit; 3 denotes moderate inflammation with redness, edema, and glazing; and 4 indicates severe inflammation, characterized by marked redness, edema, ulceration, and a tendency to spontaneous bleeding. The final score is calculated as the average across all assessed sites, yielding a global indicator of gingival health.

2.4.2. Secondary endpoints

These secondary endpoints address factors like bad breath and dental aesthetics, which can be more persuasive for young adults than traditional risk-based narratives focused on cardiovascular, cancer, or respiratory risks. This approach may better motivate smoking cessation in this demographic group.

2.4.2.1. Dental plaque and calculus accumulation by quantitative light-induced fluorescence (QLF). Dental plaque and calculus accumulation were accurately assessed using the Qraycam™ Pro (AIOBIO Co. Ltd., Seoul, Republic of Korea), a high-resolution, handheld, auto-focus quantitative light-induced fluorescence (QLF) camera. The device uses a 405 nm violet-blue excitation light source to induce red autofluorescence from porphyrins produced by bacterial metabolism. This phenomenon allows for the detection and quantification of dental plaque based on fluorescence intensity, providing a sensitive and non-invasive method for evaluating plaque accumulation and maturation. Images of the anterior teeth were captured by the same operator following the standard QLF digital photography protocol and under identical low level ambient light conditions. To standardize positioning the camera was placed as close as possible to the teeth, with a black rubber collar attached to the lens. The auto-focus function of the camera was used to focus on the maxillary lateral incisor and canine (focal depth 0.32). Both white-light and QLF images were captured automatically and recorded using Q-Ray™ software (v.1.41, Inspektor Research Systems BV, Amsterdam, NL). Fluorescence images of the vestibular surfaces of the anterior teeth (cuspid to cuspid, upper and lower jaw) were saved as bitmap files and analyzed using the proprietary software (v.1.41, Inspektor Research Systems BV, Amsterdam, NL). Two key parameters were used for the analysis: ΔR30 and ΔR120 [29]. The ΔR30 parameter indicates the percentage of the tooth surface showing at least a 30 % increase in fluorescence, corresponding to the total area of mature dental plaque. The ΔR120 represents regions with a fluorescence increase of at least 120 %, indicating areas of thicker and more mature

deposits, including calculus.

Recent clinical trials conducted at the Center of Excellence for the Acceleration of Harm Reduction (CoEHAR) have demonstrated the robustness and consistency of QLF technology in measuring dental plaque and calculus accumulation including in people who smoke [30,31]. These findings support the use of QLF as a standardized, objective alternative to conventional semiquantitative indices, offering improved reliability and reproducibility in oral health assessments [29,32–34].

2.4.2.2. Dental shade assessment by digital spectrophotometry. The SMILE study used advanced digital technologies to objectively quantify tooth color, employing digital spectrophotometry (Vita Easyshade V) for precise tooth shade measurements. Assessments were conducted under identical ambient lighting conditions, and by the same operator. The device was calibrated and operated according to the manufacturer's instructions. Color measurements targeted the central area of each anterior tooth (i.e., cuspid to cuspid, upper and lower jaw) using the "base shade determination" mode. The measuring tip of the spectrophotometer has a diameter of 5 mm, thus assessing a circular area of approximately 5 mm in diameter on the tooth surface. CIELAB color parameters were recorded, and average values per subject were calculated by summing individual tooth values and dividing by the number of teeth assessed. Tooth whiteness was further evaluated using the Whitening Index for Dentistry (WID), calculated as $WID = 0.511L^* - 2.324a^* - 1.100b^*$ [35]. Each participant's overall WID score was determined by averaging the WID values across all tested anterior teeth. Higher WID values indicate whiter teeth, while lower values suggest greater discoloration (i.e., less white teeth). Differences in WID were interpreted against the 50:50 % acceptability threshold (WAT = 2.90 ΔWID units), with ΔWID differences greater than 2.90 considered visibly noticeable [35].

Separate studies from CoEHAR have also validated the precision and repeatability of digital spectrophotometry for assessing tooth shade including in people who smoke [36,37]. This technology has proven to be a reliable and standardized method for objectively quantifying dental discoloration, surpassing the subjectivity and variability of traditional visual scoring systems.

2.4.2.3. Tooth stain scores. Extrinsic staining of the anterior teeth was assessed using the MacPherson modification of the Lobene Stain Index (MLSI) [38], a validated method for quantifying dental discoloration.

2.4.2.4. Oral health–related quality of life (OHRQoL). Oral health–related quality of life (OHRQoL) was assessed using a structured self-administered questionnaire, completed independently by each participant. This 16-item instrument was developed to capture the general population's perceptions of how oral health impacts overall quality of life [39]. The questionnaire covers three main domains: daily activities, social interactions, and communication problems.

2.4.2.5. EuroQo visual analogue scale (EQ-VAS). General health status was assessed using the EuroQo Visual Analogue Scale (EQ-VAS), a self-reported measure included in the EuroQol EQ-5D instrument. This tool is widely used in health outcomes research and provides a quantitative estimate of the respondent's perceived well-being at the time of assessment [40].

