

Original Research Article

Prevalence of Anemia, Iron deficiency during the second trimester of gestation in pregnant women: A comparative study of walled and new Lahore city population.

Abstract

Background. Anemia during pregnancy is common worldwide, and it is also reported regarding Lahore, Pakistan, but it is not well documented in women residing in the Walled City of Lahore (WC). The study was designed to compare the pregnant female population of walled and new Lahore (NC) city for the prevalence of anemia and iron deficiency. *Methods.* All the females were within the second trimester of pregnancy with World Health Organization (WHO) criteria of age between 18-45 years. 446 venous blood samples were drawn, out of which 180 belong to WC and 266 to NC. After informed consent, demographic information, including age, gravidity, education, and socioeconomic status, was collected. A complete blood count test was performed to get results for hemoglobin, MCV, and MCH. Total iron-binding capacity, ferritin, and hepcidin levels were checked on ELISA 96-wells plates. *Results.* The prevalence of anemia in WC samples is 58.3%, and in NC samples, it is 42.4%. A decrease in hepcidin is significantly ($p < 0.05$) associated with age and gravidity in both populations. *Conclusion.* It is concluded that anemia control programs should focus on WC women with extra efforts as anemia and iron deficiency are more prevalent. Making intelligent and focused efforts about this public health worry, Pakistan may succeed in achieving Sustainable Development Goals 2025 about eradicating anemia.

Keywords: Anemia, iron deficiency, pregnancy, prevalence, Walled city of Lahore

1. Introduction:

Anemia is characterized either as a low number of red blood cells or a low level of hemoglobin [1]. Anemia impacts one-third of the world's population, contributing to higher morbidity and mortality, lower job efficiency, and reduced neurological growth.

Most prevalent cause of anemia is iron deficiency although the word anemia, iron deficiency, and iron deficiency anemia are interchangeably used obscuring the need to consider the entire range of causes.[2]

While anemia is caused by a reduction in hemoglobin and red blood cell content, which lowers hemoglobin concentrations and hematocrit (used to diagnose anemia), there are various other causes of anemia. Anemia due to iron deficiency (IDA) is the most prevalent pathological cause of anemia during pregnancy, especially in developing countries, where contributions from other anemia-producing hemoglobinopathies are less important[4–7]. In a normal pregnancy, the mother absorbs 500-800 mg of iron. It is much understood about the association between pregnancy and physiological anemia iron deficiency is the most frequent and leading cause of anemia during pregnancy as iron demand is increased [8–11]. Anemia prevention is one of the World Health Assembly's (WHA) Global Nutrition Priorities for 2025, and it is included in the Sustainable Development Goals (SDGs) nutrition targets and metrics. The goals are especially focused on women of reproductive age, with a 50% decrease in anemia in this demographic group by 2025 (compared to 2012 as the baseline) [11,12]. Anemia causes fatigue, dizziness, and increased vulnerability to infections in reproductive-aged women. Still, it raises the likelihood of adverse health problems for both the fetus and mother during pregnancy, including abortion, preterm labor, intrauterine growth retardation, stillbirth and mortality [13–15]. In Pakistan, anemia prevalence was exceeding up to 51.3%. The National Nutritional Survey (NNS) 2018 showed that 56.6% of adolescent girls and 42.7% of women of reproductive age found to be anemic [16–18]. It is good that Pakistan is among the countries that are on the right track to get rid of anemia[19]. But still, a lot of work is to be done to eradicate it. A retrospective report from Pakistan in 2019 published that maternal anemia is linked to poor maternal and neonatal outcomes. There has been few studies aimed to look at maternal anemia with a women in the third trimester of pregnancy[20,21]. According to a report there are about 89% of pregnant women are anemic in rural areas of Punjab while the prevalence of anemia in Lahore is high [22–25]. One of our study group belongs to the Walled City of Lahore and the other from the new City of the Lahore. The Walled City area of Lahore lacks health maintenance and faces a lot of problems, especially about women's health There is a low literacy rate of women, and poverty is another issue that incorporates ill health[26,27]. Compared to these New City Lahore women, the population has more literacy and awareness of their health. Our study focuses on the prevalence of anemia in pregnant women of the Walled City of Lahore and compares it with pregnant women residing in New City Lahore. All of the blood sampling was done in tertiary care hospitals and rural areas of Lahore.

2. Material and Methods:

2.1. Study Design and Setting

We conducted a multicenter comparative study between two populations of Lahore. The two hospitals, including Said Mitha Teaching Hospital Lahore, and Govt, Kot Khwaja Saeed Teaching Hospital, were selected by lottery method.

