**FORMAT FOR RESEARCH PROTOCOL**
**FOR SUBMISSION TO VIREC**

*[**Guideline;** Microsoft word file(.doc/.docx); Orientation- portrait; Margins-one inch (2.54cm) on all sides; Page numbering- bottom right; Length- typically 10-20 pages; Font-Times New Roman; Font size-14(main text) & 16 bold(headings) , Title case (title) ;Spacing-1.5lines; Paragraph alignment-center alignment(title page)justified(text); Heading alignment-left aligned ;subheadings-number format (1,1.1,1.1.1); Paper-single side of A-4 size good quality bond paper; Binding-left side soft bound]*

1. **Title Page( first page)**
   - Title of the Research Project
   - Full Name of the Principal Investigator
   - Department /Course & Subject [as applicable]
   - Name of Guide(s) [for PG-Thesis/UG-Studentship Project/Research Scholar]
   - University logo [for PG-Thesis]
   - University name [for PG-Thesis]
   - Institute logo
   - Institution name
2. **Introduction (start from second page)**
   - Broad research area
   - What is known
   - Knowledge gap and Research problem
   - Justification for the study
3. **Objective**
   - Research Question(PICO format)
   - Objectives (SMART format-Specific, Measurable, Achievable, Relevant, Time-bound)
   - Secondary Objectives (if any)
   - Research Hypothesis(for Analytical study/RCT only)
4. **Materials and Methods**

**4.1. Place of study**-institution; geographical area; geographic location

**4.2. Study setting**- exact unit /dept/locality of study

**4.3. Study design**-type of study: Qualitative/Quantitative/Mixed method//(e.g., cross-sectional, cross sectional analytical, cohort, RCT, qualitative)

**4.4. Study period& timeline**

**4.5. Sample size estimation;**

- Manual/software used
- Formula used
- Assumptions and parameters
- Final sample size (for each group/total)
- Justification if any

**4.6. Sampling Technique**

- Study population;
- Case definition - Inclusion and exclusion criteria
- Probability or non-probability technique
- Recruitment method and steps

### 4.7. Study Variables

- Independent, dependent, confounding variables
- Operational definitions in tabular form
  [name of variable, type, definition, scale of measurement, unit of measurement, remarks]

### 4.8. Data Collection Tools

- Instruments used (questionnaires, observation checklists, devices, etc.)
- Pretesting/Pilot study (if any)

### 4.9. Data Collection Procedure

- Survey/Observation/Experiment/Interview
- Step-by-step plan of how data will be collected
- Study Flowchart

## 5. Data Analysis Plan

- Data entry, Data cleaning, Missing data
- Tool/Software used [SPSS, R, etc.]
- Technique- Thematic/Statistical
- Statistical tests for each objective

## 6. Ethical Considerations

- Approval from Institutional Ethics Committee
- PIS –Participant Information Sheet details[in participant's own language]
- ICF-Informed Consent Form details [in participant's own language]
- Informed consent process
- Risk categorization
- Confidentiality, Privacy
- Special provision for vulnerable group/special group

## 7. Budget & Funding

- Approximate cost estimate
- Funding source [self-finance/sponsor]

## 8. Implications

- Relevance to public health/clinical practice/academics

## 9. References

- Vancouver style
- Manual/Tools [Zotero,End Note,Mendeley,etc.]

## 10. Annexure

1. Study Tools [Study Proforma/Questionnaire/Checklists/SOP/Device/Drugs/ Algorhythm, etc.]
2. Participant Information Sheet(PIS))(English + local language)
3. Informed Consent Form(ICF)(English + local language)
4. Ethical approval letter (when obtained)

**A PROPOSAL TEMPLATE**
**Title Page** (For PG Thesis Proposal)

# Adolescent Anaemia and Menstrual Hygiene: a Cross-Sectional Analytical School-Based Survey in Sambalpur District, Odisha

SYNOPSIS

for the degree of

MD/MS (_____subject_____)

under

Sambalpur University

By

Dr. _____

Postgraduate Student

under guidance of

Prof. _____

at

Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR)
Ayurvihar, Burla, Sambalpur, Odisha-768017
==========

**Title Page**
(for Non-Thesis Research Proposal)

# Adolescent Anaemia and Menstrual Hygiene: a Cross-Sectional Analytical School-Based Survey in Sambalpur District, Odisha

PROJECT PROPOSAL

By

Dr. _____

(designation)

at

Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR)
Ayurvihar, Burla, Sambalpur, Odisha-768017

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## . A RESEARCH PROPOSAL TEMPALTE

## 1. TITLE

**Adolescent Anaemia and Menstrual Hygiene: a Cross-Sectional Analytical School-Based Survey in Sambalpur District, Odisha**

## 2. INTRODUCTION

### 2.1.Broad Research Area

Adolescence is a critical period of growth and development, especially for girls, as it marks the transition into reproductive maturity. However, it is also a phase when many health issues emerge, most notably anaemia. Globally, anaemia is a widespread public health problem, with adolescent girls among the most affected due to rapid growth, menstrual blood loss, and dietary inadequacies. According to the **NFHS-5 (2019–21)**, the prevalence of anemia among Indian adolescent girls (15–19 years) was **59.1%**, showing a concerning rise from previous years. In Odisha, the prevalence is even higher, particularly in rural and underserved areas like **Sambalpur district**, where anaemia among girls significantly impairs cognitive development, physical capacity, and reproductive health outcomes.