2.4.2.6. Simple oral hygiene (SOH) score. The QLF proprietary software integrates fluorescence intensity data to calculate the Simple Oral Hygiene (SOH) score, also referred to as the Fluorescent Plaque Index (FPI), ensuring consistency in terminology [29]. This method offers a standardized, objective, and reproducible approach to quantifying plaque in clinical and research settings [29,32,33].

2.4.3. Data management and quality procedures

To ensure standardized data collection and high-quality outcomes, all study-related procedures such as data management, assessor training, and calibration were implemented following harmonized protocols. A web-based electronic case report form (eCRF) was used for data entry, with all investigators trained in its use and monitored throughout the study by an independent data management team. Prior to participant enrollment, calibration workshops and site-specific training sessions were conducted to align the use of assessment tools (e.g., MGI, QLF, spectrophotometry). Periodic recalibration and quality control procedures were carried out during the study period, including inter-rater reliability checks, equipment calibration, and image quality verification. Full methodological details are available in **Appendix 2**.

2.4.4. Statistics

We conducted a cross-sectional analysis of data collected at enrollment (Visit 0) from 460 smokers enrolled in the SMILE Study cohort [23]. Descriptive statistics were used to compare baseline characteristics of participants recruited from four dental clinics located in Italy, Poland, Moldova, and Indonesia. Summary statistics for demographic information and variables collected at the enrollment visit are presented for the whole sample and for each study center.

Descriptive statistics were used to summarize the data: means and standard deviations were reported for normally distributed continuous variables, while medians and interquartile ranges (IQRs) were used for non-normally distributed variables. Categorical variables were presented as absolute counts and relative frequencies (percentages).

For between-group comparisons, Analysis of Variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) post hoc test was applied to normally distributed variables. For non-normally distributed variables, the Kruskal–Wallis test with multiple pairwise comparisons was used. Chi-square tests were conducted to assess differences in frequency distribution of categorical variables.

Due to substantial skewing in the distribution of certain variables (i.e., MGI, age, number of pack/yrs), normalization of non-normally distributed variables was done by mean and standard deviation, resulting in a normally distributed equivalent variable presenting the same mean and standard deviation as the original variable, and suitable for further parametric analysis.

Additional exploratory analyses were conducted using multiple regression models to evaluate the impact of baseline clinical and demographic variables on selected outcome measures. These models accounted for potential confounding factors, ensuring a more accurate estimation of associations.

The full prospective and comparative analysis of the SMILE Study, including data from subjects randomized and followed for the entire 18-month study duration, will be presented in separate publications.

The analyses were carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), all the tests were 2-sided and a p value < 0.05 was considered significant.

3. Results

Out of 460 subjects evaluated 109 (23.7 %) were of Asian ethnicity, 351 (76.3 %) White/Caucasians. Age (median[interquartile range - IQR] was 30.0[25.0–37.0] years. The overall Female/Male ratio was 25.0/75.0 %. In Table 1 the distributions of age, ethnicities, Female/Male ratios, number of cig/day, number of pack/yrs, and FTND, across sites are reported with the relevant differences between study centers.

A statistically significant difference in age distributions was observed among the study centers (Kruskal–Wallis test, $p < 0.0001$) (Fig. 1). Pairwise comparisons revealed significant differences between most centers, with the exception of "Italy vs Indonesia" and "Moldova vs Poland".

Overall WID score was (mean±SD) 14.7 ± 5.7. The difference across sites was significant (One-way ANOVA, $p < 0.0001$) (Fig. 2**)**. WID score

Table 1
Baseline demographic characteristics and smoking history of the 460 participants in the SMILE study cohort stratified by study center.

Centres	Italy	Moldova	Poland	Indonesia	p value
No. (% of total)	155 (33.7)	153 (33.3)	43 (9.3)	109 (23.7)	-
Age (median[IQR])	27.0[24.0–32.0]	33.0[27.0–41.0]	40.0[31.3–52.5]	29.0[25.0–34.0]	<0.0001§
Ethnicity (No.[%])	Wh/C 155 (100)	Wh/C 153 (100)	Wh/C 43 (100)	Asian 109 (100)	<0.0001#
F/M ratio (No.[%])	51/104 (33/67)	38/115 (25/75)	18/25 (42/58)	8/101 (7/93)	<0.0001#
No. of cigarette/day (median[IQR])	15.0 [12.0–20.0]	19.0[15.0–20.0]	20.0[15.0–20.0]	12.0[9.8–17.0]	<0.0001§
No. of pack/year (median[IQR])	7.5[6.0–12.9]	9.0[5.0–14.3]	20.0[7.6–28.0]	6.0[3.6–10.5]	<0.0001§
FTND (mean±SD)	4.9 ± 1.9	4.8 ± 1.8	5.8 ± 2.0	3.3 ± 2.0	<0.0001‡

IQR: interquartile range; Wh/C: White/Caucasians; F/M: Female/Male; FTND: Fagerstrom Test for Nicotine Dependence.
§ Kruskal-Wallis test;.
# χ^2 test;.
‡ one-way ANOVA.

