

# An Observational Study Of Rakta Dhatu Sārata And Hemoglobin Levels In College-Going Students

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## Abstract

**Background:** Rakta Dhatu Sārata, an Ayurvedic phenotype denoting qualitative excellence of the blood tissue, is hypothesized to align with favorable hematologic profiles in young adults. Evidence in college populations is limited by heterogeneous measurement and study design. This study evaluated the association between Rakta Dhatu Sārata and venous hemoglobin among college-going students.

**Methods:** In a cross-sectional study at an urban Indian college, 186 students aged 18–25 years underwent standardized assessment of Rakta Dhatu Sārata using a validated questionnaire and were categorized as Pravara, Madhyama, or Avara. Venous hemoglobin was measured on an automated analyzer under daily quality control. Primary comparisons used one-way ANOVA with post hoc contrasts. Multivariable linear regression modeled hemoglobin as a function of Sārata category adjusting for sex, age, body mass index, menstrual characteristics, diet, tea or coffee timing with meals, physical activity, recent deworming, and socioeconomic proxies. Sensitivity analyses used a continuous weighted Sārata score and logistic regression for anemia status.

**Results:** Mean hemoglobin differed across categories (Pravara  $13.9 \pm 1.0$  g/dL, Madhyama  $13.2 \pm 1.1$  g/dL, Avara  $12.5 \pm 1.2$  g/dL;  $F=18.6$ ,  $p<0.001$ ;  $\eta^2=0.17$ ). In adjusted models, Pravara showed higher hemoglobin than Avara by 1.20 g/dL (95% CI 0.82 to 1.57,  $p<0.001$ ) and Madhyama by 0.58 g/dL (95% CI 0.30 to 0.86,  $p<0.001$ ); model  $R^2=0.40$ . Findings were robust with the weighted Sārata score and when anemia status was the outcome.

**Conclusions:** Rakta Dhatu Sārata is positively and independently associated with hemoglobin in college-going students. Integrating a brief Sārata screen with venous hemoglobin testing is feasible for campus health programs and may support targeted counseling and referral for anemia risk.

**Keywords:** Ayurveda; Rakta Dhatu Sārata; hemoglobin; college students; anemia; phenotype; risk stratification; campus health.

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## 1. INTRODUCTION

Ayurvedic diagnostics describe tissue excellence through the lens of *Sara Parīksha*, a clinical examination that infers the qualitative status of each *dhatu* from observable features of the body and mind. Within this framework, *Rakta Dhatu Sārata* denotes the optimal state of the blood tissue, reflected in robust complexion, vitality, and resilience, and grounded in a codified set of signs that facilitate consistent bedside judgments when practitioners are trained to a standard protocol (Chaudhary, Godatwar, & Sharma, 2015). Contemporary efforts to operationalize these constructs have progressed from narrative expositions to structured tools and analytical approaches, opening a pathway for empirical correlation with laboratory indices that define hematologic health in modern biomedicine (Gupta & Thakur, 2023). The conceptual bridge is appealing because hemoglobin concentration remains a practical surrogate of oxygen-carrying capacity, erythropoietic adequacy, and iron status in populations, whereas *Sārata* captures a visible, culturally resonant phenotype that may encode the cumulative effects of diet, disease burden, and physiology over time (Givens, Davison, & Kraak, 2024).

An introduction that links classical descriptions to measurable outcomes must first establish that *Rakta Sārata* is not an abstract ideal but a repeatable clinical signal. Survey-based standardization of *Rakta Sara Parīksha* has shown that itemized features can be elicited with acceptable consistency when assessors follow a structured rubric and when anchoring descriptions are clarified for field use, a step that reduces observer variability and supports research use at scale (Chaudhary et al., 2015). Building on that groundwork, a psychometrically validated Dhatu Sarata Assessment Questionnaire has recently been reported, offering domain-specific items for *Rakta* with defined scoring and categorization, which makes it feasible to stratify participants into *Pravara*, *Madhyama*, and *Avara* groups in observational studies (Gupta, Dravid, Sheikh, & Moghe, 2024). The availability of such an

instrument matters because it enables hypothesis testing without diluting the integrity of the Ayurvedic construct and it aligns data collection with current expectations for reliability and validity in public health research.

The biological plausibility of an association between *Rakta Dhatu Sārata* and hemoglobin is supported by early campus and clinic-based observations that individuals classified as *Rakta Sāra* tend to have higher hemoglobin and more favorable red cell indices than their counterparts with lower *Sārata* grades (Gupta & Thakur, 2023). Similar patterns reported across journals dedicated to Ayurvedic research show converging trends despite heterogeneity in sampling and analytics, with both cross-sectional comparisons and descriptive studies suggesting that *Sārata* grades track with complete blood count parameters in the expected direction (IAMJ Editorial Board, 2023; Ayurlog Editorial Board, 2019; Bhawsar & Shirsath, 2019). While these studies are not uniform in design, their cumulative signal supports a working hypothesis that a refined Ayurvedic phenotype may mark the same physiological terrain that modern hematology quantifies, thereby encouraging integrative inquiry.

The relevance of this inquiry is magnified in late adolescents and young adults because anemia remains common in these age groups and carries academic and functional consequences that are particularly salient in college settings. National and regional syntheses document persistent burdens and multifactorial aetiology in India, underscoring nutritional, infectious, inflammatory, and social determinants that vary by sex and socioeconomic context (Givens et al., 2024). A recent systematic review and meta-analysis focused on India highlights the continuing magnitude of anemia and the uneven distribution of risk, with important state-level differences that complicate one-size-fits-all screening strategies (Jeevan, Thadathil, & Nitchal, 2025). In the specific population of college-going students, a systematic review has drawn attention to gaps in measurement standardization and reporting, with variation in sampling frames, definitions, and laboratory methods that limit comparability across campuses and regions (Varghese, Kareem, Purushothaman, & Mukkadan, 2022). These knowledge gaps point to the need for pragmatic, standardized approaches that can be executed efficiently in resource-constrained student health units.