Menstruation, a physiological milestone during adolescence, often becomes a neglected aspect of health due to widespread taboos, limited access to sanitary materials, and lack of awareness about hygienic practices. Poor menstrual hygiene has been linked with reproductive and urinary tract infections, but its possible association with nutritional status, particularly anaemia, remains underexplored. Inadequate menstrual hygiene may increase susceptibility to chronic low-grade infections, leading to inflammation and micronutrient loss, potentially aggravating anaemia among adolescent girls.

### 2.2.What is known

Several studies across India have assessed either anaemia prevalence or menstrual hygiene practices among adolescent girls independently. For example, studies in

Uttar Pradesh, Tamil Nadu, and Maharashtra have documented that while menstrual hygiene awareness is increasing, a large number of girls still rely on unhygienic methods such as old cloths, leading to health complications. Similarly, cross-sectional surveys have reported high anemia prevalence in both rural and urban school populations, often linked to poor dietary diversity and inadequate iron intake. Some emerging evidence suggests a correlation between poor hygiene during menstruation and increased anemia risk, possibly due to systemic inflammation or coexisting infections.

However, most available studies have focused on either health education outcomes or sanitary pad usage patterns. Very few have adopted an analytical approach to study the **direct association between menstrual hygiene practices and anemia status** in adolescent schoolgirls. Moreover, Odisha — particularly **Sambalpur district** — lacks district-specific, school-based data that explores this link, despite having a large population of adolescent girls vulnerable to both poor hygiene and nutritional deficiencies.

### 2.3. Knowledge Gap and Research Problem

While national-level data underline the severity of adolescent anemia, there is limited evidence on how behavioral and hygiene-related factors, especially menstrual hygiene practices, contribute to this burden. Most studies do not account for confounding factors such as socioeconomic status, dietary habits, or parental education, and few have been conducted in Odisha's western districts. Therefore, there exists a significant **knowledge gap** in understanding the multifactorial causes of adolescent anemia in specific socio-cultural and geographic contexts.

This gap is especially relevant to Sambalpur district, where socio-economic constraints, cultural beliefs, and lack of health education may lead to both poor menstrual hygiene and dietary deficiencies. Thus, the research problem is to assess whether there exists a statistically significant **association between**

**menstrual hygiene practices and anaemia status** among adolescent girls in schools, using an analytical cross-sectional approach.

### 2.4. Justification for the Study

Given the persistent burden of anaemia despite national programs like **Anaemia Mukt Bharat (AMB)** and **Rashtriya Kishor Swasthya Karyakram (RKSK)**, there is a pressing need for targeted, evidence-based interventions. This study aims to generate district-level data on menstrual hygiene and anaemia among adolescent girls in a school setting. The school environment provides a controlled, accessible platform to collect reliable data and implement future interventions.

By identifying modifiable behavioural factors like menstrual hygiene, this study has the potential to inform local health authorities and school health programs. The findings could support the design of integrated anaemia control strategies that include hygiene education, thereby improving adolescent health outcomes and contributing to national goals for women's health. Ultimately, this research aims to bridge a critical gap and offer practical insights for public health policy, clinical screening, and school-based health initiatives.

## 3. OBJECTIVES

### 3.1.Research Question

Is there an association between menstrual hygiene practices and the prevalence of anaemia among adolescent school girls in Sambalpur district?

### 3.2.Research Objectives

**Primary Objective**

1. To assess the association between menstrual hygiene practices and anaemia among adolescent school girls in Sambalpur district.

**Secondary Objectives**

1. To estimate the prevalence of anaemia among adolescent girls.
2. To evaluate the menstrual hygiene knowledge, attitudes, and practices (KAP) among the study population.
3. To identify socio-demographic and nutritional factors associated with poor menstrual hygiene and anaemia.

## 3.3. Research Hypotheses:

**Null Hypothesis ($H_0$):**
There is no significant association between menstrual hygiene practices and anemia among adolescent school girls in Sambalpur district.

**Alternative Hypothesis ($H_1$):**
There is a significant association between poor menstrual hygiene practices and increased prevalence of anemia among adolescent school girls in Sambalpur district.

# 4. METHODOLOGY

## 4.1.Place of Study:

The study will be conducted in selected government and private high schools located in Sambalpur district, in the western part of Odisha, India. Sambalpur is a culturally and demographically diverse district with both urban and rural areas, offering a representative population of school-going adolescent girls.

Sambalpur district lies between 21.27°N latitude and 83.98°E longitude. It is situated in the Mahanadi River basin region and is bordered by Bargarh, Deogarh, Angul, Jharsuguda, and Sundargarh districts.

## 4.2.Study Setting:

The study will be school-based, involving adolescent girls aged 10–19 years from Classes 6 to 12. Both urban and rural schools under the District Education Office will be selected using stratified random sampling. Data collection will be carried out within school premises, during school hours, after obtaining necessary permissions from school authorities and parental consent.

The setting allows for access to a captive, age-appropriate population, standardized procedures for haemoglobin testing and educational context to explore knowledge, attitude, and practices (KAP) regarding menstrual hygiene.The study site has access to primary healthcare centres (PHCs) and community health centres (CHCs), allowing timely referral for participants found to be anaemic.

## 4.3. Study Design

This study will adopt a **school-based cross-sectional analytical design**. A cross-sectional design is appropriate for assessing the **prevalence** of anemia and menstrual hygiene practices, as well as examining the **association** between the two at a single point in time.