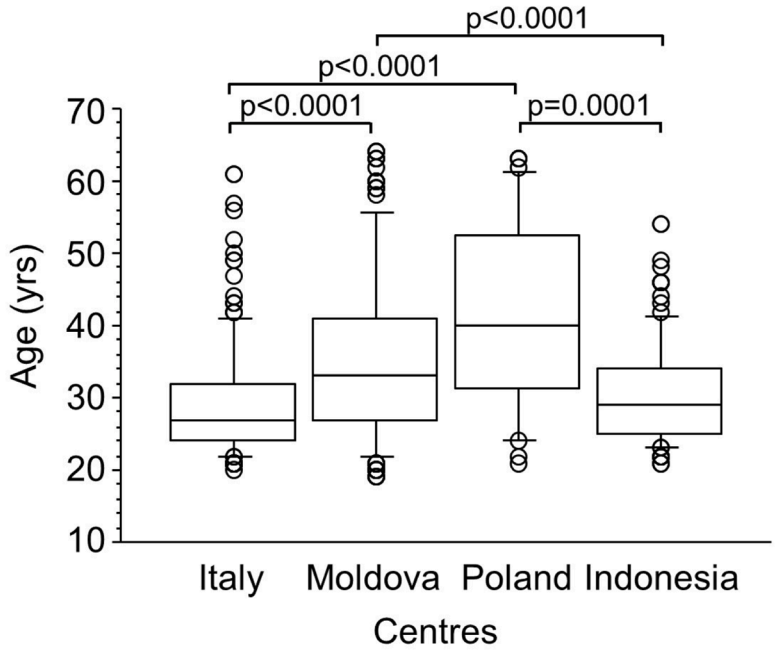

Fig. 1. Age distribution across study sites. Age was significantly different among sites (Kruskal-Wallis test, $p < 0.0001$). The pairwise comparisons were significantly different, with the exception of the Italy vs Indonesia and Moldova vs Poland comparisons. Boxplot horizontal bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

resulted significantly higher in Italy than in Moldova and Indonesia (Fisher's Least Significant Difference).

WID score resulted significantly and negatively correlated to both age and smoking history, expressed as number of pack/years (**Appendix 3**). When evaluated separately per each site, the correlations between WID Score and age were no longer significant for Italy and Poland, while remained significant for Moldova (slope −0.105, $p = 0.0007$) and Indonesia (slope −0.140, $p = 0.019$). As concerns the correlations between WID Score and number of pack/years, these were significant with the exception of Indonesia (Italy: slope −0.153, $p = 0.024$; Moldova: slope −0.203, $p < 0.0001$; Poland: slope −0.248, $p = 0.014$).

Table 2 presents a multiple linear regression model with WID score as the outcome variable. Independent variables included ethnicity, sex, age, and pack-years. Ethnicity was significantly and negatively associated with WID score, with Asian participants showing lower values compared to White/Caucasians ($B = –1.39$, $p = 0.030$; reference = White/Caucasians). Female sex was significantly and positively associated with WID score ($B = 2.81$, $p < 0.0001$; reference = males). Age showed a significant negative association ($B = –0.09$, $p = 0.003$). In contrast, pack-years were not significantly associated with WID score in the multivariable model. Although pack-years correlated to WID score in the overall sample ($p < 0.0001$), this association was no longer significant once ethnicity, sex, and age were included in the regression model. This suggests that ethnicity, sex, and age had a more substantial influence on WID scores than smoking history alone

The MGI score was (median[IQR]) 1.00[0.44–1.72]. In Fig. 3 the distribution of MGI is presented, separately per sites. MGI was significantly different among sites (Kruskal-Wallis test, $p < 0.0001$), with the Indonesian site showing the lowest score compared to all other centers ($p < 0.0001$ for all pairwise comparisons).

A significant positive correlation was observed between MGI and smoking history (expressed as number of pack-years) in the overall sample (**Appendix 4**). When this association was examined separately by study sites, it remained statistically significant only for the Italian site (slope 0.010, R^2 0.020, $p = 0.045$) and the Moldavian site (slope 0.031, R^2 0.174, $p < 0.0001$). In contrast, no significant correlation was observed between MGI and age in either the overall cohort or individual centers.

In a multiple linear regression model with MGI as the outcome variable, ethnicity, age, and smoking history (number of pack-years) were all significantly associated with MGI, whereas sex was not (Table 3). Participants of Asian ethnicity had significantly lower MGI scores compared to White/Caucasian participants ($B = −0.70$, $p < 0.0001$;