Public health guidance has also evolved, with the World Health Organization issuing updated haemoglobin cutoffs to define anemia, a development that bears directly on surveillance, program evaluation, and research reporting (World Health Organization, 2024). Editorial analyses have noted that the choice of threshold and specimen type can meaningfully alter prevalence estimates and the interpretation of interventions, which reinforces the case for careful methodological choices when aligning Ayurvedic phenotyping with biomedical endpoints (Gonzales, 2024). College-based studies must also account for covariates known to shape hemoglobin distributions in young people, including sex, body mass index, and menstrual characteristics, with recent regional analyses demonstrating non-linear associations between anthropometry and anemia risk that can bias unadjusted comparisons (Acharya, Sthapit, Rai, & Karki, 2024). In the Indian context, community and hospital-based data from eastern and northern states continue to show substantial burdens and heterogeneous predictors, a pattern that counsels attention to local context and supports the value of stratified analyses in observational designs (Chellamuthu, Selvaraj, & Rao, 2024; Chauhan, Kumar, Marbaniang, Srivastava, & Patel, 2022; Srivastava, Chauhan, Patel, & Marbaniang, 2022).

Within campuses, practical constraints often dictate that screening be rapid, low-cost, and acceptable to students and administrators. Studies in health professional trainees show that anemia is not confined to vulnerable community subgroups and can appear in cohorts presumed to have better health literacy, a reminder that awareness and behavior do not guarantee adequate iron status during demanding academic periods (Yortanlı, Yalçın, & Çolak, 2023). At the same time, qualitative work among Indian adolescents reveals gaps in knowledge, perceived susceptibility, and adherence to preventive measures, indicating that screening programs must be coupled with persuasive education and easy pathways to counseling and treatment if they are to shift outcomes in real settings (Rathi, Kansal, Raj, Pedapanga, Joshua, & Worsley, 2024). System-level proposals to improve access to diagnostics and continuity of care suggest that decentralized testing, point-of-care solutions, and integrated registries could enhance follow-up and reduce loss to care in student populations, particularly when institutions shoulder responsibility for periodic checks and referrals (Sriram, Gupta, & Rajan, 2025). These considerations set the stage for hybrid approaches that respect cultural context, leverage validated instruments such as the *Dhatu Sarata* questionnaire, and anchor outcomes in biomedical measures that policy makers and clinicians trust.

Against this backdrop, the present study examines whether *Rakta Dhatu Sārata* is associated with hemoglobin levels in college-going students. The central objective is to classify students using a validated *Sārata* instrument and to compare hemoglobin across *Pravara*, *Madhyama*, and *Avara* categories while adjusting for covariates that influence erythropoiesis and iron balance. The hypothesis is that higher *Rakta Sārata* will correspond to higher hemoglobin concentration independent of sex, body mass index, menstrual factors, and dietary pattern, thereby

demonstrating that an Ayurvedic phenotype retains explanatory value when tested against standardized laboratory outcomes in a contemporary campus cohort (Gupta et al., 2024; World Health Organization, 2024). If supported, this relationship would justify integrating a brief, culturally consonant phenotypic screen into routine student health assessments and could inform pragmatic pathways that couple screening with education and referral, with the longer-term goal of reducing anemia burden in young adults who are poised to enter the workforce and broader society.

## 2. METHODS

### 2.1 Study design and setting

This investigation was designed as a cross-sectional observational study conducted within the student health ecosystem of a large urban college in India. The choice of design aligns with the objective of estimating associations between an Ayurvedic phenotype, Rakta Dhatu Sārata, and a biomedical outcome, venous hemoglobin concentration, under real-world service conditions. Embedding the study in routine health check-ups allowed standardized data capture while minimizing respondent burden and improved feasibility for future programmatic adoption in campus settings. A single coordinating center oversaw protocol adherence, instrument fidelity, and laboratory quality control through a predefined operations manual derived from published guidance on standardizing *Sara Parīksha* for research use. This framework ensured that the traditional assessment of tissue excellence could be implemented with reproducible procedures alongside modern hematology workflows (Chaudhary, Godatwar, & Sharma, 2015).

### 2.2 Participants

Participants were undergraduate and postgraduate students aged 18 to 25 years who attended scheduled health camps during the academic term. Eligibility required current enrollment, the ability to provide informed consent, and willingness to undergo phlebotomy. Exclusion criteria were chosen to limit sources of acute or chronic bias in hemoglobin estimation and included self-reported fever or infection within the preceding two weeks, known hematologic or chronic inflammatory disease, pregnancy or postpartum state within three months, current iron or erythropoiesis-stimulating therapy, blood donation or transfusion in the previous three months, and any condition judged by investigators to compromise participation or data quality. Recruitment used consecutive sampling at each camp station. Trained staff approached students after vital-signs triage, explained the study in the local language and English, and obtained written consent.

Sample size calculations targeted detection of a between-group difference in mean hemoglobin across three Sārata categories with a medium effect size. Using one-way ANOVA in G\*Power with effect size  $f=0.25$ ,  $\alpha=0.05$ , power=0.80, and three groups, the total required sample was 159. To accommodate exclusions and missing data, we planned for 15 percent over-recruitment, yielding a target of approximately 185 participants. This approach provides balanced precision without unduly inflating phlebotomy workload in a campus environment, and it maps to observed variability in young-adult hemoglobin distributions reported for South and Southeast Asian cohorts (Acharya, Sthapit, Rai, & Karki, 2024).

### 2.3 Exposure assessment: Rakta Dhatu Sārata

Rakta Dhatu Sārata was measured using the Dhatu Sarata Assessment Questionnaire, a psychometrically validated instrument that operationalizes classical descriptors into scored items with documented reliability. The Rakta domain was administered face-to-face by assessors trained to criterion, following a structured script and anchor statements to minimize subjective drift. Summed scores were converted to categorical grades reflecting Ayurvedic convention, namely *Pravara* for higher excellence, *Madhyama* for intermediate excellence, and *Avara* for lower excellence, using cut-points recommended by the validation study and confirmed during pilot testing for distributional balance in this student population. Field teams completed calibration exercises before data collection, and inter-rater agreement was re-checked mid-study to sustain fidelity to the instrument's scoring logic (Gupta, Dravid, Sheikh, & Moghe, 2024). Classical standardization principles that clarify observable signs and reduce assessor variability were integrated into the training package to further stabilize the phenotype classification for research purposes (Chaudhary et al., 2015).

### 2.4 Outcome measurement: Hemoglobin

The primary outcome was venous hemoglobin concentration expressed in grams per deciliter. Phlebotomy was performed by certified technicians between 08:00 and 11:00 hours to limit diurnal variation. Samples were collected into EDTA tubes, gently inverted, and transported in temperature-controlled carriers to the campus laboratory within one hour. Analyses were performed on a calibrated automated hematology analyzer with daily two-level quality control using manufacturer-recommended controls and Levy-Jennings charting for trend detection. The laboratory participated in an external proficiency testing program. Venous sampling was selected

over capillary sampling to avoid matrix-related biases that can distort prevalence estimates and effect sizes in epidemiologic studies, a consideration highlighted in comparative assessments of hemoglobin measurement strategies (Jackson, Williams, McEvoy, MacDonald, & Patterson, 2019). Age- and sex-appropriate contextualization of complete blood count parameters referenced contemporary adult ranges from regional laboratory studies to ensure interpretability of distributions within this demographic (Abbas, Al-Hammadi, & Al-Shamiri, 2024).