## 4.4.Study Period

### 4.4.1. Duration:

The project will be conducted during Jun-2025–Sep-2027 for approximately 27 months during the post graduation period.

### 4.4.2. Timeline:

| Activity | Duration (Period) | Month |
|---|---|---|
| 1. Proposal Preparation & Review | Month 1-3(3months) | May-Jul-2025 |
| 2. Ethical Clearance | Month 3 (1months) | Aug-2025 |
| 3. Protocol Submission & Registration | Month 3 (1months) | Aug-2025 |
| 4. Tool Development, Pre-testing, Training of Investigators, Field Preparation, & Permissions | Month 4-5(1month) | Sept-Oct-2025 |
| 5. Data Collection Data Entry | Months 6-18(13months) | Nov-2025-Dec-2026 |
| 6. Data Cleaning Data Analysis Data Interpretation | Month 19-21(3months ) | Jan-Mar-2027 |
| 7. Report Writing | Month 22-23 (2months ) | Apr-May-2027 |
| 8. Draft Corrections & Finalization | Month 24-26(3 months) | Jun-Aug-2027 |
| 9. Thesis Progress Review | Every Six-monthly | End-term-2,3,4,5 |
| 10. Thesis Submission | Month-27(1month) | Sep-2027 |

### 4.5.Sample Size estimation:

### Manual/Software

The sample size for this study has been calculated using the standard formula for cross-sectional studies estimating a proportion:

## Formula Used:

$n=Z^2 \cdot p \cdot q/d^2$

Where:

- **n** = required sample size
- **Z** = Z-score for desired confidence level (typically 1.96 for 95% confidence)
- **p** = estimated prevalence or proportion (in decimal)
- **q** = 1 – p
- **d** = allowable margin of error (precision level)

## Assumptions:

### 1. Confidence Level (Z = 1.96)

- A 95% confidence level is standard in public health research. The corresponding Z-score is **1.96**, which reflects that we are 95% confident that the true population parameter will lie within the estimated confidence interval.

### 2. Estimated Prevalence (p = 0.50)

Data from previous studies like **NFHS-5 (Odisha)** reports **59.1% prevalence of anemia** among adolescent girls aged 15–19 years. Another recent study in rural Odisha by Patra et al., 2020 reported **poor menstrual hygiene practices in 45–60%** of adolescent girls.

Since this is an **analytical study** exploring the association between menstrual hygiene and anaemia, and local prevalence data specifically linking the two variables are scarce, a conservative estimate of **p = 50%** is used. This provides the **maximum variability** and therefore the **largest sample size**, ensuring adequate power regardless of the actual event rate.

Thus, **p = 0.50** (i.e., 50%) is a justified midpoint in the absence of a precise joint prevalence.

**3. Complement of Prevalence (q = 1 – p = 0.50)**

When p = 0.50, q = 0.50 to account for the proportion not exhibiting the trait.

**4. Precision (d = 0.05 or 5%)**

A **5% margin of error** is selected, which is acceptable for most public health surveys and ensures a reliable estimate.

**5.Non-response:**

Given that some students may be absent on the day of the survey, decline participation, or have incomplete responses, a **10% non-response rate** is assumed.

**Calculation:**

$n=Z^2 \cdot p \cdot q/d^2$

$=(1.96)^2 *(0.5)*(0.5)/(0.05)^2$

$=3.84*0.25/0.0025$

$=0.96/0.0025$

$=384$

Adjusting for non-response ;

$n=384+10\%*384=384+38=422$

**Final Sample Size:**

Thus, **minimum required sample size** is approximately 422 **participants**to ensure adequate statistical power and reliability of findings.

## 4.6. Sampling Technique

### 1. Study Population

The study population will consist of **adolescent school girls aged 10 to 19 years** who have attained menarche, enrolled in government and private schools across selected urban and rural areas of **Sambalpur district, Odisha**.

### 2. Case Definition

For this study, a "case" is defined with the following criteria;

**Inclusion Criteria**

1. Enrolled in selected schools within Sambalpur district.
2. Girls aged between **10 and 19 years**.
3. Those who have **attained menarche**.
4. Provided **written informed assent**, and **parental consent** (if under 18 years of age).

**Exclusion Criteria**

1. Girls with known chronic hematologic disorders (e.g., thalssemia, sickle cell anaemia).
2. Those who are absent on the scheduled day of data collection.
3. Those who has donated /received blood or blood products in last three months.

### 3. Sampling Technique

A **probability-based multistage stratified random sampling technique** will be used to ensure a representative sample of adolescent girls across urban and rural settings of Sambalpur.

**Stage 1: Stratification by Area**

The district will be stratified into **urban** and **rural blocks** to ensure diversity in socio-demographic backgrounds.

**Stage 2: School Selection**

From each stratum (urban and rural), a list of eligible schools (government and private) will be obtained from the **District Education Office**.

**Simple random sampling** will be used to select 4 **schools** from each stratum.

**Stage 3: Participant Selection**

A list of all adolescent girls aged 10–19 years who have attained menarche will be obtained from the school attendance registers.

From each selected school, the required number of participants will be selected using **systematic random sampling**, proportional to the school's eligible population.