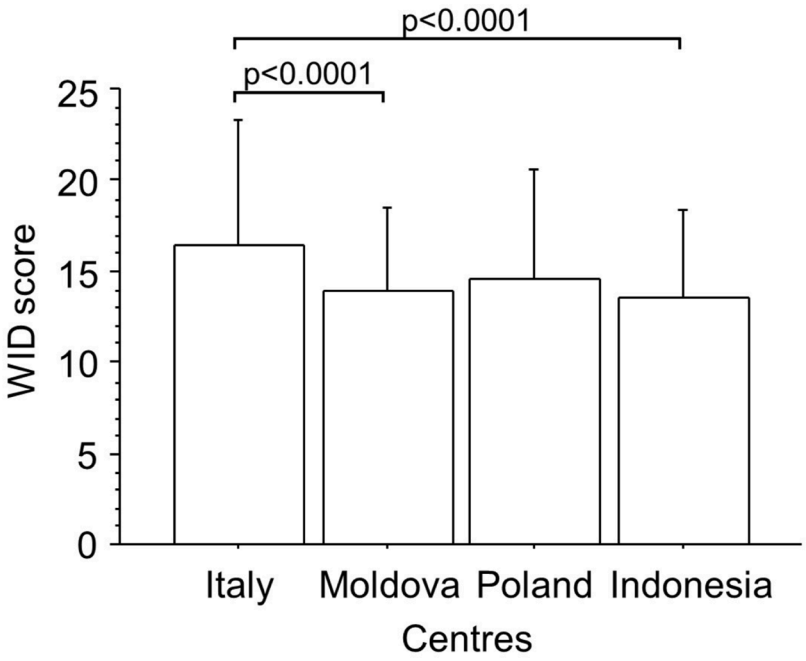

Fig. 2. Whitening Index for Dentistry (WID) scores across study sites. WID score was significantly higher in Italy than in Moldova and Indonesia. No significant difference was found among Moldova, Poland and Indonesia (one-way ANOVA and Fisher's Least Significant Difference). Bars indicate mean+standard deviation (SD).

Table 2
Multiple linear regression model for WID score. B values and relevant 95 % confidence interval (95 %CI) are reported for each independent variable.

Variable	B	95 % CI	p value
Ethnicity (Asian, Ref. White/Caucasian)	−1.39	−2.65/−0.14	0.030
Sex (Female, Ref. Male)	2.81	1.58/4.04	<0.0001
Age (normalized)	−0.09	−0.16/−0.03	0.003
No. of Pack/yrs (normalized)	−0.05	−0.12/0.02	0.155

Adjusted R^2: 0.118.

reference group: White/Caucasian). Age showed a small but statistically significant negative association with MGI ($B = -0.01$, $p = 0.003$). Smoking history (pack-years) showed a small but statistically significant positive association ($B = 0.02$, $p < 0.0001$). No significant association was found between sex and MGI in the multivariable analysis.

The distribution of R30 values differed significantly across study sites (Kruskal–Wallis test, $p < 0.0001$; Fig. 4**A**). In pairwise comparisons, only the Indonesia center exhibited significantly lower median R30 values compared to Italy and Moldova, while no significant difference was observed with Poland. In contrast, R120 values did not differ significantly between sites (Fig. 4**B**). In simple linear regression models, normalized R30 resulted weakly positively correlated to number of pack/years (slope 0.078, R^2 0.012, $p = 0.021$). Normalized R120 was also significantly weakly positively correlated to number of pack/years (slope 0.047, R^2 0.013, $p = 0.018$).

The distribution of MLSI - Buccal scores was significantly different across sites (Kruskal-Wallis test, $p < 0.0001$, Fig. 5**A**). Pairwise comparisons revealed that all center-to-center differences were statistically significant, except for Italy vs. Moldova and Moldova vs. Poland. The distribution of MLSI - Lingual scores also differed significantly across sites (Kruskal-Wallis test, $p < 0.0001$, Fig. 5**B**). In this case, Italy vs. Poland and Moldova vs. Poland were the only pairwise comparisons that were not statistically significant, whereas all other comparisons showed significant differences.

The SOH score differed significantly across study sites (Kruskal–Wallis test, $p < 0.0001$), and higher in Moldova compared to Indonesia (Fig. 6**A**). Similarly, OHQOL scores also varied significantly across sites (Kruskal–Wallis test, $p < 0.0001$); the Moldova site reporting the highest median OHQOL score compared to all other participating centers and the Indonesia site had a significantly higher median OHQOL score compared to Italy (Fig. 6**B**). Finally, the QoL EQ VAS Score also showed a statistically significant difference across sites (Kruskal–Wallis test, $p < 0.0001$). The Italian site had a significantly lower median QoL EQ VAS score compared to Indonesia and Moldova (Fig. 6**C**).

In Table 4 and **Appendices 5–7**, the individual characteristics of oral hygiene habits are presented: frequency of domiciliary oral hygiene (Table 4), type of toothbrush usage (**Appendix 5**), frequency of mouthwash use (**Appendix 6**), and frequency of dental floss use (**Appendix 7**). In all the evaluation the frequency distributions were significantly different across study sites (χ^2 test, $p < 0.0001$).

4. Discussion

The SMILE study stands as one of the largest and most comprehensive investigations into the oral health and dental aesthetics of smokers to date [23]. By enrolling participants from four culturally and socioeconomically distinct countries (i.e. Italy, Poland, Moldova, and Indonesia), this study provides valuable insights into how ethnicity, age, smoking habits, and local healthcare contexts may influence oral health markers in smokers. The high smoking prevalence in all study sites enhances the relevance of the findings to real-world smoking populations.