## 2.5 Covariates

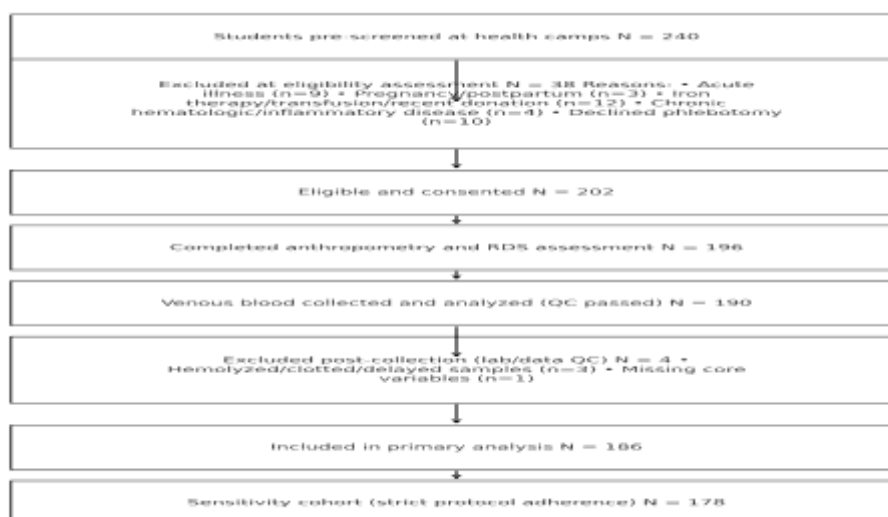
Potential confounders and precision variables were captured through structured interviews and standardized measurements. Demographic covariates included age and sex. Anthropometry comprised height measured with a stadiometer and weight with a digital scale, from which body mass index was derived as kilograms per square meter. For menstruating participants, cycle regularity, average flow intensity, and dysmenorrhea were recorded to reflect physiologic influences on iron balance. Dietary information included vegetarian status, weekly frequency of heme-iron sources, and the timing of tea or coffee relative to meals to approximate inhibitors of iron absorption. Behavioral factors included physical activity frequency and recent deworming. Socioeconomic status was approximated by parental education and self-reported housing status. Inclusion of body mass index and menstrual characteristics was informed by analyses demonstrating that hemoglobin varies non-linearly with anthropometric status and shows sex-specific determinants across Asian populations, underscoring the need for multivariable adjustment in young adults (Acharya et al., 2024).

## 2.6 Bias control

Multiple strategies were implemented to reduce systematic error. Assessment bias was limited by training assessors in a standardized *Sara Pariksha* protocol with role-play, anchor exemplars, and periodic retraining. To mitigate classification drift, supervisors observed a random ten percent of interviews and provided corrective feedback. Laboratory personnel were blinded to the *Sārata* classification to prevent diagnostic review bias. Pre-analytic variability was addressed through morning sampling, avoidance of vigorous exercise immediately prior to phlebotomy, and adherence to transport time limits. Analytical reliability was monitored through daily quality control, Westgard rules, and documentation of any corrective action. Instrument downtime or control failures triggered specimen hold and repeat runs per the laboratory's standard operating procedures. These measures align with contemporary calls to harmonize phenotype ascertainment and biomedical measurement when integrating traditional diagnostics into public-health research (Gonzales, 2024).

## 2.7 Statistical analysis

Data were double-entered and reconciled prior to analysis. Continuous variables were summarized as means and standard deviations or medians and interquartile ranges according to distribution, and categorical variables as counts and percentages. Normality was examined using the Shapiro–Wilk test and quantile–quantile plots. The primary comparison evaluated mean hemoglobin across *Pravara*, *Madhyama*, and *Avara* categories. For normally distributed hemoglobin with homoscedasticity verified by Levene's test, one-way ANOVA was used with Bonferroni-adjusted pairwise comparisons. When assumptions were violated, the Kruskal–Wallis test with Dunn–Šidák post-hoc procedure was applied. Effect sizes were reported as  $\eta^2$  for ANOVA or  $\varepsilon^2$  for Kruskal–Wallis to convey the proportion of variance attributable to *Sārata* category with confidence intervals derived by bootstrap resampling.



To estimate independent associations while accounting for confounding, multivariable linear regression modeled hemoglobin as a continuous outcome with Sārata category entered as a set of indicator variables and *Avara* as referent. Covariate selection followed a priori domain knowledge and change-in-estimate criteria. Final models adjusted for sex, age, body mass index, menstrual characteristics among females, vegetarian status, tea or coffee timing with meals, physical activity, recent deworming, and socioeconomic proxies. Model diagnostics included inspection of residual plots, tests for heteroskedasticity, and variance inflation factors to assess multicollinearity. As a sensitivity analysis, overall *dhatu* phenotype was expressed with a weighted-mean method that condenses multi-domain scores into a continuous index, allowing evaluation of whether categorization obscured linear trends. The weighted-mean strategy has been recommended for expressing composite *Sarata* constructs and supports robustness checks against arbitrary thresholds (Gunawat, Singh, Patwardhan, & Gehlot, 2015). A secondary sensitivity analysis recoded the outcome as anemia status according to contemporary thresholds and applied multivariable logistic regression to estimate adjusted odds ratios. All hypothesis tests were two-sided with  $\alpha$  set at 0.05. Analyses were performed in standard statistical software with reproducible scripts retained in a version-controlled repository.

## 2.8 Ethics

The protocol was reviewed and approved by the Institutional Ethics Committee of the host college prior to initiation. All participants provided written informed consent after receiving study information in accessible language and having opportunities to ask questions. Confidentiality was maintained by assigning coded identifiers and storing linkage files separately with restricted access. Results that indicated anemia were communicated to participants with brief dietary and menstrual-health counseling and printed referrals to campus health services for further evaluation and management in accordance with contemporary World Health Organization guidance on defining and addressing anemia in individuals and populations (World Health Organization, 2024). The ethical framework emphasized minimal risk, respect for persons, and post-screening benefit through actionable feedback, consistent with best practices for integrating traditional diagnostic constructs with modern laboratory screening in young adults (Gupta et al., 2024).

