



# Common Hematological Reference Indices Among Healthy Reproductive Age Indian Women-Data Subset from Nationwide Study

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**Abstract** Despite the negative implications on women's health, pregnancy, and fetal outcomes, population-based studies on hematological indices among reproductive age women in India have received inadequate attention. This study aimed to generate normative ranges for various hematological parameters among these women. After ethics approval, apparently healthy (n = 5884) women (aged 18–40 years) were recruited from six eco-geographic zones

of India. After various exclusions (n = 5412), including women having anemia, data of clinically, and biochemically healthy women (n = 472) was analysed to generate centiles (2.5 and 97.5th) and correlations. The mean age and mean BMI of women was 29.3 ± 6.5 years and 23.25 ± 3.26 kg/m<sup>2</sup> with BP of 112.26 ± 8.9/74.04 ± 6.7 mmHg. The reference intervals for hemoglobin (12–15.1 gm/dl), RBC (3.68–5.55 millions/μl), WBC (4.1–11.26\*10<sup>9</sup>/L), platelet count (1.32–4.42\*10<sup>5</sup>/μl), and erythrocyte sedimentation rate (4.35–41.65 mm/hr) were different from currently used reference values (p < 0.05). However, these haematological indices did not vary among various age categories, geographical zones, ethnicities and rural or urban origins. Pearson's correlation revealed a statistically significant association between ESR, WBC, monocytes, and platelets with homeostasis model assessment of insulin resistance (HOMA-IR). Women with HOMA-IR > 2 displayed a statistically significant differences in parameters like MCV, ESR, eosinophil and platelet counts as compared to the women with HOMA-IR < 2. This study

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provides a pioneering reference data of hematological indices among women of reproductive age in India. Despite the small sample size results can be extrapolated to the national population given the representative sampling of various geographical zones. This may pave way for future comprehensive large-scale studies on the subject.

**Keywords** Normative data · Centile · Hematological indices · India · Reproductive age

## Introduction

The reproductive years constitute a critical juncture in women's life, marked by constant interplay of dynamic physiological, psychological, and societal changes [1]. It is a phase of heightened vulnerability to a plethora of hormonal, reproductive, and metabolic disorders that are of paramount concern. Given that this phase has a direct impact on neonatal and pregnancy outcomes [2] emphasizing the ramifications of maternal health on intrauterine and fetal development, it is imperative to establish reliable population-specific normative data with precise definitions, measurements, and appropriate inferences. Haematological parameters such as RBC, WBC, platelet counts and their respective distribution widths and mean volumes significantly contribute to meaningful clinical consequences such as predictors of endothelial dysfunction, low grade inflammation, insulin resistance (IR) [3].

Country-specific reference intervals for haematological parameters have been established in many countries around the world [4, 5]. Recent studies from Africa, Saudi Arabia and Bemenda reported that significant variation in hemogram compared to standard reference ranges [6, 7]. Most of the normative ranges used for Indian population are derived from Western cohorts that are likely to be different from Indian population with regard to dietary patterns, ethnicity, life style, socio economic status, environmental factors etc. [8], hence raising a demand of having our own norms. In this direction, the data generated by few studies from India are either hospital-based or regional displaying variations against western populations [9–12]. Thus, there is paucity of data on a truly national representative data on healthy Indian women particularly in the reproductive age. To address this issue, we took the opportunity of using the sub-study data generated on control subgroup of women participating in the ICMR-national PCOS task force programme beyond the objective of defining reference intervals. This study represents

a pioneering normative data that can conveniently be used to draw comparisons in hemogram among reproductive age women.

