

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation | Page No |
|---------------------------|---------|--|---|
| Title and abstract | 1 | (a) Indicate the study’s design with a commonly used term in the title or the abstract | Not included |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | Page 3 |
| Introduction | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | Objective on page 3 and Introduction page 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | End objective page 3 and end Introduction page 4 |
| Methods | | | |
| Study design | 4 | Present key elements of study design early in the paper | Methods section page 5 starts with selection countries, then data on prevalence in countries, data on population serum folate levels and folic acid supplementation. Elements of this method is described in last para of introduction page 4 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | Methods section pages 4-6 |
| Participants | 6 | (a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants | Methods section on selection countries ,prevalence data and serum folate levels pages 4-6 |
| | | (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case | N/A |

| | | | |
|---------------------------|----|---|--|
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | Outcome of number of NTDS defined in Data source – prevalence of NTD pregnancies page 5 |
| 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 | <i>NTD prevalence page 5</i> <i>Serum folate levels page 6</i> <i>Folic acid supplementation page 6</i> |
| Bias | 9 | Describe any efforts to address potential sources of bias | Sensitivity analysis of no supplementation vs 25% supplementation page 6 |
| Study size | 10 | Explain how the study size was arrived at | N/A |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | Described Calculation of Number of Neural Tube Defects Prevented page 7 |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | Described Calculation of Number of Neural Tube Defects Prevented page 7 |
| | | (b) Describe any methods used to examine subgroups and interactions | N/A |
| | | (c) Explain how missing data were addressed | Described page 7 |
| | | (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy | N/A |
| | | (e) Describe any sensitivity analyses | Page 6 No supplementation vs 25% supplementation. Page 6 two alternative methods of calculating population serum folate |

Continued on next page

| 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 analysed | Table 1 |
| | | (b) Give reasons for non-participation at each stage | N/A |
| | | (c) Consider use of a flow diagram | N/A |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | Table 2 |
| | | (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount) | Table 1 |
| Outcome data | 15* | <i>Cohort study</i> —Report numbers of outcome events or summary measures over time | <i>1st para results page 7</i> |
| | | <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure | |
| | | <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures | |
| 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 | Table 3 |
| | | (b) Report category boundaries when continuous variables were categorized | N/A |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | N/A |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | Table 3 and last para results page 8 |
| Discussion | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 1 st para discussion page 8 |
| 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 | Discussion page 8,9 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | Discussion page 9 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | Page 9 |
| 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 | Not funded |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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 www.strobe-statement.org.