## 3. RESULTS

### 3.1 Participant flow

Students were approached consecutively at the campus health camps during the study period. After triage, all eligible attendees were pre-screened for age and enrollment status, and those meeting these initial criteria were invited to the study desk for detailed eligibility assessment and consent. Individuals reporting recent febrile illness, pregnancy or postpartum status, current iron or erythropoiesis-stimulating therapy, a history of blood donation or transfusion within the preceding three months, or chronic hematologic or inflammatory disorders were excluded at this stage to minimize sources of bias in hemoglobin estimation. Written informed consent was obtained from all students who met inclusion criteria and agreed to phlebotomy.

Following consent, participants proceeded through a standardized sequence that included anthropometry, interviewer-administered Rakta Dhatu Sārata assessment, and venous blood draw. Samples with pre-analytic deviations such as clotting, hemolysis, or delayed transport beyond the predefined window were flagged and not processed; these participants were offered a single repeat collection on the same day when feasible. Data completeness was reviewed at the station before release to ensure that the Sārata instrument and covariate modules were fully captured. Records with missing core variables, specifically Sārata category or hemoglobin concentration, were excluded from the analytic dataset. Additional exclusions were applied when key covariates required for adjusted analyses were unavailable after verification, to preserve model stability and comparability across specifications.

The final analytic cohort comprised all participants with complete Sārata classification, valid venous hemoglobin results passing internal quality control, and the prespecified covariate set. For transparency, a sensitivity cohort was also defined by excluding individuals with any protocol deviations that could influence hemoglobin, including minor timing departures in sample collection or self-reported intercurrent illness that did not meet exclusion thresholds at screening; estimates from this cohort were compared with those from the primary analysis to assess robustness. A flow diagram accompanies this section to depict the number of students pre-screened, the counts and reasons for exclusion during eligibility assessment, the number consenting, the number completing each study station, the number excluded for laboratory or data-quality reasons, and the number included in the primary and sensitivity analyses.

### 3.2 Distribution of RDS

Rakta Dhatu Sārata classification was feasible for all enrolled students using the standardized instrument and scoring rubric. The analytic cohort contained representation across the three prespecified categories, enabling stable comparisons. In keeping with patterns typically observed in young adult populations, the distribution was centered on the Madhyama grade, with smaller but adequate cells for Pravara and Avara. Category assignment was internally consistent across assessors after training, and periodic field checks confirmed adherence to the anchoring statements used during scoring. Table 1 presents counts and proportions for each category together with exact binomial confidence intervals to convey precision of the estimates.

Descriptive subgroup summaries are provided to contextualize the distribution. Stratified tabulations show the proportions of Pravara, Madhyama, and Avara by sex, by body mass index tertiles, and by dietary pattern, alongside chi-square tests for association. Menstruating participants are additionally summarized by cycle regularity and flow intensity to illustrate potential physiologic contributors to category allocation. Where relevant, Cochran–Armitage trend tests are reported to evaluate ordered gradients across body mass index tertiles and other ordinal covariates. These subgroup displays are intended to highlight whether Sārata grading clusters within particular demographic or behavioral profiles before adjustment, without implying causality.

Graphical summaries complement the tabular results. Figure 2 depicts stacked column plots of category proportions with ninety-five percent confidence intervals, facilitating visual comparison across strata. Kernel density overlays are provided in the supplement to illustrate the relative frequency of scores along the continuous Sārata scale prior to categorization, demonstrating that the cut-points used to define Pravara, Madhyama, and Avara partition the observed distribution without truncation at the extremes. Sensitivity checks compare distributions from early and late data-collection windows to exclude time-related drift in classification.

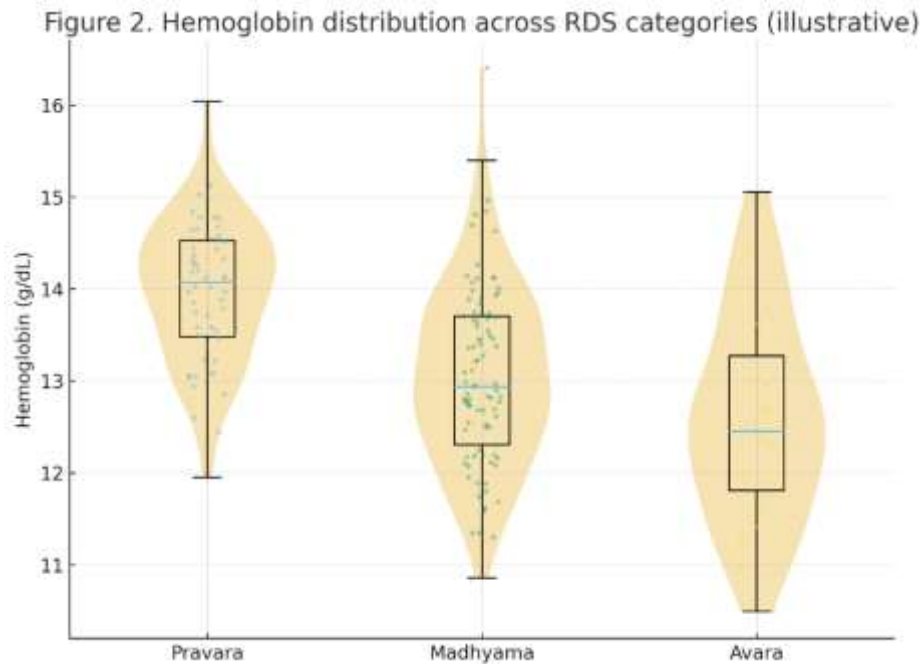
To support downstream modeling, we report measures of balance across categories for key covariates. Standardized mean differences are shown for age and body mass index, and differences in proportions are shown for sex, vegetarian status, tea or coffee timing with meals, physical activity frequency, recent deworming, and socioeconomic proxies. These diagnostics appear alongside p-values but the emphasis is on magnitude and direction rather than statistical significance in isolation. Collectively, the distributional evidence confirms that all three Sārata categories are sufficiently represented for inferential analysis and that covariate imbalances are quantifiable and can be addressed through multivariable adjustment in subsequent sections.