## Material and Methods

### Study Design, Sites, and Population

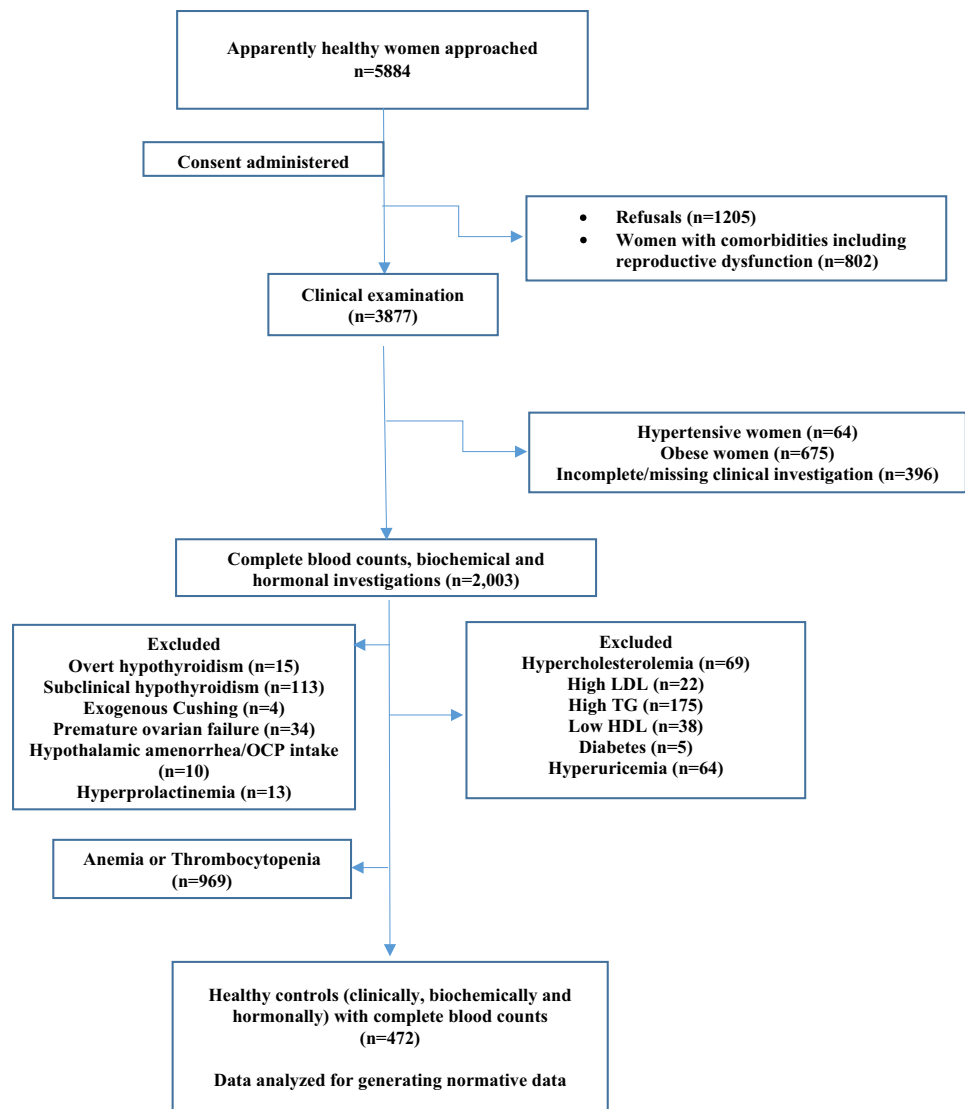
This cross-sectional study involved enrolment of women aged 18–40 years from Oct 2018 to Sept 2022 across 10 sites from various geographical zones (North, South, East, West, North East and Central) of the country India using a multistage sampling technique involving selected polling booths from urban and rural areas. The study was conducted according to the Helsinki Declaration of 1975 and was approved by all the respective institutional Ethics Committees (IEC) with ethics committee numbers as 131/IEC-SKIMS/2017-101-SKIMS-Srinagar; PGI/IEC/2017/47-PGIMER- Chandigarh; IEC-34/09.02.2017-AIIMS-New Delhi; IEC/2017/057-IPGIMER-Kolkata; NEIGR/IEC/2018/02-NEIGRIHMS-Shillong; ECR/300/Inst/AP/2017-Osmania Medical College-Hyderabad; IEC/MHRT/302-MHRT-Hyderabad;173/IEC-AIIMSRPR/2017-AIIMS-Raipur;D/ICEC/Sci-33/37/2017-NIRRH- Mumbai and IEC.No.07/09/2017/MCT-GMC-Thiruvananthapuram. The apparently healthy reference population (n = 5884) comprised of women recruited from community dwelling women based on stringent inclusion and exclusion criteria. The following were the criteria for inclusion: women aged 18–40 years who were permanent residents of the area for more than one year, and signed an informed consent. Pregnant or lactating women and those with cognitive limitations, physical limitations, or both that prevented them from answering the questionnaire were excluded from the study. Women with a history of drug intake such as steroids, androgens, oral contraceptives, antiepileptics, or drugs known to hamper glucose or lipid metabolism were also excluded. Clinicians captured the information using a validated questionnaire that included medical history, drug and diet intake, brief physical examination including anthropometry and blood pressure measurement. The data of women (n = 472) after exclusion of clinical, hematological, biochemical and hormonal derangements were analysed in the study (Fig. 1).