2.2. Participants and Scheme of Sampling:

10 cc blood was drawn from the pregnant females during the second trimester of pregnancy from 13-26 weeks. Informed consent was obtained from every participant of the research, and privacy rights were highly observed. The WHO criteria for age 18-45 years were followed. Both the primigravida and multigravida were recruited for sampling. Females with any co-morbid condition were excluded from the sampling.

Total number of women receiving the OPD visit during second trimester of pregnancy n=1278

Women who did not give consent to give sample n= 579

These women are subjected for the diagnosis of duration of pregnancy and evaluated for any comorbid condition
Sample n= 699

Women with less duration of pregnancy n= 60
Women with more than 26 weeks of pregnancy n= 81
Women with any comorbid condition n= 38
Women those taking iron supplements n= 74

Final sample of the
women n= 446

Figure 1: Schemes of studies for the sample selection procedure.

2.3. Procedures for data and sample collection

After the prior consent, the information about location, gravidity, education, and socioeconomic status were collected. One time a ten-cc blood sample was drawn from each participant by an expert phlebotomist. Two ccs of blood were placed in CBC vials, and the seven-cc blood transferred to gel tubes for serum separation. All the aliquots were transported from OPD to the lab in a cold chain.

2.4. Biochemical Procedures

Complete blood count (CBC) was performed for each sample on an automated hematology analyzer called Sysmex-KX21, Sysmex-XP-100 three-part differential. Serum iron was measured using Fairbank et al. 1987 Stookey et al. 1970 methods[26] while ferritin, total iron-binding capacity (TIBC), and hepcidin-25 were measured with ELISA by using antibody-coated 96-wells plates (Science glory)[28–30]. Standard curves for all the mentioned assays are calibrated, and then samples are performed by observing the absorbance.

2.5. Anemia and iron deficiency

WHO defined anemia in pregnant women according to the hemoglobin levels. Hemoglobin < 11g/dL is subcategorized in mild <10 g/dL, moderate < 9 g/dL and severe < 7 g/dL anemia. [31–33]. Iron deficiency was diagnosed with less serum iron and serum ferritin concentrations [34]. A newly emerged marker for iron homeostasis hepcidin is also used for the detection of anemia during pregnancy [28,35].

2.6. Statistical Analysis

Sample size was determined by using Raosoft® with a margin of error of 3%, and the confidence level was 95%. We performed a descriptive analysis for all the demographic and biochemical parameters. To detect correlations between discrete variables and outcomes of significance (anemia). Qualitative variables were represented as absolute and relative frequencies, while quantitative variables are represented as means, standard deviations, and

amplitude ranges (minimum and maximum). In the event of a meaningful correlation, the risk and respective 95% confidence interval were determined. To compare two associated dichotomous variables, McMemar's chi squared test was used. Cochran's Q test was used to compare variation in correlated proportions of a dichotomous effect. A *p* value of 0.05 was deemed significant. The hemoglobin and hepcidin showed a strong relationship with hematological markers of iron deficiency.

3. Results

In total, 446 samples were subjected to analysis. 266 samples belonged to new Lahore city, and 180 were from the Walled City of Lahore. The prevalence of anemia 58.3% in WC and 42.4% in NC. Iron deficiency prevalence is minor than anemia, and it is 23.3% in WC and 29.3% in NC. Few samples from each group fall in the category of non-iron deficiency, and in WC, it is 18.3%, and in NC, it is 28.1%. (Fig 1)

All the biochemical parameters are explained concerning further sub-grouping of samples into non-iron deficiency(N-ID), iron deficiency (ID), and Iron deficiency anemia (IDA). The samples with normal serum iron levels, hemoglobin, ferritin, and hepcidin are placed in N-ID. In contrast, the samples with normal hemoglobin values but low values of serum iron are placed into the ID group[34,36].The anemic samples are characterized by low serum iron, low ferritin, and low hemoglobin levels (Table 1) [29,37,38].

Age and gravidity the essential demographic characteristics. These were also influencing the hematological biomarkers of iron deficiency and iron-deficiency anemia. Hpcidin, serum iron, and hemoglobin are influenced by age as these are low in the age group from 31-45 years compared to the 18-30 years of age. Multigravida is observed with moderate to severe deficiency, which is indirectly proportional to the number of gravidities.