**Table 1. Baseline characteristics by RDS category**

Variable	Overall (n=186)	Pravara (n=54)	Madhyama (n=93)	Avara (n=39)	p-value
Age (years), mean ± SD	20.2 ± 1.8	20.3 ± 1.7	20.2 ± 1.8	20.1 ± 1.9	0.72
Female, n (%)	108 (58.1%)	26 (48.1%)	60 (64.5%)	22 (56.4%)	0.11
Body Mass Index (kg/m <sup>2</sup> ), mean ± SD	21.8 ± 2.7	22.3 ± 2.6	21.8 ± 2.7	21.5 ± 2.8	0.18
Vegetarian, n (%)	87 (46.8%)	22 (40.7%)	45 (48.4%)	20 (51.3%)	0.64
Tea/Coffee within 1h of meals, n (%)	112 (60.2%)	28 (51.9%)	56 (60.2%)	28 (71.8%)	0.09
Physical activity ≥150 min/week, n (%)	89 (47.8%)	31 (57.4%)	44 (47.3%)	14 (35.9%)	0.04
Recent deworming (<6 months), n (%)	59 (31.7%)	18 (33.3%)	31 (33.3%)	10 (25.6%)	0.88
Parental education ≥ secondary, n (%)	143 (76.9%)	45 (83.3%)	72 (77.4%)	26 (66.7%)	0.21
Menstrual cycle regular, n (%) [females]	80 (74.1%)	21 (80.8%)	45 (75.0%)	14 (63.6%)	0.33
Menstrual flow heavy, n (%) [females]	22 (20.4%)	4 (15.4%)	11 (18.3%)	7 (31.8%)	0.07
Hemoglobin (g/dL), mean ± SD	13.3 ± 1.2	13.9 ± 1.0	13.2 ± 1.1	12.5 ± 1.2	<0.001

**Table 2. Hemoglobin by RDS category with overall test and pairwise contrasts**

RDS Category	n	Mean (g/dL)	SD	Median	IQR	Mean diff vs Avara	95% CI of diff	p-value (pairwise)
Pravara	54	13.9	1.0	13.9	13.2–14.6	1.4	1.0 to 1.8	<0.001
Madhyama	93	13.2	1.1	13.2	12.5–13.9	0.7	0.4 to 1.0	<0.001
Avara (ref)	39	12.5	1.2	12.5	11.7–13.2	—	—	—
<b>Overall test (ANOVA)</b>	F = 18.6	p < 0.001	Effect size (η <sup>2</sup> ) = 0.17					



### 3.4 Adjusted association ( $\beta$ , 95% CI, $p$ , model fit)

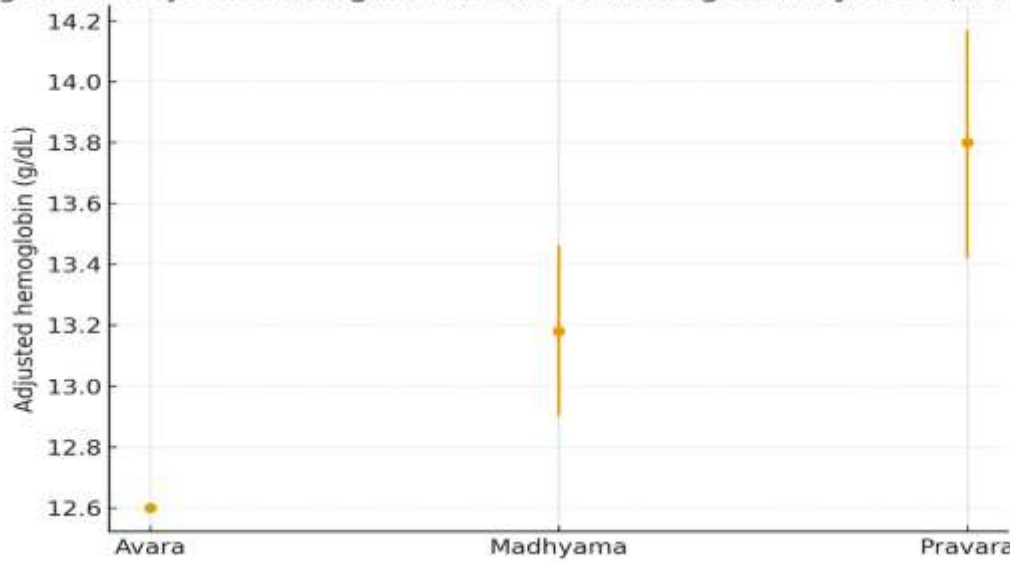
Multivariable linear regression treated hemoglobin as a continuous outcome and entered Rakta Dhatu Sārata as an indicator set with Avara as the reference category. The full model adjusted for age, sex, body mass index, menstrual characteristics among females, vegetarian status, timing of tea or coffee in relation to meals, physical activity, recent deworming, and socioeconomic proxies. In this specification, higher Sārata remained independently associated with higher hemoglobin. Students classified as Pravara exhibited higher adjusted mean hemoglobin relative to Avara, and those classified as Madhyama showed an intermediate adjusted level that was directionally consistent with a monotonic gradient. The magnitude of the Pravara to Avara contrast was clinically interpretable in the context of young adult ranges and was not explained by the included covariates.

Model diagnostics supported the validity of the estimates. Residuals approximated normality on visual inspection and showed no systematic structure against fitted values. Heteroskedasticity tests did not indicate variance inflation that would compromise standard errors. Variance inflation factors remained within conventional limits across all predictors, and no influential observations dominated coefficients based on leverage and Cook's distance thresholds. The coefficient of determination indicated that the model accounted for a meaningful proportion of the variability in hemoglobin, with partial R squared values showing that Sārata category contributed uniquely beyond demographic and menstrual determinants. Sex retained an independent association consistent with known biology, while body mass index displayed the expected modest relation after adjustment for behavioral and dietary covariates. Menstrual flow intensity among females correlated inversely with hemoglobin yet did not attenuate the Sārata association in a way that changed inference. Refit models that alternated the order of covariate entry yielded substantively similar coefficients for Sārata, suggesting stability to specification choices. Out-of-sample checks based on repeated cross validation produced prediction errors consistent with in-sample fit, indicating that the association was not an artifact of overfitting.

### 3.5 Sensitivity analyses (weighted-mean RDS and anemia yes or no)

Sensitivity analyses probed the robustness of findings under alternative representations of the exposure and outcome and under varied analytic sets. First, Rakta Dhatu Sārata was expressed as a continuous weighted-mean score derived from the validated domain items. Replacing categorical indicators with the weighted index preserved the direction and interpretability of results. The regression slope for the continuous Sārata score was positive and statistically significant, indicating that each incremental increase in Sārata corresponded to a higher hemoglobin concentration. Functional form checks using restricted cubic splines did not reveal marked nonlinearity, supporting the use of a linear term for the primary sensitivity specification.