### Laboratory Evaluation

All women were subjected to assessment of fasting sampling for blood counts, LFT, KFT, OGTT, hormones and abdominal USG. All laboratory investigations were carried out in accordance with standard operating procedures (SOPs) followed by good laboratory practices

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**Fig. 1** Pert chart presenting the study overview and flow of subjects



(GLP) uniformed by common training and certification program. In addition, all centres participated in external quality program (EQAS) conducted by national and international agencies. For hemogram approximately 5.0 ml peripheral venous blood was collected with standard 5 ml K3-EDTA BD vacutainer tubes and the blood specimens were processed at the respective site following uniform protocol on automated haematology analysers (Swelab alfa plus basic). The following analytes were investigated: Hemoglobin (Hb), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, WBC differential count (neutrophils, eosinophils, lymphocytes, basophils, monocytes) and the platelet count.

## Statistical Analysis

The statistical analysis was accomplished using SPSS software package version 26.0 (IBM). Normality distribution of the data was tested by the Kolmogorov–Smirnov test in addition to Q-Q plot visual inspection and outliers were removed by the Dixon and Reed method. The data was found to show non-normal distribution hence presented as median and interquartile range (IQR). The median and 95% confidence intervals were determined by using 2.5 and 97.5th percentiles of each hematological parameter with descriptive statistics. Kruskal–Wallis tests with the Dunn post hoc tests were performed for differences between the age categories (18–25, 26–33, 34–40 years), BMI and region. Pearson’s correlation was used to check the relationship between various hematological parameters and HOMA-IR. A two-sided *p* value of <0.05 was considered statistically significant.

## Results

Out of a total of 5884 apparently healthy women approached, 3877 consented and were assessed clinically. 2003 women underwent biochemical and hormonal evaluations among whom 562 were excluded due to various metabolic and hormonal disorders. Of remaining 969 were anemic or had thrombocytopenia and were thereof excluded from the final analysis (Fig. 1). Hematological normative ranges were determined in 472 reproductive age clinically and biochemically healthy women. The mean age of the study population was  $29.3 \pm 6.5$  years. The mean age and mean BMI of women was  $29.3 \pm 6.5$  years and  $23.25 \pm 3.26$  kg/m<sup>2</sup> with BP of  $112.26 \pm 8.9/74.04 \pm 6.7$  mmHg. There were 107(22.66%), 149(31.56%), and 218(45.75%) in age groups 18–25, 26–33 and 34–40 years respectively.

### RBC Parameters

The RBC count and Hb were as  $4.6 \pm 0.45$  (millions/ $\mu$ l) and  $13.09 \pm 0.82$  gm/dl respectively in our study population as

shown in Table 1. The reference ranges were wider for RBC (3.68–5.55 millions/ $\mu$ l), MCH (21.96–34 pg), and MCHC (28–35.55 g/dl) than the standard limits. The RBC parameters for the age categories (18–25, 26–33, 34–40 years) are summarized in Table 2. No statistically significant differences were observed in RBC parameters among these subgroups. At the same time no significant differences were noted among rural and urban-related (as shown in Table 2).

### White Blood Cell (WBC) Parameters

The mean  $\pm$  SD and RI for WBC were ( $6.98 \pm 1.84$  and  $4.1$ – $11.26 \times 10^9/L$ ), lymphocyte ( $3.25 \pm 0.83$  and  $1.64$ – $5.01 \times 10^3$ ), and Monocyte ( $0.49 \pm 0.23$  and  $0.1$ – $0.9 \times 10^3$ ) respectively (Table 1). In particular, statistically significant difference in reference intervals of WBC indices were not observed in age subgroups (Table 2). Even though a wider reference intervals for WBC was observed among rural women than urban ones ( $4.0$ – $12.07$  vs.  $4.3$ – $10.63 \times 10^9/L$ ), however the difference among the groups

**Table 1** Showing the mean, standard deviation (SD), median and all (2.5 to 97.5) percentiles of selected haematological parameters among study population