**Stage 4: Recruitment**

1. **Permission & Coordination-**Prior permission will be obtained from the **District Education Officer (DEO)** and respective school principals.
2. **Awareness Sessions-**Brief health education sessions will be conducted to inform students and teachers about the study's purpose and importance.
3. **Assent and Consent Process-**Written **informed assent** will be obtained from each participant. For minors, **parental/guardian consent** will be collected through information sheets sent home in advance.
4. **Enrolment-**Eligible students will be enrolled based on inclusion/exclusion criteria. Each participant will be assigned a **unique study ID code** to maintain anonymity.

This structured sampling technique ensures that the study captures **representative, unbiased, and generalizable data** on adolescent girls' menstrual hygiene practices and anaemia status in Sambalpur district.

## 4.7. Variables

### Dependent Variable

1. Hemoglobin level categorized per WHO cut-off (<12 g/dL – anaemic)

### Independent Variables

1. Menstrual hygiene practices (frequency, absorbent type, hand hygiene, disposal methods)
2. Knowledge about menstruation
3. Diet and socio-demographic factors

### List of Study Variables with Operational Definitions & Classification

| Sl. No. | Variable Name | Type | Operational Definition | Measure-ment Scale | Unit | Dependent / Independent | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | Age | Continuous | Age of participant at last birthday | Ratio | Years | Independent | As reported by participant |
| 2 | Class/ Grade | Categorical | Educational class the student is currently studying in | Ordinal | N/A | Independent | Verified from school records |
| 3 | Residence | Categorical | Whether participant lives in a rural or urban area | Nominal | N/A | Independent | Rural = 1, Urban = 2 |
| 4 | Socioeconomic status (SES) | Ordinal Composite | Based on Modified BG Prasad Scale | Ordinal | Categories | Independent | SES I to V |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | Mother's education level | Categorical | Highest level of formal education completed by mother | Ordinal | N/A | Independent | No schooling to Graduate+ |
| 6 | Dietary habits | Categorical | Vegetarian or mixed diet | Nominal | N/A | Independent | Self-reported |
| 7 | Iron-rich food intake frequency | Ordinal | Frequency of consuming iron-rich foods | Ordinal | Times/week | Independent | Based on recall |
| 8 | Menstrual status | Categorical | Whether participant has attained menarche | Nominal | N/A | Independent | Yes = 1, No = 2 |
| 9 | Age at menarche | Continuous | Age (in years) at menarche | Ratio | Years | Independent | If menstruating |
| 10 | Type of absorbent used | Categorical | Cloth/sanitary pad/other | Nominal | N/A | Independent | Cloth = 1, Pad = 2, Other = 3 |
| 11 | Frequency of absorbent change | Continuous | Number of times absorbent is changed daily | Ratio | Times/ day | Independent | As reported |
| 12 | Handwashing during menstruation | Categorical | Whether handwashing is practiced before/after changing absorbent | Nominal | N/A | Independent | Yes = 1, No = 2 |
| 13 | Disposal practice of absorbent | Categorical | Method of disposing used absorbent | Nominal | N/A | Independent | Burn = 1, Bury = 2, etc. |
| 14 | Privacy at school during menstruation | Categorical | Availability of private space for MHM at school | Nominal | N/A | Independent | Yes = 1, No = 2 |
| 15 | Awareness about menstrual hygiene | Categorical | Adequate knowledge assessed via a scoring tool | Nominal | N/A | Independent | Adequate = 1, Inadequate = 2 |
| 16 | Hemoglobin level | Continuous | Measured using finger-prick and validated device | Ratio | g/dL | Independent (continuous), contributes to dependent outcome | Used to define anaemia status |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 17 | Anaemia status | Categorical | Hb < 12 g/dL = Anaemic, ≥12 g/dL = Normal (WHO criteria) | Nominal | N/A | Dependent | Primary outcome variable |
| 18 | Referral for anaemia | Categorical | Whether participant was referred for care | Nominal | N/A | Dependent (secondary) | Based on anaemia status |

## 4.8. Study Tools

**1. Semi-structured Pre-tested Questionnaire:**

A carefully designed semi-structured questionnaire will be used to collect data on socio-demographic details, knowledge, attitudes, and practices (KAP) related to menstrual hygiene, and dietary patterns relevant to anaemia. The questionnaire will be developed in English and then translated into Odia for ease of understanding by participants. It will be pre-tested in a pilot study involving a small group of adolescent girls from a similar demographic but outside the study area, to ensure clarity, appropriateness, and cultural sensitivity of the questions. The questionnaire will include the following sections:

A. **Socio-demographic Information:** Age, class, residence (rural/urban), socioeconomic status (using modified BG Prasad scale), parental education, and family details.

B. **Knowledge about Menstrual Hygiene:** Questions related to awareness of menstruation, hygiene practices, use of sanitary materials, and awareness of anaemia.

C. **Attitudes towards Menstrual Hygiene:** Beliefs, taboos, and social stigmas related to menstruation.

D. **Practices related to Menstrual Hygiene:** Type and frequency of absorbent use, handwashing habits, disposal practices, privacy at school and home.

E. **Dietary Patterns:** Frequency of consumption of iron-rich foods, vegetarian vs. non-vegetarian diet, and any supplementation taken.

The questionnaire will be interviewer-administered by trained female data collectors to ensure comfort and accuracy of responses.

2. **Hemoglobin Estimation Tool:**

Hemoglobin levels will be measured using a portable and reliable device such as the **Sahli's method**. **Sahli's Method** involves collecting a blood sample via finger prick and comparing the color intensity against a standard haemoglobin comparator. It is a widely used, cost-effective method for anaemia screening in resource-limited settings.