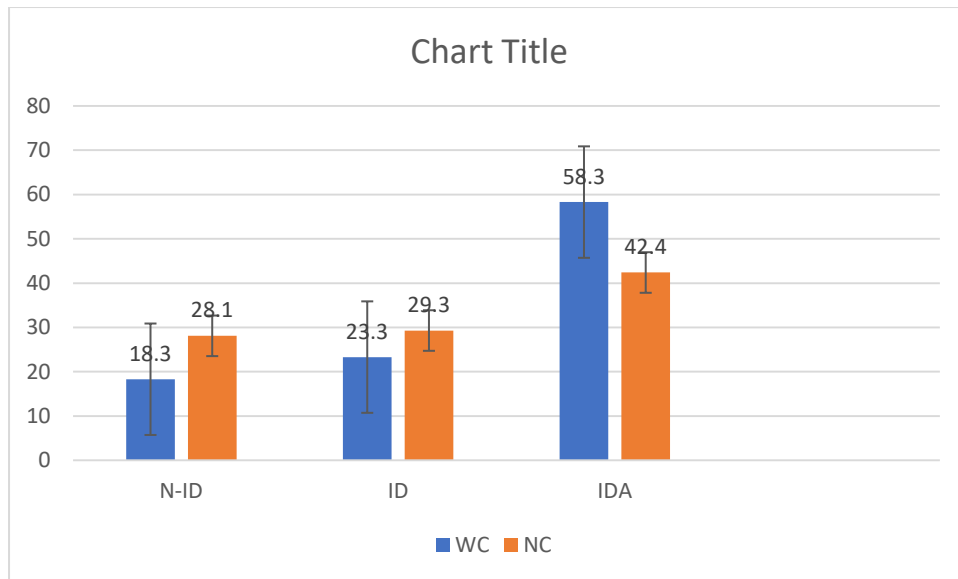


Figure 2: Prevalence of anemia and iron deficiency between the WC and NC populations $n=446$, the total samples evaluated and divided into sub-groups i.e. N-ID, ID and IDA.

Table 1: Comparison of biochemical and hematological markers of non-iron deficiency, iron deficiency, and iron deficiency anemia group of pregnant between women of two different areas. $n=446$

Biochemical Parameter	Population belongs to	N-ID	ID	IDA
Hepcidin $\mu\text{g/L}$	WC	48.70 \pm 15.6	19.42 \pm 2.78	18.00 \pm 2.32
	NC	26.18 \pm 4.12	23.13 \pm 5.24	15.77 \pm 3.28
Ferritin ng/ml	WC	83.61 \pm 5.31	19.86 \pm 1.56	20.69 \pm 1.79
	NC	79.44 \pm 3.8	29.88 \pm 11.6	15.75 \pm 1.43
TIBC $\mu\text{g/dl}$	WC	319.30 \pm 13.1	522.11 \pm 5.12	480.51 \pm 7.31
	NC	330.16 \pm 5.76	504.79 \pm 11.6	501.10 \pm 9.27
TS %	WC	26.95 \pm 2.31	3.84 \pm 0.35	4.82 \pm 0.52
	NC	24.19 \pm 1.19	6.25 \pm 1.09	3.33 \pm 0.35
Serum iron $\mu\text{g/dl}$	WC	87.69 \pm 7.82	28.50 \pm 2.41	20.96 \pm 1.17
	NC	70.24 \pm 3.82	26.73 \pm 3.14	19.67 \pm 0.78
Hemoglobin g/dL	WC	13.13 \pm 0.26	12.70 \pm 0.06	8.08 \pm 0.15
	NC	13.03 \pm 0.11	12.56 \pm 0.11	8.19 \pm 0.22
Hct %	WC	39.39 \pm 0.79	38.10 \pm 0.18	24.26 \pm 0.44
	NC	39.11 \pm 0.33	37.69 \pm 0.33	24.58 \pm 0.66
RBCs $\times 10^6/\mu\text{L}$	WC	4.44 \pm 0.79	4.22 \pm 0.02	2.69 \pm 0.05
	NC	4.40 \pm 0.05	4.18 \pm 0.02	2.73 \pm 0.07
MCV fL	WC	86.90 \pm 1.65	84.30 \pm 0.62	56.12 \pm 0.88

	NC	85.03±0.84	84.74±1.04	53.57±1.41
MCH %	WC	29.60±0.43	30.00±0.01	30.00±0.00
	NC	29.71±0.27	30.00±0.01	30.00±0.00

Table 2: Comparison of Biochemical parameters with demographic variables, age and gravidity in anemic women $n=446$.