Analytes	Mean $\pm$ SD (n=422)	Percentiles								
		2.5	5	10	25	50	75	90	95	97.5
Hemoglobin (gm/dl)	$13.09 \pm 0.82$	12	12	12.13	12.5	12.9	13.6	14.2	14.8	15.1
RBC (millions/ $\mu$ l)	$4.6 \pm 0.45$	3.68	3.9	4.1	4.3	4.6	4.89	5.19	5.32	5.55
HCT%	$40.57 \pm 3.25$	35.53	36.34	37.04	38.2	40.13	42.52	44.64	46.13	47.72
MCV (fL)	$88.88 \pm 10.6$	72.15	74.52	78.4	83.28	88.42	92.99	98.52	101.46	108.67
ESR (mm 1st h)	$19.37 \pm 9.51$	4.35	5	8	12	17	26	32.6	35	41.65
MCH (pg)	$28.14 \pm 2.87$	21.96	22.66	24.4	26.7	28.3	29.9	31.2	32.49	34
MCHC (g/dl)	$32.36 \pm 1.77$	28	29.4	30.12	31.5	32.5	33.2	34.2	35	35.55
WBC ( $\times 10^9/L$ )	$6.98 \pm 1.84$	4.1	4.3	4.6	5.6	6.8	8	9.68	10.45	11.26
Neutrophil ( $\times 10^3$ )	$5.95 \pm 0.94$	4.1	4.38	4.65	5.37	6	6.6	7.17	7.44	7.8
Eosinophil ( $\times 10^3$ )	$0.3 \pm 0.16$	0.1	0.1	0.1	0.17	0.28	0.4	0.5	0.6	0.61
Basophil ( $\times 10^3$ )	$0.07 \pm 0.13$	0.0	0	0	0	0.02	0.06	0.2	0.3	0.5
Lymphocyte ( $\times 10^3$ )	$3.25 \pm 0.83$	1.64	1.87	2.18	2.69	3.2	3.8	4.21	4.6	5.01
Monocyte ( $\times 10^3$ )	$0.49 \pm 0.23$	0.1	0.11	0.14	0.32	0.5	0.62	0.8	0.88	0.9
Platelets ( $10^5/\mu$ l)	$2.71 \pm 0.81$	1.32	1.5	1.7	2.12	2.68	3.2	3.78	4.03	4.2

\*Data is presented as Mean  $\pm$  SD and percentiles

RBC Red blood cells; HCT Haematocrit; MCV Mean corpuscular volume; ESR Erythrocyte sedimentation rate; MCH Mean Corpuscular Hemoglobin; MCHC Mean corpuscular hemoglobin concentration; WBC White blood cell

**Table 2** Showing Mean ± SD, median and reference intervals (2.5–97.5) percentiles of selected haematological parameters among study population based on area of residence (rural vs. urban)

Analytes	Rural (n = 229)		Urban (n = 243)		P value
	Median (2.5–97.5 percentile)	Mean ± SD	Median (2.5–97.5 percentile)	Mean ± SD	
Hemoglobin (gm/dl)	12.9(12–15.2)	13.02 ± 0.84	13(12–15.1)	13.15 ± 0.79	0.88
RBC (millions/μl)	4.59(3.72–5.59)	4.61 ± 0.43	4.6(3.59–5.53)	4.6 ± 0.46	0.98
HCT%	39.7(36.18–47.67)	40.42 ± 2.99	40.63(34.66–47.92)	40.7 ± 3.47	0.33
MCV(fL)	88.29(71.68–105.83)	88.22 ± 7.88	88.56(72.13–122.51)	89.5 ± 12.63	0.84
ESR (mm 1st h)	18(5–44)	20.28 ± 9.87	17(4–36)	18.53 ± 9.11	0.14
MCH (pg)	28.25(22–34.79)	28.14 ± 2.69	28.4(20.04–33.06)	28.15 ± 3.03	0.47
MCHC (g/dl)	32.4(28–35.49)	32.28 ± 1.62	32.7(27.99–36.96)	32.43 ± 1.9	0.29
WBC (*10 <sup>9</sup> /L)	6.9(4–12.07)	7.05 ± 1.99	6.69(4.3–10.63)	6.91 ± 1.69	0.55
Neutrophil (*10 <sup>3</sup> )	6(4.2–7.8)	6.0 ± 0.95	5.96(4–7.56)	5.9 ± 0.93	0.31
Eosinophil (*10 <sup>3</sup> )	0.27(0.1–0.61)	0.29 ± 0.17	0.3(0.1–0.61)	0.31 ± 0.16	0.21
Basophil (*10 <sup>3</sup> )	0.01(0–0.52)	0.06 ± 0.12	0.03(0–0.5)	0.08 ± 0.13	0.12
Lymphocyte (*10 <sup>3</sup> )	3.21(1.48–4.9)	3.18 ± 0.82	3.2(1.78–5.26)	3.31 ± 0.83	0.26
Monocyte (*10 <sup>3</sup> )	0.48(0.1–0.9)	0.47 ± 0.22	0.5(0.1–0.9)	0.51 ± 0.23	0.05
Platelets (10 <sup>3</sup> /μl)	2.6(1.3–4.18)	2.65 ± 0.77	2.76(1.28–4.51)	2.75 ± 0.84	0.22

