Evaluation of haematological indices, neutrophils and platelets in pregnant women attending tertiary care centre

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Abstract
Objective: The present study was undertaken keeping in view the fact that pregnancy produces several changes in the haematological profile of women. It has been documented by many workers that these changes are fairly significant and hence it was theorized that they could be used to predict the onset of unfavorable circumstances during the various phases of pregnancy. The objective of the study was to analyze these changes especially in neutrophils and platelets and to determine their usefulness as surrogate markers for impending pathological changes during pregnancy.

Materials and Methods: This case-control design study included 400 healthy pregnant women sub-divided into three groups 1st, 2nd and 3rd trimesters, and 400 healthy non pregnant women. Questionnaire interviews were applied Hematological profiles were assessed. Data were computer analyzed using SPSS program version 18.

Results: The present study is a cross sectional design. The study population comprised 400 normal pregnant women and 400 healthy non pregnant women (control group). Neutrophils are significantly increased in pregnant women compared to non-pregnant women and Platelet count of the study population was a significant decrease in platelet count in pregnant women compared to non-pregnant women.

Conclusions: 1. The mean ages of controls and pregnant women in the first, second and third trimesters. 2. Unemployment and lower family income were more prevalent among pregnant women. 3. Medical history of the study population showed that the frequency of the previous pregnancy in controls was significantly lower than that in cases. 4. White blood cells and neutrophils were progressively increased whereas lymphocyte count, RBC count, hemoglobin, hematocrit, MCHC and platelet were decreased in pregnant women compared to non-pregnant women as pregnancy advanced.

Key words: Haematological profile, neutrophils, pregnant women.

Introduction
Pregnancy is associated with normal physiological changes that assist nurturing and survival of the fetus. Haematological parameters reflect these adaptive changes which become very important in the event of complications. Haematological profile is considered one of the factors affecting pregnancy and its outcome. Anemia is the most common haematological problem in pregnancy, followed by thrombocytopenia. The Leukocytosis is almost always associated with pregnancy 1,2. This study is designed to evaluate the overall mean values of seven major haematological parameters and their mean values at different trimesters of pregnancy. Haematological profile is measured all over the world to estimate general health, because it is a reliable indicator and is a simple, fast and cost-effective test. During pregnancy, changes occur and can be observed in haematological indices such as red blood cell (RBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and white blood cell (WBC) count. Some of these are decreased for example, RBC and PLT counts-partly as a result of the physiological hemodilution that occurs in pregnancy, while others are increased, such as the WBC count. There are subtle and substantial changes in haematological parameters during pregnancy and the puerperium, total blood volume increases by about 1.5 liter mainly to supply the needs of the new vascular bed. Pregnancy put extreme a stress on the haematological system. It therefore is very essential that understanding of the physiological changes is obligatory in order to interpret any need for therapeutic intervention. 3 Haematological parameters reflect these adaptive changes which become very important baseline parameters to evaluate all impending complications during pregnancy. Any abnormality from the normal baseline in pregnancy of various parameters like Hemoglobin reflect anemia, red blood cells indices reflect type of anemia, morphological changes in RBC and WBC also reflect type of anemia and infection, platelet count will reflect impending eclampsia in pregnancy. It is proposed that present study will bring out various haematological changes in pregnant women with special reference to the neutrophils. The deviation in parameter may be used as surrogate markers for impending pathophysiological changes in mother and fetus.
Review of Literature

1. There are subtle and substantial changes in hematological parameters during pregnancy and the puerperium total blood volume increases by about 1.5 liter mainly to supply the needs of the new vascular bed.

2. Pregnant places extreme stresses on the hematological system and understanding of the physiological changes that result as obligatory in order to interpret any need for therapeutic intervention.

3. Iron deficiency is the most common cause of anemia in pregnancy.

4. As consequences various quantitative and qualitative hematological changes occur during pregnancy including cell counts, hemoglobin levels, hematocrit, leucocytes, thrombocytes, red blood cells indices, morphological changes and reticulocyte production index.

5. One of the study was carried out to determine the overall mean values for hematological indices in pregnancy and the trimester specific mean values for hematological indices in pregnant women. They found that a progressive decline in Hb concentration from the first to the third trimester, but a drop from first to the second trimester. There was a slight rise in the PCV in the third trimester.

6. Milman N et al (2007) did a study to report reference intervals for haematological variables during normal pregnancy and postpartum. The series comprised 434 healthy ethnic Danish women with a normal pregnancy or =37 wk duration and a normal delivery with newborns weight >2500 g. The lower reference value for Hb during pregnancy was 6.45 mmol/L (105 g/L) and 7.3 mmol/L (118 g/L) postpartum. The lower reference value for Hct was 0.31 in pregnancy and 0.35 postpartum. There was a gradual decline in the lower reference value for erythrocyte folate during pregnancy and postpartum from 0.46 to 0.29 micromol/L and in plasma folate from 6 to 4 mmol/L. Lower reference value for plasma cobalamin declined during pregnancy from 96 to 71 pmol/L, but increased postpartum to 148 pmol/L. Upper reference value for plasma homocysteine increased gradually during pregnancy and postpartum from 11.0 to 20.6 micromol/L. Geometric mean serum ferritin at 18 wk gestation was 32 microg/L. Plasma creatinine values were low during pregnancy and displayed a significant increase postpartum. The characteristic changes occurring in haematological indices during pregnancy and postpartum are described in this study. The results may be used as reference values in the assessment of health status of pregnant women with a similar socio-economic and racial background.