Cigarette consumption patterns varied significantly across countries in the SMILE study, mainly reflecting cultural norms, behavioral patterns, and product availability. World Bank data show Indonesia with one of the world's highest male smoking rates, 71.4 % in 2020 [41]. This high prevalence is distinct with widespread use of clove ('kretek') cigarettes, chemically different from conventional ones, and lower reported daily consumption. Baseline data (Table 1) confirm this, with the Indonesian cohort showing the lowest median cigarettes per day and cumulative exposure. These factors (reduced daily intake and use of unconventional products) may underlie the more favorable baseline clinical profiles in this group, particularly for gingival inflammation and plaque accumulation.

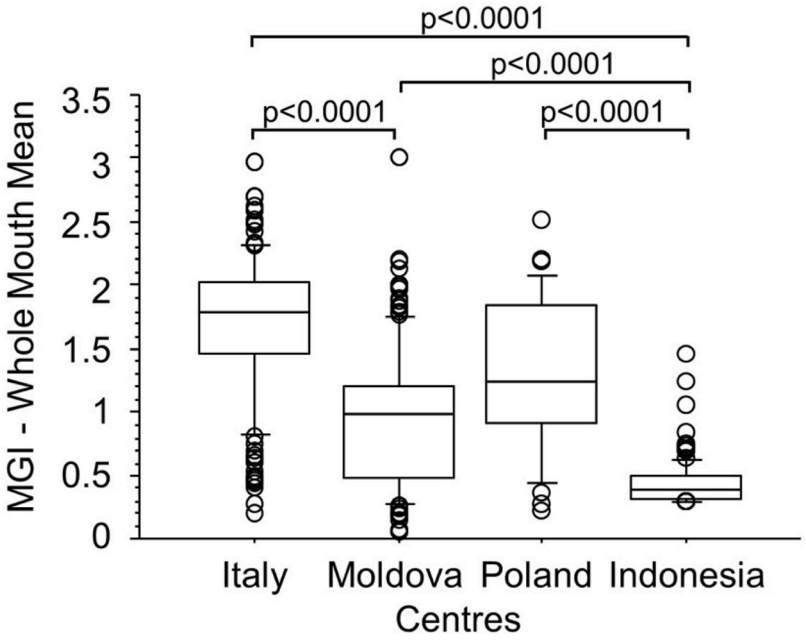

Fig. 3. Modified Gingival Index (MGI) score distribution across study sites. The pairwise comparisons were significantly different (Kruskal-Wallis test, $p < 0.0001$), with the exception of Poland vs Moldova and Poland vs Italy comparisons. Boxplot horizontal bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

Table 3
Multiple linear regression model for normalized Modified Gingival Index (MGI). B values and relevant 95 % confidence interval (95 %CI) are reported for each independent variable.

Variable	B	95 % CI	p value
Ethnicity (Asian, Ref. White/Caucasians)	−0.70	−0.84/−0.56	<0.0001
Sex (Female, Ref. Male)	0.01	−0.14/0.13	0.922
Age (normalized)	−0.01	−0.02/0.00	0.003
No. of Pack/yrs (normalized)	0.02	0.01/0.03	<0.0001

Adjusted R^2: 0.285.

The observed differences in participant age, sex distribution, ethnicity, and cigarette consumption across centers reflect the distinct recruitment strategies employed at each site. For instance, the younger age in Italy and Indonesia may be attributed to active recruitment among university students and healthcare staff, whereas older participants in Poland were mostly sourced from hospital patient databases.

Sex distribution was notably skewed, especially in Indonesia, where 93 % of participants were male. This reflects national smoking prevalence data [41] and highlights the need to consider socio-cultural smoking patterns when interpreting baseline oral health and aesthetic outcomes, as sex-related biological and behavioral factors may affect both smoking behavior and oral health. However, sex did not emerge as a significant predictor in the multivariable models for MGI, although it was strongly associated with tooth color WID, with females reporting significantly higher WID values. This finding is consistent with literature indicating that women often exhibit better oral hygiene practices and are more concerned with dental aesthetics, potentially leading to higher tooth whiteness scores [42,43].

To the best of our knowledge, no prior studies have reported on differences in tooth shade across populations. In addition, this is one of the first multicenter trials to implement the WID in the clinical evaluation of smokers, providing a standardized and objective measure of dental discoloration. In the SMILE study, baseline analysis of the WID by digital spectroscopy revealed substantial variation between sites, with