\*Data is presented as Mean ± SD and median (2.5–97.5) percentiles

RBC Red blood cells; HCT Haematocrit; MCV Mean corpuscular volume; ESR Erythrocyte sedimentation rate; MCH Mean Corpuscular Hemoglobin; MCHC Mean corpuscular hemoglobin concentration; WBC White blood cell

was insignificant. Similar trends were observed in other leukocytes parameters as depicted in supplementary Table 1.

**Platelets Parameters**

The median and 2.5th and 97.5th percentile for platelet in our study group were 2.68 \*10<sup>5</sup>/μl and (1.32–4.2\*10<sup>5</sup>/μl) respectively as depicted in Table 1. On stratifying the data into various age and region categories, no statistically significant difference was observed among these subgroups suggesting the same reference intervals may be used across the selected age group of women irrespective of place of residence. (Table 2 and supplementary Table 1).

**Association of Haematological Parameters with IR**

As depicted in supplementary Table 2, women with HOMA-IR > 2 displayed a statistically significant difference in MCV (83.11 ± 12.24 vs. 89.44 ± 9.7fL, p = 0.02), ESR (2.78 ± 0.88 vs. 18.44 ± 9.66 mm 1st hour, p = 0.04),

eosinophils (0.28 ± 0.17 vs. 0.31 ± 0.16\*10<sup>3</sup>, p = 0.03) and platelet count (18.44 ± 9.66 vs. 2.61 ± 0.86 10<sup>5</sup>/μl, p = 0.01) as compared to women with HOMA-IR < 2. Pearson’s correlation analysis as presented in Table 3 revealed a statistically significant correlation between ESR, WBC, monocytes, and platelets with HOMA-IR. No correlation was found between the other CBC parameters.

**Discussion**

Given the significant disparity in blood counts reported across diverse ethnic groups [13, 14], population specific data from different geographic regions are warranted. There is paucity of such data in Indian setting particularly in the context of the particularly vulnerable reproductive age group. In this study, we furnish the haematological indices from a cohort of women of childbearing age representative of all major geographical zones of India which may serve as potentially valuable reference ranges. These

**Table 3** Showing correlation between haematological parameters and HOMA-IR among reproductive age women

CBC analytes	Hb (g/dl)	RBC (millions/ $\mu$ l)	HCT	ESR (mm 1st hour)	MCH (pg)	MCHC (g/dl)	WBC ( $\times 10^9$ /L)	Neutrophil ( $\times 10^3$ )	Eosinophil ( $\times 10^3$ )	Basophil ( $\times 10^3$ )	Lymphocyte ( $\times 10^3$ )	Monocyte ( $\times 10^3$ )	Platelets ( $10^5$ / $\mu$ l)
r	-0.01	-0.05	-0.05	.091*	0	0.05	.115**	-0.01	0.05	-0.02	0.04	-.082*	.124**
P	0.77	0.14	0.14	0.02	0.99	0.17	0.00	0.70	0.17	0.68	0.25	0.02	0.00

Spearman's rho correlation coefficient statistically significant \*  $p < 0.05$ , \*\*  $p < 0.01$

RBC Red blood cells; HCT Haematocrit; MCV Mean corpuscular volume; ESR Erythrocyte sedimentation rate; MCH Mean Corpuscular Hemoglobin; MCHC Mean corpuscular hemoglobin concentration; WBC White blood cell

hematological parameters generated in cohort of healthy women after stringent exclusion are presented in accordance to the guidelines of Clinical and Laboratory Standards Institute (CLSI) in an interval between 2.5 and 97.5% of the data distribution [15]. A significant prevalence of anemia (51.6%), was recorded among these apparently healthy women and were thereof excluded from the final analysis.