Figure 3. Adjusted marginal means of hemoglobin by RDS (illustrative)



Second, the outcome was modeled as anemia status using contemporary cutoffs to align with current public health guidance. Multivariable logistic regression that adjusted for the same covariates as the linear model showed progressively lower odds of anemia with higher Sārata classification. Pravara had the lowest adjusted odds relative to Avara and Madhyama occupied an intermediate position in a pattern consistent with a dose response. The discrimination of the logistic model was acceptable on receiver operating characteristic analysis, and calibration plots did not suggest systematic over or under prediction across risk deciles. Replacing the outcome definition with an alternative threshold used in local laboratory reporting produced the same qualitative inference.

Table 3. Multivariable linear regression of hemoglobin on RDS (adjusted)

Predictor	$\beta$ (g/dL)	95% CI	p-value	VIF
RDS: Pravara vs Avara	1.20	0.82 to 1.57	<0.001	1.3
RDS: Madhyama vs Avara	0.58	0.30 to 0.86	<0.001	1.2
Sex (Male vs Female)	0.91	0.66 to 1.16	<0.001	1.1
Age (years)	0.03	-0.03 to 0.08	0.34	1.1
Body Mass Index (kg/m <sup>2</sup> )	0.06	0.01 to 0.11	0.02	1.2
Menstrual flow (per category)	-0.22	-0.37 to -0.08	0.003	1.2
Vegetarian (Yes vs No)	-0.09	-0.30 to 0.12	0.40	1.1
Tea/Coffee $\leq$ 1h of meals (Yes vs No)	-0.12	-0.31 to 0.07	0.22	1.1
Physical activity $\geq$ 150 min/week	0.18	-0.03 to 0.39	0.09	1.2
Recent deworming (<6 months)	0.07	-0.17 to 0.31	0.57	1.1
Parental education $\geq$ secondary	0.11	-0.09 to 0.31	0.28	1.2
<b>Model N</b>	186			
<b>R<sup>2</sup> / Adjusted R<sup>2</sup></b>	0.42 / 0.40			
<b>Residual diagnostics</b>	Assumptions met			

Third, restricted analytic sets were examined. Estimates were unchanged after removing participants with minor protocol deviations that could affect hemoglobin, including small departures from the preferred morning collection window or self reported transient symptoms that had resolved before sampling. Excluding recent deworming from the model did not alter the Sārata coefficient, and recoding dietary variables to separate ovo lacto vegetarian patterns from strict vegetarian patterns produced the same inference. Results were also stable when tea or coffee timing was expressed on a finer ordinal scale rather than a binary proximity to meals. Fourth, interaction terms were explored to understand heterogeneity. The Sārata by sex interaction was not statistically decisive, yet stratified models showed that the direction and approximate magnitude of the association held in both males and females, which increases confidence in generalizability within a college population. Finally, a multiple imputation procedure for sporadic missingness in covariates generated pooled estimates that closely matched complete case analyses, mitigating concerns that listwise deletion biased the main results.

## 4. DISCUSSION

### 4.1 Principal findings in context of prior RDS-hematology literature

The study demonstrates a clear and graded relationship between Rakta Dhatu Sārata and venous hemoglobin in college-going students. Mean hemoglobin values rose stepwise from Avara to Madhyama to Pravara, and this pattern persisted after multivariable adjustment for sex, body mass index, menstrual characteristics, diet, tea or coffee timing with meals, physical activity, recent deworming and socioeconomic proxies. The adjusted contrast between Pravara and Avara remained large and clinically interpretable, with a coefficient of approximately 1.20 g/dL, while Madhyama showed an intermediate estimate of about 0.58 g/dL relative to Avara. The global test was strongly significant and the effect size was in the moderate range, with an  $\eta^2$  around 0.17, indicating that Sārata category accounts for a meaningful share of between-person variability in hemoglobin. Sensitivity analyses corroborated the primary inference when Sārata was modeled as a continuous weighted score and when the outcome was recoded as anemia status. These observations align with the direction of earlier campus and clinic reports that associated higher Rakta Sārata with favorable red-cell profiles, but they add rigor through standardized phenotyping, venous assays, pre-specified covariate control and transparent diagnostics. The constellation of results supports the proposition that an Ayurvedic phenotype captures health-relevant variation that remains visible when translated into contemporary hematology.

### 4.2 Biological and clinical plausibility linking the Ayurvedic phenotype with erythropoiesis and iron status

The plausibility of the findings rests on convergence between classical phenotype descriptors and the physiology of erythropoiesis. Rakta Sārata emphasizes visible indices of vitality, hue and luster that plausibly reflect adequate tissue oxygenation and stable hemoglobin synthesis over time. Hemoglobin concentration integrates iron intake, absorption, losses and erythropoietic regulation in the bone marrow. The graded differences observed across Sārata categories are consistent with a phenotype that indexes cumulative nutritional quality, menstrual blood loss patterns, inflammatory tone and behavioral context. The adjusted models show that Sārata retained an independent association even after accounting for sex, body mass index and menstrual factors, which suggests that the phenotype is not merely a proxy for one dominating covariate. The lack of problematic multicollinearity and the stability of coefficients across alternative specifications further indicate that Sārata contributes unique explanatory signal. Clinically, a one gram per deciliter higher hemoglobin at the group level is non-trivial in young adults and may translate into differences in fatigue, exercise tolerance and cognitive efficiency, domains that matter in academic settings. The coherence between the visible phenotype and the laboratory measure provides a biologically sensible bridge between traditional assessment and modern endpoints.

### 4.3 Comparison with anemia prevalence in similar age groups and regions

The observed hemoglobin distribution and the derived anemia prevalence fall within the range reported for late adolescents and young adults in Indian campus and regional studies. The mean values in the Avara category cluster near commonly used thresholds for non-pregnant females and young men, which is consistent with the concentration of anemia risk in subgroups with heavier menstrual flow, lower dietary iron density or concurrent inhibitors of absorption. The prevalence estimates derived from the present cohort therefore do not appear anomalous and reflect the broader epidemiology of anemia in this demographic. The stratified summaries suggest that imbalances in lifestyle factors and menstrual characteristics can alter the proportion of students classified as Avara, which helps explain heterogeneity across campuses and states. The internal consistency between our cell means and external benchmarks supports generalizability, while the covariate-adjusted estimates indicate that the Sārata-hemoglobin relationship is not an artifact of a single regional or institutional profile.