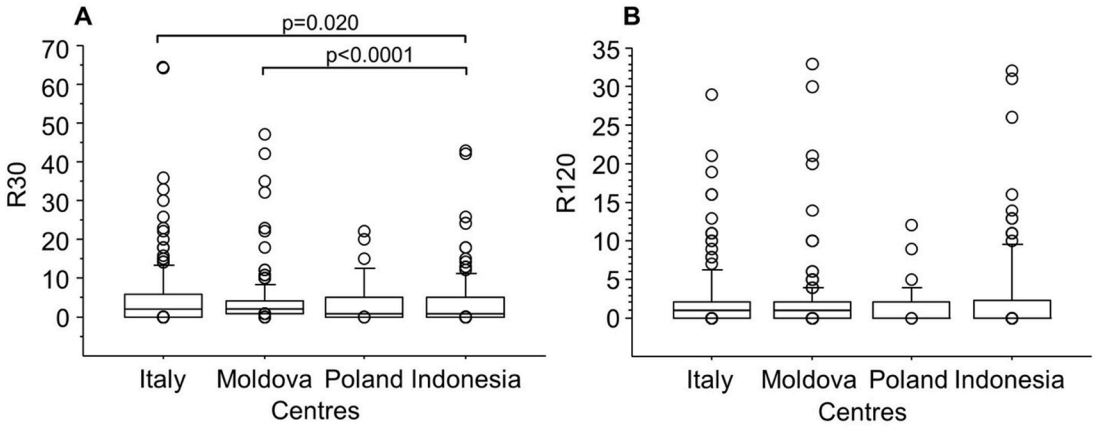

Fig. 4. Distribution of R30 (panel A) and R120 (panel B) among study sites. R30 was significantly different across sites (Kruskal-Wallis test, $p < 0.0001$). For R120 no difference was found in variable distribution across sites. Boxplot horizontal bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

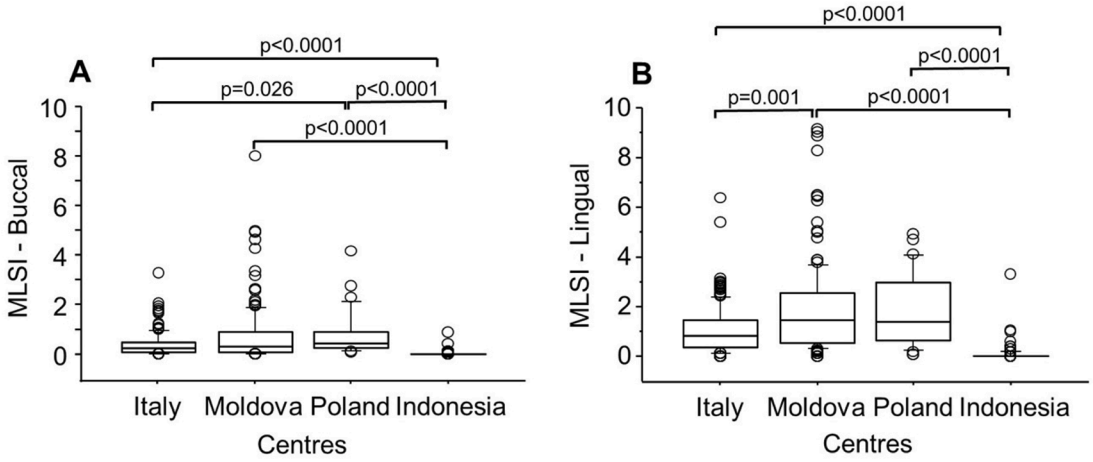

Fig. 5. Distribution of MLSI - Buccal (panel A) and MLSI - Lingual (panel B) across study sites. Both MLSI - Buccal and MLSI - Lingual were significantly different among across sites (Kruskal-Wallis test, $p < 0.0001$). Boxplot horizontal bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

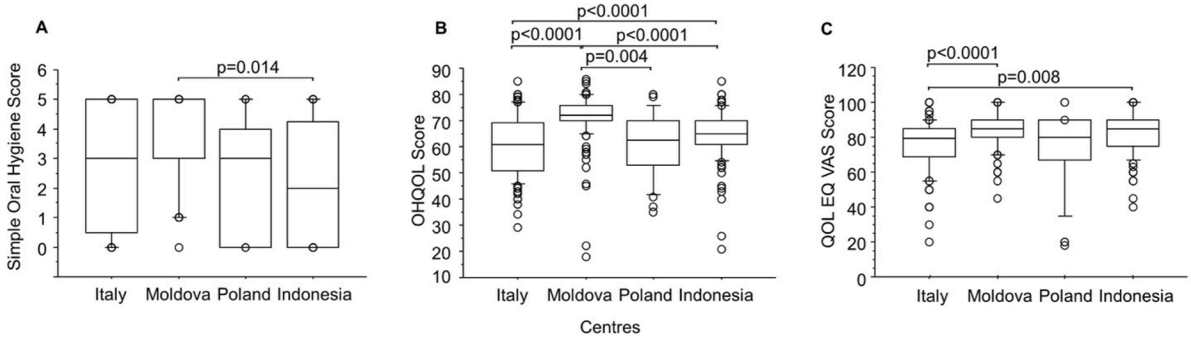

Fig. 6. Distribution of Simple Oral Hygiene Score (panel A), OHQOL Score (panel B) and QoL EQ VAS Score (panel C) across study sites. All the three indexes were significantly different among centres (Kruskal-Wallis test, $p < 0.0001$). Boxplot horizontal bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

Table 4
Frequency distribution of the type of frequency of domiciliary oral hygiene in different centres.