Noteworthy deviances in the normative values of various haematological parameters were identified in this study in comparison to established standards or findings from prior research. However, the fact remains that no study specifically focusing on women of reproductive age was identified for direct comparisons. The normative range for haemoglobin was (12–15.1 gm/dl) as against reported earlier [8]. The reference ranges from our study group were wider for RBC, MCH, MCHC, and ESR than the standard limits which are primarily derived from western cohort, however, similar to that reported in other Indian studies [9]. It was observed that normative ranges of RBC (3.68–5.55 vs. 3.5–5.2 millions/ $\mu$ l) count ranges were comparable with the data in female gender examined in other studies across India [8, 9, 16]. The median values of Hb, and RBCs, however were similar and lower to that found among West African and Iranian populations respectively but contrarily, reference limit values of both the parameters were comparatively narrow in our population [17]. According to our study normative platelet count range (1.32–4.2  $\times 10^5$ / $\mu$ l) were found to be lower than the standard reference intervals (1.5–4.5  $\times 10^5$ / $\mu$ l). Similar results have been earlier reported in Kashmiri, Assam and West Bengal population [18–20]. These results are also in concordance to that found in the African [7] with a noticeable difference when compared to American, Chinese, Oman and Malaysian studies which depicted lower platelet ranges as compared to our study [21, 22]. With respect to leukocytes, our study population depicted much higher WBC limits when compared to female populations of Africa, Chinese, Oman, though similar to America and Malaysian population [5, 7, 21, 22]. The total WBC count, and lymphocyte percentage were higher among our study population and our results are in concordance with another Indian study by Subhashree et. al. [9] who also reported higher values for the mentioned parameters and attributed it to high susceptibility of study population to infections. However, in the current study the normative values for haematological parameters didn't vary between different selected geographical regions or urban versus rural areas signifying no regional differences. Since, the health status of reproductive age manifests in women during pregnancy and childbirth and has a direct bearing on foetal development [23], this data may be used for reference purposes to avert any adverse consequences of wrong classification or misdiagnosis.

Furthermore, a significant proportion, nearly half (48%), of the study population exhibited IR (HOMA-IR > 2.0), a finding of considerable significance in the context of pregnancy outcomes among our study group. The maintenance of optimal glycemic control, as a means of averting hyperglycaemia and its associated adverse pregnancy outcomes assumes paramount importance in the Indian context [24]. It has been previously documented that RBC count bears a positive correlation with IR, which is thought to be mediated by alterations in rheological properties and impaired tissue perfusion [25]. However, in the current study, we did not find any significant correlation between RBC count and IR. The subjects with IR exhibited a relatively higher BMI, diastolic blood pressure, ESR, MCV, and eosinophil count as compared to the non-insulin resistance group. Conversely, the WBC count, known to initiate an inflammatory response, was found to have a significant correlation with IR, as previously reported in other studies [26, 27]. Consistent with the results of prior studies [28], we also observed a higher mean platelet count among women displaying IR compared to those with HOMA-IR < 2, and a positive correlation between platelet count and IR. It is possible that platelet count may serve as an indicator of the severity of IR [29] and the heightened risk of gestational diabetes mellitus among women. The present study propounds that the screening of reproductive age women for IR is crucial in order to prevent gestational diabetes mellitus and its potential ramifications on the course of pregnancy and fetal development, particularly in women who otherwise appear healthy.

To the best of our knowledge, our study constitutes the most extensive examination of its kind with stringent exclusions with a comprehensive panel of hematological parameters, featuring multisite data collection, and representative of community from all major geographical regions of India. The utilization of standard laboratory techniques, and uniform protocols lends credence to the reliability and robustness of the outcomes. In addition, this is the first study to unravel the presence of IR among healthy women. In light of the impact of maternal health status on neonates and pregnancy, the data garnered from this high-risk reproductive age group holds unique and significant value. There are several limitations in the present study, such as representativeness of a single gender and cross-sectional design of the study, which precludes the ability to conclude whether IR was a result of an increase in specific hemogram parameters or vice versa.

## Conclusion

This is the first study proving representative data to generate reference data on various blood counts among reproductive age Indian women. Besides, these indices exhibit

notable variations in comparison to the reference indices obtained from Western populations, thereby emphasizing the requirement of normative ranges specific to Indian women. Rampant prevalence of anemia and IR was also observed in the study and hence calls for intervening at key windows of reproductive age to avert the clinical implications of IR and anemia on pregnancy and fetal outcomes, thereby warranting well designed intervention studies.

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**Data availability** Upon rational request, and subject to review, the corresponding author may provide the data that support the findings of this study.

## Declarations

**Conflict of Interests** Authors disclose no disclose financial or non-financial interests directly or indirectly related to this work.

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