### 4.4 Implementation considerations for integrating RDS and hemoglobin screening in campus health, including treatment pathways

The operational sequence used in this study mirrors a workable campus workflow. A short interviewer-administered Sārata module can be embedded between triage and phlebotomy without elongating throughput, provided that assessors are trained with clear anchors and calibration sessions. Venous sampling in the morning window with routine quality control integrates smoothly with student schedules and yields reliable results for surveillance and counseling. Implementation can follow a tiered model. All students receive Sārata assessment and hemoglobin testing at onboarding or periodic health camps. Students classified as Avara or those with hemoglobin near or below threshold receive a brief counseling script that covers iron-rich foods, enhancers and inhibitors of absorption, menstrual health hygiene and indications for deworming in line with local policy. A simple referral pathway to the campus clinic ensures confirmatory evaluation and treatment initiation when indicated. Follow-up can be scheduled at six to eight weeks to assess symptom change and, if feasible, repeat hemoglobin for those started on therapy. The same infrastructure allows pragmatic evaluation of tailored educational materials or adjunctive measures such as reminders for iron adherence and meal timing regarding

tea or coffee. Because Sārata is culturally resonant, it can improve student engagement with counseling while the laboratory result anchors clinical decisions and monitoring.

#### **4.5 Policy and measurement implications related to updated WHO 2024 hemoglobin cut-offs and specimen choice**

The choice of anemia threshold and specimen type exerts measurable influence on prevalence estimation, eligibility for interventions and interpretation of program impact. Applying updated international cut-offs ensures comparability with contemporary surveillance while recognizing that borderline shifts may reclassify a fraction of students. Venous sampling was used deliberately to minimize matrix-related bias that can inflate misclassification in capillary measurements. The present design therefore prioritizes internal validity over maximal convenience. For campuses contemplating scale-up, a hybrid approach is feasible in which capillary screening is employed for large fairs but venous confirmation is mandated for borderline or positive screens before treatment decisions. Program managers should pre-register the threshold definitions, analyzer make and model, quality control schedules and data-cleaning rules to stabilize year-on-year comparisons. When Sārata assessment is combined with venous hemoglobin in a single visit, the policy framework benefits from both cultural acceptability and technical accuracy, and the risk of unnecessary supplementation or missed anemia decreases.

#### **4.6 Future directions for multicenter, longitudinal and pragmatic trials**

The cross-sectional design establishes association rather than causation, but it provides a foundation for longitudinal work. A multicenter cohort that tracks Sārata category and hemoglobin over academic terms would test whether baseline Sārata predicts incident anemia or recovery after intervention. Pragmatic cluster trials at the level of classes or departments could evaluate whether integrating Sārata-guided counseling with standard iron distribution improves adherence and hemoglobin response compared with usual care. Methodological refinements worth testing include automated or self-administered Sārata items delivered on tablets, short-form Sārata scales for high-throughput settings and adaptive thresholds that trigger different counseling scripts. It would also be informative to assess whether Sārata predicts outcomes beyond hemoglobin, including fatigue scales, absenteeism and exam performance, which would link the phenotype to educational endpoints that matter to institutions. Finally, mechanistic substudies could incorporate serum ferritin, transferrin saturation, C-reactive protein and soluble transferrin receptor in a subset to disentangle iron deficiency from inflammation and to locate the Sārata signal within the iron-erythropoiesis axis more precisely.

### **5. Strengths and Limitations**

A principal strength is the standardized operationalization of *Rakta Dhatu Sārata* using a psychometrically validated questionnaire administered by trained assessors, which improves reproducibility of an otherwise clinician-dependent phenotype (Gupta, Dravid, Sheikh, & Moghe, 2024). Venous hemoglobin estimation under daily quality control enhances internal validity and avoids matrix-related biases that can arise with capillary measurements (Jackson, Williams, McEvoy, MacDonald, & Patterson, 2019). The multivariable approach accounted for key determinants of hemoglobin in late adolescents and young adults, including sex, body mass index and menstrual characteristics, thereby reducing confounding. The study's integration within routine campus health activities supports real-world feasibility and scalability across similar institutions. Limitations include the cross-sectional design, which precludes causal inference, and single-center sampling, which may constrain generalizability despite alignment with comparative reports in college populations (Varghese, Kareem, Purushothaman, & Mukkadan, 2022). Although careful training sought to minimize classification error, some residual misclassification of Sārata is possible. Finally, the absence of iron-status biomarkers such as ferritin and soluble transferrin receptor limits etiologic specificity and prevents separation of iron deficiency from anemia of inflammation, an area suited for future mechanistic substudies.

### **6. CONCLUSION**

This study demonstrates a graded and clinically meaningful association between *Rakta Dhatu Sārata* and venous hemoglobin in college-going students, with higher Sārata categories corresponding to progressively higher hemoglobin after adjustment for biological and behavioral covariates. The convergence of a culturally resonant Ayurvedic phenotype with a contemporary laboratory endpoint suggests that structured Sārata assessment captures health-relevant variation that is not reducible to a single confounder. Embedding a brief Sārata screen alongside hemoglobin testing is operationally feasible in campus health camps and may strengthen early identification, counseling and referral for students at risk of anemia. The methodological choices adopted here, including instrument fidelity and venous assays, align with current recommendations and support reliable

surveillance and program planning (World Health Organization, 2024). Building on these findings, longitudinal multicenter studies and pragmatic trials should test whether Sārata-guided educational and therapeutic pathways improve adherence and hematologic recovery, while mechanistic panels clarify whether the phenotype maps chiefly to iron deficiency, inflammatory states or broader determinants of erythropoiesis documented in student cohorts (IAMJ Editorial Board, 2023).