Frequency of oral Hygiene	Italy (N[%])	Moldova (N[%])	Poland (N[%])	Indonesia (N[%])
Once/day	11 (7)	76 (50)	5 (17)	16 (15)
Two or more/day	142 (93)	77 (50)	25 (83)	93 (85)

The difference among centres was significant ($\chi 2$ test, $p < 0.0001$).

the highest scores observed in the Italian cohort. The WID scores were found to be inversely correlated with increasing age and cumulative smoking exposure (pack-years), in line with findings reported in previous studies [44–46]. However, in multivariable analysis, the effect of pack-years was no longer significantly, suggesting that age, sex, and ethnicity may play a more prominent role on tooth whiteness than smoking exposure alone. These findings are biologically plausible because with aging, teeth undergo structural changes such as increased dentin deposition and enamel thinning, which contribute to a darker appearance over time [47]. Moreover, exposure to tobacco combustion products leads to progressive extrinsic enamel staining, further decreasing WID scores [48,49].

Gingival health, as measured by the MGI, also varied significantly across study centers. The Indonesian cohort showed the lowest MGI scores, a finding that persisted even after adjusting for age, ethnicity, and smoking exposure (pack-years). Multivariate regression confirmed that MGI was independently associated with ethnicity (lower in Asians), age (marginal inverse effect), and pack-years (positive association). These findings reinforce the well-established role of smoking in gingival inflammation and suggest that cumulative exposure, rather than current smoking alone, is a more reliable predictor of early inflammatory changes in smokers [4,7]. There is broad consensus among periodontists on the detrimental impact of smoking on gingival health [50,51]. Microvascular constriction induced by acute smoking may reduce visible signs of inflammation such as bleeding on probing (BOP) [52,53]; yet, this does not preclude the persistence of underlying immune-inflammatory processes [54]. To mitigate the vasoconstrictive effects of recent smoking, participants in our study were instructed to refrain from smoking for at least two hours prior to clinical examination.

Although no significant association was observed between MGI and sex, a marginal inverse relationship emerged with age. This finding may reflect the cumulative nature of exposure to risk factors over time, such as smoking, plaque retention, and suboptimal oral hygiene practices. With increasing age, the progressive deterioration of periodontal tissues and potential shifts in the host immune response (immune senescence) may contribute to higher levels of gingival inflammation, even in the absence of overt clinical symptoms [55,56].

The lower MGI scores observed in the Asian cohort may, in part, reflect the characteristics of the recruited sample, which included a high proportion of healthcare and university staff - populations typically more engaged in preventive health behaviors, including oral hygiene and much reduced cigarette consumption. This hypothesis is supported by the lower R30 scores (i.e., indicative of reduced plaque formation) observed in the Indonesian group. Additional factors unique to the

Indonesian cohort may also have played a role. One is the high prevalence of gingival melanosis, more common in individuals with darker skin tones and may mask clinical signs of gingival inflammation [57]. Another is the widespread use of clove-containing kretek cigarettes, which are distinct from conventional tobacco products. Clove oil, a key ingredient in kreteks, contains eugenol and other antioxidants [58], which have demonstrated anti-inflammatory effects in vitro [59]. These observations suggest that clove oil may partially mask gingival inflammation in kretek smokers. Finally, dietary practices specific to the Indonesian population may also contribute to the lower gingival inflammation levels observed in this group. These interpretations should be viewed as plausible contextual explanations rather than direct conclusions from the present data.

While R30 values, reflecting total plaque coverage, were significantly lower in Indonesia, R120 values (i.e., indicative of more mature plaque or calculus) did not vary across sites. A weak but significant positive correlation between R120 and pack-years suggests that long-term smoking may contribute to advanced plaque maturation. The lower plaque accumulation in the Indonesian cohort aligns with their lower MGI scores, hypothetically reflecting better oral hygiene practices, widespread use of clove-containing kretek cigarettes, or dietary factors that inhibit plaque formation. In addition, these data support the potential utility of QLF imaging as a sensitive and standardized tool for detecting subtle differences in plaque dynamics in large-scale studies [29].

Tooth stain scores varied significantly between sites for both buccal and lingual surfaces, although no consistent pattern emerged. Differences might be explained by a combination of smoking behavior, oral hygiene practices, and cultural norms surrounding dental aesthetics. In particular, studies have shown that smoking status and frequency all contribute to tooth stain severity [18,60]. The observed differences in staining scores between buccal and lingual surfaces are not unexpected and likely reflect differences in accessibility during routine oral hygiene practices. Brushing is generally more effective on buccal surfaces due to their easier visibility and reach, while lingual areas, particularly in posterior teeth, are more difficult to access and clean thoroughly. This reduced mechanical plaque removal may facilitate greater stain accumulation on lingual surfaces over time. The use of a validated visual index (i.e., Macpherson-modified Lobene) provides a robust baseline for future longitudinal comparisons in participants switching to combustion-free nicotine alternatives [38].

The analysis revealed marked variability between centers in scores related to hygiene status (SOH score), oral health–related quality of life (OHQOL), and general quality of life (EQ-VAS). Moldova consistently reported higher scores, possibly reflecting cultural attitudes toward oral health, healthcare access, or participant motivation. Interestingly, Italy - despite having higher WID scores - showed lower EQ-VAS, suggesting that tooth appearance alone may not translate into broader perceptions of well-being. This underscores the multidimensional nature of oral health, influenced not only by clinical factors but also by psychological, cultural, and social elements [61].