Biochemical parameter	Age		Gravidity			
	18-30 years	31-45 years	1	2	3	> 3
Hepcidin $\mu\text{g/L}$	19.59±1.59	22.81±3.49	23.31±2.51	20.39±2.79	16.44±2.69	19.52±4.65
Ferritin ng/ml	20.15±1.23	19.53±1.31	47.12±3.23	22.68±1.82	20.47±2.35	16.02±1.59
Serum Iron $\mu\text{g/dl}$	32.72±1.58	26.47±2.27	44.82±2.83	26.55±1.89	23.13±1.69	18.10±1.12
Hbg g/dL	10.40±0.15	9.90±0.26	11.17±0.23	10.28±0.22	9.541±0.29	8.791±0.32
TS %	8.03±0.56	6.37±0.79	13.2±1.02	5.06±0.50	4.83±0.74	3.44±0.36
RBCs $\times 10^6/\mu\text{L}$	2.73±0.07	2.69±0.05	3.74±0.08	3.43±0.07	3.19±0.09	2.93±0.10

4. Discussion:

In the present study, we used multiple hematological markers to compare the burden of iron deficiency and iron-deficiency anemia between the two populations of different locations. Our studies found that pregnant women residing in the Walled City of Lahore have a high prevalence of anemia, 58.3%. In comparison, the women who belong to new Lahore city have 42.4% anemia prevalence. Iron deficient women are 29.3% in NC and 23.3% in WC. As for our knowledge, this is the first report about the anemia prevalence in WC pregnant women during the second trimester of gestation. Increased prevalence of anemia in WC women is linked to the fewer health facilities, poverty, lack of education, and awareness [39,40]. This prevalence of anemia in pregnant women can result in adverse pregnancy outcomes and can also affect the fetus and maternal life [9,21,23].

According to the WHO, iron deficiency is responsible for almost half of all anemia cases and is the "single" most significant contributor to the anemia load [41,42]. Our studies found less prevalence of iron deficiency NC-29.3% and WC-23.3%, significantly less than expected [43]. This may also be due to the progressive rise of iron absorption during the second trimester of gestation and its relation with hepcidin as a critical player of iron homeostasis during pregnancy [35,38,44,45].

Hepcidin has been reported in many studies mainly focused on its role during pregnancy, but here we are saying its relation with age and gravidity (Table 2). Hepcidin is higher in the 31-45 years of age compared to the age group having participants between 18-30. Hepcidin levels decline during pregnancy[38]. Lower hepcidin values may be associated with age, especially in iron deficiency and iron-deficiency anemia.

Gravidity and less duration between two pregnancies have always been associated with iron deficiency and anemia based on hemoglobin levels [46,47]. Here we found the low hepcidin levels with an increase in gravidity during women's second trimester of pregnancy. Other than hepcidin and hemoglobin markers of iron deficiency, including ferritin, serum iron, TS, and total iron-binding capacity, is not directly associated with the number of pregnancy [48].

In our study, the prevalence of anemia is less than the previously published data about Lahore, Pakistan. Ullah *et al.* printed that 57.7% of women are anemic in Lahore[23]. In the latest research of 2020, it is reported that many socioeconomic factors pivotal in developing anemia among pregnant women of Lahore [24]. In the current study, we found an overall prevalence of 49.5 % in pregnant women. It's a way leading towards achieving WHO Sustainable Development Goals (SDG) 2025 for the health of pregnant women[17] as according to Ayoub *et al.*, Pakistan is on the right track to achieve these goals [19].

The present study has many strengths—biochemical analysis of pregnant women in the hard-to-reach area that is the Walled City of Lahore. Due to a lack of awareness and research culture, women are reluctant to give samples. All these results of the study draw the attention of health departments toward the equal distribution of resources for eradicating anemia both in the walled city and new city of Lahore. After getting data from Beau of Statistics about the population number of women of reproductive age, the handsome sample number was calculated using Rao software [49].

However, the study has many limitations. We agree that analyzing additional biomarkers, such as soluble transferrin receptors and C reactive proteins, would have added practical value. About the fact that the analysis of Hgb concentrations needs adjustments we couldn't make changes due to lack of resources [50]. It would be better if hepcidin levels were compared throughout the pregnancy by doing follow-up sampling. But this couldn't be possible for unavoidable reasons.

Finally, misclassification and the complex etiology of anemia would not have affected our results.

5. Conclusion:

During the second trimester of gestation, pregnant women who belong to the Walled City of Lahore have a high prevalence of anemia compared to the women of the new city Lahore. The overall study findings suggest that the anemia control programs should be equally focused on all Lahore, Pakistan. Less duration between two pregnancies and gravidity are the possible risk factors. By launching focused and specific programs, we can achieve Sustainable Development Goals SDGs 2025 about women's health to decrease 50% anemia in reproductive age women considered 2012 as a baseline.

6. Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Research Ethics Committee of the University of Central Punjab, Lahore via letter-number UCP/FLS/2020/136.

Informed Consent Statement: Informed consent was obtained from all the participants of the study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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