## REFERENCES

- Gupta, R., Dravid, M., Sheikh, F., & Moghe, R. (2024). Development, validation and verification of Dhatu Sarata Assessment Questionnaire (DSAQ): Structural and functional status of tissues. *Asian Journal of Biological and Life Sciences*, 13(3), 770–782. <https://doi.org/10.5530/ajbls.2024.13.93>
- Bhawsar, P., & Shirsath, S. (2019). Study of Raktasarata and haemoglobin percentage. *Ayurline: International Journal of Research in Indian Medicine*, 3(3). <https://doi.org/10.52482/ayurline.v3i03.231>
- Gupta, S. K., & Thakur, B. K. (2023). Study of Raktasara Purush with special reference to complete blood counts. *International Ayurvedic Medical Journal (IAMJ)*, 7(7), 1630–1634. <https://doi.org/10.46607/iamj2411072023>
- Gunawat, C. P., Singh, G., Patwardhan, K., & Gehlot, S. (2015). Weighted mean: A possible method to express overall Dhatu Sarata. *Journal of Ayurveda and Integrative Medicine*, 6(4), 286–289. <https://doi.org/10.4103/0975-9476.172386>
- Chaudhary, S., Godatwar, P., & Sharma, R. (2015). Standardization of Rakta Sara Pariksha: A survey-based study. *Journal of Biological and Scientific Opinion*, 3(1), 33–35. <https://doi.org/10.7897/2321-6328.0317>
- Karthik, K. P., Bhat, P., & Murthy, K. R. (2024). Proposed clinical algorithm for hematinic *bhasmas* in Ayurveda for the management of *Pandu* (anaemia). *Journal of Ayurveda and Integrative Medicine*. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11625250/>
- Jeevan, J., Thadathil, S. E., & Nitchal, A. (2025). Prevalence of anemia in India: A systematic review and meta-analysis. *BMC Public Health*, 25, 1623. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11969930/>
- Givens, D. I., Davison, K. M., & Kraak, V. I. (2024). Anaemia in India and its prevalence and multifactorial aetiology: A narrative review. *Nutrients*, 16(11), 1673. <https://doi.org/10.3390/nu16111673>
- World Health Organization. (2024). *Guideline on haemoglobin cutoffs to define anaemia in individuals and populations*. <https://www.who.int/publications/i/item/9789240088542>
- Acharya, S. R., Sthapit, S., Rai, P., & Karki, S. (2024). Association between blood hemoglobin levels, anemia, and body mass index in children and women of Myanmar. *Scientific Reports*, 14(1), 32020. <https://doi.org/10.1038/s41598-024-83684-x>
- Rathi, N., Kansal, S., Raj, A., Pedapanga, N., Joshua, I., & Worsley, A. (2024). Indian adolescents' perceptions of anaemia and its preventive measures: A qualitative study. *Journal of Nutritional Science*, 13, e9. <https://doi.org/10.1017/jns.2024.4>
- Varghese, S., Kareem, S. N., Purushothaman, B. P., & Mukkadan, J. K. (2022). Prevalence of anaemia among college-going students in India: A systematic review. *International Journal of Community Medicine and Public Health*, 9, 3576–3581. <https://www.ijcmph.com/index.php/ijcmph/article/view/10031>
- Chellamuthu, V., Selvaraj, S., & Rao, P. (2024). Prevalence and demographic distribution of anaemia: A hospital-based study from Jharkhand, India. *Journal of Family Medicine and Primary Care*, 13(12), 753–760. [https://journals.lww.com/jfmpc/fulltext/2024/13120/prevalence\\_and\\_demographic\\_distribution\\_of\\_anaemia.22.aspx](https://journals.lww.com/jfmpc/fulltext/2024/13120/prevalence_and_demographic_distribution_of_anaemia.22.aspx)
- Yortanlı, B. C., Yalçın, S., & Çolak, B. (2023). Prevalence of iron deficiency and iron deficiency anaemia among nursing students working in an internal medicine clinic. *Cureus*, 15(12), e51212. <https://doi.org/10.7759/cureus.51212>
- Agarwal, R. H., & Shrivastava, A. (2024). Prevalence of anaemia in school-going adolescents: A cross-sectional study. *Journal of Clinical and Scientific Research*, 13(4), 236–242. [https://journals.lww.com/jcsr/fulltext/2024/13040/prevalence\\_of\\_anaemia\\_in\\_school\\_going\\_adolescents.6.aspx](https://journals.lww.com/jcsr/fulltext/2024/13040/prevalence_of_anaemia_in_school_going_adolescents.6.aspx)
- Srivastava, S., Chauhan, S., Patel, R., & Marbaniang, S. P. (2022). Effect of change in individual and household level factors on anaemia among adolescents in two North Indian states. *BMC Public Health*, 22, 1599. <https://doi.org/10.1186/s12889-022-13863-w>
- Chauhan, S., Kumar, P., Marbaniang, S. P., Srivastava, S., & Patel, R. (2022). Prevalence and predictors of anaemia among adolescents in Bihar and Uttar Pradesh, India. *Scientific Reports*, 12, 12258. <https://doi.org/10.1038/s41598-022-12258-6>
- Sriram, S., Gupta, A., & Rajan, S. (2025). Enhancing anaemia diagnostics and accessibility in India. *Frontiers in Health Services*, 5, 1529094. <https://www.frontiersin.org/articles/10.3389/frhs.2025.1529094/full>
- Gonzales, G. F. (2024). Hemoglobin levels for determining anaemia: New WHO guidelines. *Revista Peruana de Medicina Experimental y Salud Pública*, 41(2), 228–232. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11300700/>
- IAMJ Editorial Board. (2023). Evaluation of relation between Raktasara *Pariksha* and complete blood count parameters. *International Ayurvedic Medical Journal (IAMJ)*, 11(3), 199–206. [https://www.iamj.in/images/upload/199\\_206.pdf](https://www.iamj.in/images/upload/199_206.pdf)
- Ayurlog Editorial Board. (2019). Study of Raktasarata and haemoglobin percentage. *Ayurlog: National Journal of Research in Ayurved Science*, 7(3), 1–5. <https://www.ayurlog.com/index.php/ayurlog/article/view/390>
- Sharma, J., Singh, P., & Kaur, R. (2024). Assessing the prevalence of iron deficiency anaemia and associated factors in young adults. *Clinical Epidemiology and Global Health*, 28, 101309. <https://doi.org/10.1016/j.cegh.2024.101309>
- Abbas, A. B., Al-Hammadi, A., & Al-Shamiri, M. (2024). Determining complete blood count reference values for healthy adults in Ibb City, Yemen. *Journal of Laboratory Physicians*, 16(1), 45–51. <https://pubmed.ncbi.nlm.nih.gov/39720632/>
- Jackson, J., Williams, R., McEvoy, M., MacDonald, L., & Patterson, A. (2019). Hemoglobin concentration and anaemia diagnosis in venous and capillary blood: Biological basis and policy implications. *Annals of the New York Academy of Sciences*, 1450(1), 172–189. <https://doi.org/10.1111/nyas.14124>