The SMILE study is among the largest multicenter RCT in oral health. It explores oral health and dental aesthetics in smokers, with a rigorous design, standardized imaging technologies, and robust baseline characterization across four culturally diverse countries. The SMILE study design incorporates several innovative features. Across multiple international sites, the challenge was to select a primary endpoint that is simple, reproducible, and standardized. The MGI was chosen as a validated, noninvasive, and sensitive measure of gingival health [24]. Its simplicity enables examiner calibration across centers, reducing variability and allowing reliable comparisons. The absence of probing further minimizes operator bias and enhances sensitivity. Moreover, we selected secondary endpoints focused on dental aesthetics and oral health, more immediate drivers of behavior change for smokers, particularly when cardiovascular or cancer risks are less persuasive. This trial is the first to clinically test these objectives outcomes using digital spectrophotometry for tooth shade and quantitative light-induced fluorescence for plaque detection, both validated in prior CoEHAR studies [31,37].

Although some degree of inter-site variability is inherent to multicenter studies, several measures were implemented to minimize clinic-related bias, as explained in **Appendix 2**. Briefly, all investigators underwent centralized training and calibration sessions before study initiation, and Standard Operating Procedures (SOPs) were applied across sites for participant selection, equipment use, image acquisition, and data entry. Identical instruments were employed in all clinics, and periodic quality control audits ensured continued methodological consistency. Data were recorded in a centralized electronic case report form (eCRF) with built-in logic and range checks. Moreover, statistical analyses included site as a covariate, confirming that the observed differences were not driven by any single center.

When interpreting the results of the study, some factors and limitations need consideration. First, the recruitment strategies varied across sites, ranging from university populations to hospital databases, which may have introduced selection bias and contributed to differences in age distribution, sex ratio, and socioeconomic background. Such variability may limit comparability between centers and could influence oral health outcomes independently of smoking behavior. Second, although standardized protocols were implemented, unmeasured dietary factors (including consumption of foods and beverages known to affect enamel color or plaque accumulation) may have influenced tooth shade and gingival indices. Last, oral hygiene behaviors (e.g., brushing frequency, flossing, use of mouthwash) varied significantly between countries and participants, potentially affecting plaque accumulation, stain scores, and gingival health. While data on hygiene practices were collected, residual confounding is likely, given the self-reported nature of these measures. Fourth, the current paper focuses on baseline oral health and dental aesthetic outcomes, while behavioral and socioeconomic data - collected within the same study - will be discussed further in forthcoming publications dedicated to these dimensions. Finally, these cross-country comparisons should be interpreted as exploratory and descriptive, intended to illustrate real-world variability rather than to test specific hypotheses. They were not powered a priori, as the SMILE trial was primarily designed to evaluate longitudinal changes in oral health and aesthetic outcomes between randomized study arms. However, post-hoc analyses confirmed that the study had very high statistical power (>95 % at $\alpha = 0.05$) for both WID and MGI at baseline.

While the heterogeneity observed across centers may reflect local recruitment strategies (e.g. university staff versus hospital patients) and population characteristics, it does not compromise the internal validity of the SMILE trial. Randomization ensured a balanced distribution of potential confounding variables between the intervention and control arms. In addition, inclusion and exclusion criteria were standardized across all sites, as detailed in the published protocol, to guarantee that participants met consistent eligibility requirements and represented smokers with comparable oral health profiles. During the longitudinal phase, both within- and between-subject analyses will be adjusted for relevant covariates, ensuring a robust and unbiased assessment of treatment effects.

5. Conclusions

The SMILE study baseline findings provide a detailed characterization of oral health and aesthetic parameters in smokers across diverse cultural settings. The variability reported in WID and MGI reflects demographic (age, sex, ethnicity), behavioral (smoking history and patterns), and cultural influences. This heterogeneity should be viewed as a strength, enhancing the external validity and generalizability of the results. By capturing variation across demographic, cultural, and healthcare contexts, the study offers a comprehensive view of smoking-related oral health outcomes and informs tailored preventive strategies. Importantly, the use of standardized, objective cutting-edge

technologies provides robust benchmarks for evaluating smoking cessation and harm reduction strategies in future longitudinal analyses, while supporting clinicians in delivering personalized interventions.

Ethical statement

The SMILE study has been approved by the ethics committees of the four participating dental sites and is registered on ClinicalTrials.gov with the identifier NCT04649645 (https://clinicaltrials.gov/ct2/show/NCT04649645) and on the International Registered Report Identifier (IRRID) with the identifier DERR1–10.2196/53,222. Further information can be obtained on the website https://smilestudy.coehar.org. The study adhered to the Principles of Good Clinical Practice (GCP) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants included in the study.

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CRediT authorship contribution statement

Jan Kowalski: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Giusy Rita Maria La Rosa:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation. **Fabio Cibella:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Salvatore Urso:** Writing – original draft, Validation, Methodology, Conceptualization. **Riccardo Polosa:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2025.106229.

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