The procedure will be explained to each participant beforehand, and strict aseptic precautions will be followed to avoid infection or discomfort. The results will be recorded confidentially, and participants found to be anaemic will be referred to local healthcare facilities for further evaluation and treatment.

1. The questionnaire will be administered by trained female investigators in privacy.
2. Hemoglobin estimation (via finger prick) will follow aseptic protocols.

## 4.9. Data Collection Procedure

### 1. Pre-Data Collection Phase

### a) Permissions & Approvals

Official permission will be obtained from the District Education Officer (DEO) and school heads of selected schools.

Ethical approval has already been or will be sought from the Institutional Ethics Committee (VIREC), VIMSAR.

Informed parental consent and participant assent will be obtained in advance for all students below 18 years, as per ethical norms.

**b) Training of Data Collection Team**

- A team of trained female health workers or research assistants will be recruited and trained.
- Training will cover:
    - Study objectives and ethical considerations
    - Interview techniques and administration of the questionnaire
    - Use of Hemocue/Sahli's method for hemoglobin estimation
    - Maintaining participant privacy and data confidentiality
    - Managing distress or refusal during interviews

**c) Pre-testing of Tools**

**i. Pre-testing the Questionnaire**

**Purpose:**

To check the clarity, relevance, flow, and cultural appropriateness of questions before the actual data collection begins.

**Procedure:**

1. Select a small group (usually 10-15) of adolescents similar to your study population but outside the study sample.
2. Administer the questionnaire as if it were the real survey.
3. Observe if respondents understand the questions as intended, if any questions are confusing, ambiguous, or sensitive.
4. Collect feedback from respondents about wording, length, and difficulty.

5. Take notes on any problems faced by the interviewers in asking questions.
6. Make necessary changes to improve clarity, remove redundant questions, or add important missing items.

**ii. Pilot Testing**

**Purpose:**
To test the entire data collection process, including questionnaire administration, Hb testing procedures, logistics, and timing.

**Procedure:**

1. Conduct a small-scale version of the full study on 5-10% of the intended sample size, preferably in a school or area similar to but different from the main study site.
2. Train data collectors and supervisors on the finalized questionnaire and Hb testing protocol.
3. Carry out all procedures exactly as planned in the main study, including informed consent/assent, questionnaire filling, Hb testing, and referral.
4. Record the time taken per participant, data entry workflow, and any operational challenges.
5. Review the pilot data to check for consistency, missing data, or errors.
6. Use pilot results to fine-tune the questionnaire, data collection procedures, and logistics.

## 6.3 Data Collection at Schools

Data collection will be conducted within school hours, in a private and hygienic setting arranged in coordination with school authorities. The process will involve the following steps:

**a) Participant Orientation-**A short session will be held for selected participants to explain Purpose of the study, Voluntary nature of participation, Confidentiality assurance and Approximate duration (~15-20 minutes per participant)

**b) Consent/Assent Process-** Written informed assent will be obtained from all participants. Parental consent forms will be collected beforehand through the school.

**c) Administration of Questionnaire-**The semi-structured questionnaire will be administered by trained female interviewers in Odia/local language. Interviews will be conducted one-on-one, ensuring privacy and comfort. Participants may skip any question they feel uncomfortable answering.

**d) Hemoglobin Estimation-**After the interview, a finger-prick blood sample will be collected using a sterile lancet. Estimation will be by Sahli's method as per SOP annexed. The hemoglobin level will be recorded immediately. E)

### 6.4.Data Recording and Management

Data from the questionnaire will be recorded on paper forms and later entered into Microsoft Excel for cleaning and validation. Hemoglobin readings will be linked using unique ID codes only. Confidentiality will be maintained by de-identifying all records. All hard copies will be stored in a locked cabinet; digital files will be password protected.

### 6.5.Referral and Follow-Up

Any participant found to be moderately/severely anaemic or needing medical advice will be referred to the nearest PHC/CHC with a referral slip and guidance.Basic health education materials will be provided to all participants regardless of anemia status.

### 6.6.Duration-

Data collection is expected to span 12 months (as per the project timeline), allowing time for school vacations, logistics, and unplanned delays.

### 6.7.Monitoring and Quality Control-

A supervisory team (including the PI/co-investigator) will conduct random spot checks to ensure consistency and protocol adherence.Daily debriefing will be held with the field team to resolve

## 5.Data Analysis

### Data Entry

All collected data will be entered into **Microsoft Excel** using a structured and validated data entry template designed in line with the study questionnaire. The entry process will be carefully monitored to ensure consistency, and **double data entry** or random cross-checking of 10–15% of entries will be performed to minimize entry errors.

**Data Cleaning**

Before beginning any statistical analysis, the dataset will undergo thorough **data cleaning** in both Excel and SPSS. This process will include:

- Checking for inconsistencies (e.g., out-of-range values, invalid codes).
- Verifying logical coherence between variables (e.g., age vs. school class).
- Removing duplicate entries (if any).
- Converting all categorical variables to appropriate **numerical codes** as per a pre-defined codebook.

**Handling of Missing Data**

Any **missing data** will be identified and quantified during the cleaning process. The approach to handling missing data will include

- **Descriptive analysis** of the extent and pattern of missingness.
- If missing data is <5%, **list wise deletion** may be applied.
- For variables with ≥5% missing values, appropriate **imputation techniques** (e.g., mean/mode substitution for non-sensitive variables, or multiple imputation if required) will be considered depending on the nature and extent of the data loss.

