

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No./ Explanation
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	Title page, Abstract (p.1–2) – The design is described as “longitudinal”.
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract (p.2) – The abstract provides a concise and balanced summary of the study design, key findings, and conclusions
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction (p.3–4) – Discusses challenges in SHCN oral health and rationale for microbial analysis.
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction (last paragraph, p.4) – Objective: evaluate impact of oral hygiene management on periodontal microbiota across disability types.
Methods			
Study design	4	Present key elements of study design early in the paper	Materials and Methods (p.5) – Described as a prospective longitudinal cohort study with repeated GCF sample collection over three visits among SHCN individuals receiving periodontal treatment.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Materials and Methods (p.5–6) – The study was conducted at a university-based dental hospital between 2021 and 2023, with participant recruitment and periodontal treatment occurring during routine visits, and GCF samples collected at three timepoints over the follow-up period.
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	p.5–6; Supplementary Table S1. – Inclusion and exclusion criteria are detailed in Supplementary Table S1. Participants were SHCN individuals visiting the clinic up to three times over approximately six months, with follow-up intervals described in the Methods section.
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable. This study did not use a matched design.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	p.6–8, The primary outcome was bacterial detection levels in GCF assessed by qPCR. Key variables included brushing habits, caregiver support, and dietary practices. These were considered as potential predictors or confounders affecting

			microbial changes.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	p.6–8, Supplementary Tables S2–S8. Sources and measurement methods for questionnaire variables, GCF collection, and qPCR analysis are detailed. Identical assessment protocols were applied across all disability groups to ensure comparability.
Bias	9	Describe any efforts to address potential sources of bias	p.6-8, To minimize bias, standardized protocols were applied across all groups. GCF samples were collected by a single examiner to minimize inter-examiner variability. The prospective design with repeated sampling also helped reduce recall and temporal biases.
Study size	10	Explain how the study size was arrived at	p.8, Post-hoc power analysis using G*Power conducted to justify sample size.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	p.7, Quantitative variables (e.g., alpha diversity, bacterial abundance) analyzed via continuous measures or relative quantification; brushing frequency categorized; stratified by disability type.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	p.7, Kruskal–Wallis, Wilcoxon signed-rank, and PERMANOVA used; adjusted for disability type, brushing status, and visit number.
		(b) Describe any methods used to examine subgroups and interactions	p.5, Supplementary Figure S2–S3 – Subgroup analysis conducted by disability group, brushing status, and caregiver involvement.
		(c) Explain how missing data were addressed	p.5, Reasons for missing samples explained (e.g., missed visits); only cases with ≥2 visits included. Supplementary Figure S1 – Missing samples visualized.
		(d) If applicable, explain how loss to follow-up was addressed	p.5, Reasons for missed second or third visits described; only participants with ≥2 visits were included in longitudinal analysis.
		(e) Describe any sensitivity analyses	Not conducted.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and	p.8, Supplementary Figure S1 – Number of participants included and analyzed across 1st, 2nd, and 3rd visits reported.

		analysed	
		(b) Give reasons for non-participation at each stage	p.5, 8 – Reasons for missing follow-up visits (e.g., poor cooperation, appointment issues) described.
		(c) Consider use of a flow diagram	Supplementary Figure S1 – Flow diagram included to visualize participant tracking and sample inclusion.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	p.8-9, Supplementary Table S4 – Participant demographics, disability type, brushing habits, caregiver support, and systemic conditions detailed.
		(b) Indicate number of participants with missing data for each variable of interest	p.8, Supplementary Figure S1 – Missing values in questionnaire and GCF collection noted and visualized.
		(c) Summarise follow-up time (eg, average and total amount)	p.5,9, Follow-up intervals described; no explicit person-time calculation.
Outcome data	15*	Report numbers of outcome events or summary measures over time	p.9–10, Supplementary Table S5 – Bacterial abundance over time (qPCR results for each visit); Supplementary Figure 2–3, Longitudinal patterns stratified by disability type and oral hygiene behavior.
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	p.9–10; Supplementary Table S5 – Descriptive summaries stratified by disability type, oral hygiene status, and caregiver involvement were presented using p-values for intergroup comparisons. No multivariable models or adjusted estimates were reported.
		(b) Report category boundaries when continuous variables were categorized	p.7; Supplementary Table S2 – Brushing habits, diet, and caregiver support were categorized using predefined boundaries (e.g., brushing frequency $\geq 2/\text{day}$ vs. $< 2/\text{day}$), as described in the questionnaire and Methods.
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable – No relative risk estimates were calculated.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	p.10–11; Figures 2–3, Subgroup analyses conducted based on disability type, brushing habits, and caregiver support. No sensitivity analyses were conducted.
Discussion			
Key results	18	Summarise key results with reference to study	p.11–13, Key microbial and clinical

		objectives	findings summarized in relation to oral hygiene management and disability type, addressing study objectives.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p.13–14, Study limitations discussed, including small sample size, heterogeneity of disabilities, self-reported hygiene data, and limited generalizability. Potential bias direction noted.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p.13–14, Findings interpreted in light of clinical diversity, prior literature, and study limitations; implications for individualized oral health care in SHCN populations discussed.
Generalisability	21	Discuss the generalisability (external validity) of the study results	p.14, Generalisability discussed in terms of SHCN populations in institutional settings; limitations in broader applicability acknowledged.
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p.15 – Funding source disclosed; no role in study design, data collection, analysis, or manuscript preparation.

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.