**Data Analysis**

The cleaned and coded dataset will be imported into **IBM SPSS Statistics version 25** for analysis. The analysis will be conducted in two broad phases:

1. **Descriptive Statistics**
    - Categorical variables (e.g., type of absorbent used, handwashing practices, presence of anemia) will be summarized using **frequencies and percentages**.

- Continuous variables (e.g., age, hemoglobin levels) will be expressed as **means with standard deviations (SD)**.
- Graphs, charts, and tables will be used to illustrate key findings.

2. **Inferential Statistics**

The **Chi-square test** will be used to assess the association between menstrual hygiene practices and anemia status (categorical outcome).

For adjustment of potential confounders (e.g., age, socioeconomic status, dietary patterns), **binary logistic regression** will be used to compute adjusted odds ratios (AOR) and 95% confidence intervals.

Model fitness will be tested using **Hosmer-Lemeshow goodness-of-fit** test, and multicollinearity will be checked before final model reporting.

All statistical tests will be interpreted at a **significance level of $p < 0.05$**.

Following entry, the dataset will be exported to **IBM SPSS Statistics software version 25** for analysis. The data analysis will include both **descriptive** and **inferential statistical methods**.

**Descriptive statistics** will be used to summarize the socio-demographic characteristics, menstrual hygiene practices, and anemia status of the participants. This will include calculation of **frequencies and percentages** for categorical variables (e.g., type of sanitary material used, presence of anemia) and **mean with standard deviation (SD)** for continuous variables (e.g., age, hemoglobin level).

**Inferential statistics** will be applied to examine associations between menstrual hygiene practices and adolescent anemia. The **Chi-square test** will be employed to test for statistical association between categorical variables, such as hygiene practices (e.g., cloth vs sanitary pad use) and anemia status (anaemic vs non-anaemic).To control for potential confounding factors (such as age, dietary habits, socioeconomic status, etc.), **binary logistic regression analysis** will

be conducted. This will help determine the adjusted odds of anemia in relation to various menstrual hygiene behaviours while accounting for covariates.

Statistical significance will be determined using a **p-value threshold of less than 0.05** ($p < 0.05$). All results will be presented in the form of tables, charts, or graphs wherever appropriate for better visualization and interpretation.

## 6.Ethical Considerations

### 1. Approval from Institutional Ethics Committee (VIREC)

Before commencing the study, ethical clearance will be obtained from the **VIREC (VIMSAR Institutional Research and Ethics Committee)**, Burla. The complete research protocol—including objectives, methodology, participant information sheet (PIS), and consent/assent forms—will be submitted for review.

### 2. Informed Assent and Parental Consent

The study involves **minors (participants under 18 years of age)**, which necessitates a dual-consent process:

**Informed Assent:**

All adolescent participants will receive detailed information about the study in simple, understandable language (in **Odia and English**) via a **Participant Information Sheet (PIS)**. The study purpose, procedures, duration, risks, and benefits will be explained verbally and in writing. Only those participants who provide **voluntary written assent** will be enrolled.it will be written for those between 12 -18years and verbal for below 12 years of age.

**Parental/Guardian Consent:**

A separate **parental/guardian consent form** will be sent home in advance. Only students with signed consent from their parent/guardian will be allowed to participate in the study. For participants aged 18 or above, direct written informed consent will be obtained.

**Risk categorisation**

These safeguards collectively uphold the ethical obligation to **protect and respect minors** while allowing their participation in valuable public health research.

The primary procedures include answering a structured questionnaire and undergoing a simple finger-prick test for haemoglobin estimation, both of which involve minimal physical or psychological burden.

Emotional discomfort may arise when discussing personal menstrual hygiene practices, but participants will have the option to skip any questions they are not comfortable answering.

All interactions will be conducted in a private, respectful, and age-appropriate manner, by trained female data collectors.

No invasive procedures or interventions will be performed beyond the finger prick, and appropriate referral will be provided for those found to be anaemic.

Since this study involves **children and adolescents**, including participants **under 18 years of age**, it raises important ethical considerations due to their classification as a **vulnerable population**. Special protections are necessary to ensure their rights, safety, and well-being throughout the research process.

Ethically, the study will follow the **guidelines set by the ICMR and international ethical standards** for research involving minors. This includes obtaining **dual consent**: written **parental or guardian consent** and **informed assent** from the adolescent participants themselves, using language appropriate to their age and comprehension. All information about the study's purpose, procedures, risks, and benefits will be clearly communicated. Care will be taken to conduct interviews and hemoglobin testing in a **safe, private, and respectful environment**, led by **trained female investigators**. Participation is entirely **voluntary**, and participants can withdraw at any point without any consequences. Ensuring **anonymity, data confidentiality, and psychological comfort** are key ethical priorities. The study design intentionally limits risks to **minimal levels**, and any student found to be anemic will be **referred for medical care**, adding potential direct benefit.

The study shall be categorized as “low  risk” due to the vulnerable group of the participants even though the study design allows for a case of “ no more than minimal risk” protocol.

Overall, the ethical safeguards—including informed assent/consent, data confidentiality, and participant autonomy—ensure that any potential risk is minimized and well-managed.

**3. Data Confidentiality and Anonymity Maintained**

Participant privacy and data confidentiality will be rigorously upheld. Each participant will be assigned a **unique identification number**; no personal identifiers (name, address, roll number) will be used in analysis or publications. Physical forms and digital data will be stored securely in **password-protected systems** and **locked cabinets**, accessible only to the research team. Data will be used **exclusively for academic and public health purposes**. Any presentation or publication of findings will be done in **aggregate format**, ensuring no individual is identifiable.

**4. Participants Found Anaemic Will Be Referred for treatment**

Hemoglobin testing will be done on-site via **finger-prick capillary blood method** using standard equipment (e.g., Hemocue).Participants with haemoglobin values indicating **mild, moderate, or severe anaemia** (as per WHO classification) will be informed in private, given **written referral slips** to the nearest **Primary Health Centre (PHC)** or **Community Health Centre (CHC), p**arents/guardians and school authorities will also be informed, with guidance on seeking timely medical care. This ensures that participants receive **immediate, actionable health support**, even though no treatment is directly provided through the study.

**5. No Financial Compensation but Health Awareness Provided**

Participants will not receive any **monetary compensation** for taking part in the study, as the procedures are minimal-risk and non-invasive (except for finger-prick testing).However, to ensure ethical reciprocity, all participants will receive **educational materials** (posters or leaflets) on **anemia prevention**, **nutritious diets**, and **menstrual hygiene**. A **short interactive health awareness session** post data collection to empower them with actionable knowledge.

## 7.Study Implications

The findings of this study are expected to have important implications across policy, societal, and clinical domains. At the **policy level**, the study can inform programs like Anaemia Mukt Bharat and Rashtriya Kishor Swasthya Karyakram by highlighting the need to integrate menstrual hygiene education with anemia prevention strategies in schools, and may advocate for improved access to sanitary products and regular screening of adolescent girls. On the **societal front**, the study can contribute to breaking taboos surrounding menstruation, promoting awareness among adolescents, teachers, and families, and encouraging healthy discussions around menstrual health and nutrition. In terms of **clinical and public health practice**, the study reinforces the importance of holistic adolescent health screening, encourages early referral of anemic students to primary healthcare facilities, and supports the development of targeted educational materials and interventions that jointly address menstrual hygiene and nutritional deficiencies in adolescent girls.

## 8. Budget Estimate and Funding:

### a. Indicative Budget:

| **Item** | **Estimated Cost (INR)** |
|---|---|
| Printing and Stationery | ₹5,000 |
| Travel/transport | ₹10,000 |
| Hemoglobin Estimation Supplies | ₹15,000 |
| Data Entry & Analysis | ₹5,000 |
| Miscellaneous | ₹5,000 |
| **Total Estimated Budget** | **₹40,000** |

### b. Funding source:

It shall be borne by the Principal Investigator out of own pocket.

## 9. References


1. World Health Organization. Adolescent Nutrition: A Review of the Situation in Selected South-East Asian Countries. WHO; 2021.
2. Patra S et al. Menstrual hygiene practices and anaemia among adolescent girls in Odisha. *J Family Med Prim Care.* 2020.
3. National Family Health Survey-5 (NFHS-5), Ministry of Health and Family Welfare, Govt. of India.

4. Nair M et al. Effect of menstrual hygiene on reproductive health: A systematic review. *BMC Women's Health*. 2021.
5. Ministry of Health & Family Welfare. WIFS Guidelines. Government of India; 2013.

## 10. Annexure:

1. PIS-Participant Information Sheet-English;
2. PIS-Participant Information Sheet-Odia;
3. ICF-Informed Consent Form-English;
4. ICF-Informed Consent Form-Odia;
5. Questionnaire-English;
6. Questionnaire-Odia;
7. SOP- Hb-Estimation;
8. VIREC approval letter;

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## Annexure-5;
## QUESTIONNAIRE:

**[Instructions for Interviewer:** Ask all questions respectfully and without judgment. Ensure privacy and comfort for the participant. Explain questions when needed. Allow skipping of any question if the participant is uncomfortable. Maintain strict confidentiality and do not record names.]

**Study ID**: _____

**School Name**: _____

**Date of Interview**: __ / __ / 202__

**Interviewer's Name**: _____

**Section A: Socio-Demographic Information**

1. Name of the participant (optional): _____
2. Age (in completed years): _____
3. Class/Grade: _____
4. Residence: ☐ Rural ☐ Urban
5. Religion: ☐ Hindu ☐ Muslim ☐ Christian ☐ Other: _____
6. Father's education: ☐ Illiterate ☐ Primary ☐ Secondary ☐ Graduate & above
7. Mother's education: ☐ Illiterate ☐ Primary
   ☐ Secondary ☐ Graduate & above
8. Type of family: ☐ Nuclear ☐ Joint
9. Socioeconomic status (as per BG Prasad scale): _____
10. Number of siblings: _____

**Section B: Menstrual History**

11. Have you attained menarche? ☐ Yes ☐ No
12. If yes, age at menarche: _____ years
13. Length of menstrual cycle (days): _____
14. Duration of bleeding (in days): _____
15. Do you experience any discomfort during periods? ☐ Yes ☐ No
    If yes, specify: ☐ Pain ☐ Headache ☐ Fatigue ☐ Other: _____

**Section C: Menstrual Hygiene Practices**

16. What do you use to manage your menstruation?
    ☐ Sanitary pads ☐ Cloth ☐ Both ☐ Other: _____
17. How many times do you change your absorbent per day during menstruation? _____
18. Where do you usually change your absorbent at school?
    ☐ Toilet with privacy ☐ Open area ☐ Not applicable ☐ Other: _____

19. Do you wash your hands before/after changing?
☐ Always ☐ Sometimes ☐ Never
20. How do you dispose of the used absorbent?
☐ Dustbin ☐ Burn ☐ Bury ☐ Flush ☐ Other: _____
21. Do you face restrictions during menstruation?
☐ Yes ☐ No
If yes, specify: ☐ Religious ☐ Social ☐ School ☐ Dietary ☐ Other: _____
22. Do you feel comfortable discussing menstruation with family?
☐ Yes ☐ No

**Section D: Knowledge About Menstruation and Hygiene**

23. Did you receive any education about menstruation before menarche?
☐ Yes ☐ No
If yes, from whom? ☐ Mother ☐ Teacher ☐ Friends ☐ Other: _____
24. Do you think menstrual hygiene is important? ☐ Yes ☐ No
25. What are the correct hygiene practices during menstruation?
☐ Changing absorbent regularly
☐ Hand washing
☐ Use of clean materials
☐ Proper disposal
☐ Bathing daily
26. Have you ever heard of anaemia? ☐ Yes ☐ No
27. Do you know what causes anaemia? (Tick all applicable)
☐ Poor diet
☐ Heavy menstrual bleeding
☐ Worms
☐ Don't know
☐ Other: _____

**Section E: Dietary Habits**

28. What type of diet do you follow? ☐ Vegetarian ☐ Non-vegetarian
29. How often do you eat the following?

| Food Item | Daily | 2–3 times/wk | Weekly | Rarely | Never |
|---|---|---|---|---|---|
| Green leafy vegetables | ☐ | ☐ | ☐ | ☐ | ☐ |
| Meat (chicken, mutton, etc.) | ☐ | ☐ | ☐ | ☐ | ☐ |
| Eggs | ☐ | ☐ | ☐ | ☐ | ☐ |

| Fruits | ☐ | ☐ | ☐ | ☐ | ☐ |

| Milk or curd | ☐ | ☐ | ☐ | ☐ | ☐ |

30. Do you take iron supplements? ☐ Yes ☐ No

**Section F: Health & Anaemia Screening**

31. Do you often feel tired or weak? ☐ Yes ☐ No
32. Have you ever been diagnosed with anemia? ☐ Yes ☐ No ☐ Don't know
33. Are you currently taking treatment for any health condition? ☐ Yes ☐ No
    If yes, specify: _____

**Section G: Hemoglobin Measurement**(☐ To be filled by data collector)

34. Was hemoglobin measured today? ☐ Yes ☐ No
35. Hb value (g/dL): _____
36. Anemia status: ☐ Normal ☐ Mild ☐ Moderate ☐ Severe
37. Referred for medical care: ☐ Yes ☐ No

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## Annexure-2;
## STANDARD OPERATING PROCEDURE (SOP) FOR HEMOGLOBIN ESTIMATION USING SAHLI'S METHOD:

**Materials Required:**

1. Sahli's hemoglobinometer (Haemoglobin comparator)
2. Acid hematin solution (1 N HCl)
3. Glass mixing tubes (graduated)
4. Dropper/pipette
5. Distilled water
6. Sterile lancets
7. Alcohol swabs
8. Cotton/gauze pads
9. Gloves (disposable)
10. Sharps disposal container

**Procedure:**

1. **Preparation:**
    - Explain the procedure clearly to the participant and obtain verbal assent/consent.
    - Wash hands thoroughly and wear disposable gloves.
    - Clean the fingertip (usually middle or ring finger) with an alcohol swab; allow it to air dry.
2. **Sample Collection:**
    - Use a sterile lancet to prick the fingertip.
    - Wipe away the first drop of blood with cotton to avoid tissue fluid contamination.
    - Collect blood drops into the graduated glass mixing tube of the Sahli's hemoglobinometer until the blood level reaches the 20 µL mark (or as specified by the device).
3. **Conversion to Acid Hematin:**
    - Add 1 mL of 1 N hydrochloric acid (acid hematin solution) into the tube containing blood.
    - Mix the contents thoroughly using the dropper or pipette by shaking gently to convert haemoglobin to acid hematin.
4. **Color Matching:**
    - Place the tube against the comparator's color scale.
    - Slowly add distilled water dropwise while mixing until the brownish color of the acid hematin matches the standard color on the comparator.

- The haemoglobin concentration is read directly from the graduated scale where the color match.

5. **Recording Results:**
   - Note the haemoglobin value immediately on the data sheet along with participant ID, date, and time.
   - Maintain confidentiality of participant data.
6. **Post-procedure Care:**
   - Apply gentle pressure on the fingertip using cotton/gauze to stop bleeding.
   - Dispose of lancet and other waste in designated sharps and biohazard containers.
   - Remove gloves and sanitize hands.
7. **Precautions:**
   - Ensure accurate blood volume collection for consistent results.
   - Avoid contamination of blood sample with tissue fluids.
   - Use freshly prepared acid hematin solution to ensure reliability.
   - Perform regular calibration of the Sahli's hemoglobinometer.
   - Follow universal precautions to prevent infections.
   - Use disposable lancets and microcuvettes only once per participant.
   - Properly dispose of all biohazardous waste.
8. **Limitations:**
   - Sahli's method is less precise than automated methods but acceptable for field surveys.
   - Operator skill influences accuracy; proper training is essential.

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