7. Chandra S et al (2012) did a study on physiological changes in hematological parameters during pregnancy. They found that pregnancy is a state characterized by many physiological hematological changes, which may appear to be pathological in the non-pregnant state. The review highlights most of these changes along with the scientific basis for the same, as per the current knowledge, with a special reference to the red blood and white blood cells, platelets and hemostatic profile.

8. Akimbami AA et al (2013) did a study on hematological profile of normal pregnant women in Lagos, Nigeria. There was designed to evaluate the overall mean values of seven major hematological parameters and their mean values at different trimesters of pregnancy. Overall, the values obtained were (mean±standard deviation): hematocrit level, 30.16%±5.55%; hemoglobin concentration, 10.94±1.86 g/dL; white blood cells, 7.81±2.3×109; platelets, 228.29±65.6×109; cell volume 78.30±5.70 fl, corpuscular hemoglobin, 28.57±2.48 pg; and corpuscular hemoglobin concentration, 36.45±1.10 g/dL. When grouped by trimester, the mean±SD value of packed cell volume at first trimester was 32.07±6.80%; of second trimester, 29.76±5.21%; and of third, 33.04±3.88%. The mean±SD hemoglobin concentration values were 11.59±2.35 g/dL, 10.81±1.72 g/dL, and 10.38±1.27 g/dL for women in their first, second, and third trimester, respectively. Mean ± SD white blood cell concentration for first, second, and third trimesters were 7.31±2.38 ×109, 7.88±2.33 ×109, and 8.37±2.15 ×109, respectively, while the mean±SD platelet values for first, second, and third trimesters were 231.50±79.10 ×109, 227.57±63 ×109, and 200.82±94.42 ×109, respectively. A statistically significant relationship was found to exist between packed cell volume and white blood cell count with increase in gestational age (P = 0.010 and 0.001, respectively). However, there was no statistically significant association between platelet count and increase in gestational age (P = 0.296).

9. Elgari MM et al (2013) did a study entitled Evaluation of Hematological Parameters of Sudanese Pregnant Women attending at Omdurman Al Saudi Maternity Hospital. This was a case control study in which 100 pregnant women were enrolled as study group and 50 non pregnant healthy women as control subjects. The study revealed that there were significant decreased in RBCs count, hemoglobin (Hb) and packed cell volume (PCV) of pregnant women compared to non pregnant women (P value <0.05) and significant decreased in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of pregnant women (P value <0.05). TWBCs count was increased significantly (P value < 0.050) in contrast platelets count significantly.
lower than the normal control (P, value <0.05). On bases of blood picture we classified anemia’s of pregnancy as normocytic normochromic 37 (37%) with RPI mean value of 0.49±0.2, microcytic hypochromic 52(52%) with RPI mean value of 0.76±0.6, and dimorphic picture 11 (11%) with RPI of mean value 2.1±0.88.

10. Hill HR et al (1974) did a study on hyperactivity of neutrophil leukotactic responses during active bacterial infection to determine if changes in neutrophil leukocyte function occur during active bacterial infection, the neutrophils of 25 patients with active bacterial infection and 25 age-matched controls were compared for leukotactic activity, random mobility, and nitroblue tetrazolium reduction. The neutrophil leukocytes of patients with bacterial infection were hyperactive in unidirectional movement toward a chemotactic stimulus as measured in the leukotactic assay and usually had increased nitroblue tetrazolium reduction. The mean leukotactic index was 165±56 in patients with bacterial infection and 70±11 in controls (P < 0.001). After 7-10 days of appropriate therapy with clinical and bacteriological response, leukotactic activity returned to normal values. A hyperactive leukotactic response continued, however, in patients with persisting bacterial infection. The hyperactive leukotactic response of circulating neutrophils appears to be an early and sensitive event in the inflammatory cycle stimulated by bacterial infection and may aid in the localization of invading bacteria9.

11. Myers B et al (2009) did a study on thrombocytopenia in pregnancy. They found that thrombocytopenia occurs in 8–10% of all pregnancies. In pregnancy it was usually mild and benign. Rare causes could be associated with severe complications for mother and baby. They recommended that cases thought to be due to immune thrombocytopenic purpura or microangiopathic processes should be managed in a specialist centre. Found that thrombocytopenia occurs in 8–10% of all pregnancies. In pregnancy it was usually mild and benign. Rare causes could be associated with severe complications for mother and baby. They recommended that cases thought to be due to immune thrombocytopenic purpura or microangiopathic processes should be managed in a specialist centre10.

Aims
The aim of this study was to assess RBCs with haematological indices, neutrophils and platelets in pregnant women in a tertiary care centre. The changes in these parameters were be analysed to use as surrogate markers to identify impending danger to mother or/and fetus.

Objectives
To study the haematological parameter in all pregnant women in different trimester of pregnancy and compare them with healthy non pregnant women.
To analyse pattern of changes in neutrophils specifically in pregnant women and compare with healthy non pregnant women, so that it can be established as surrogate marker of impending pathophysiological changes during pregnancy.
To study the changes in platelets both qualitative and quantitative in all pregnant and compare with non-pregnant women of same age group.

Materials and Methods
Source of data: The study was carried out on pregnant women attending Outpatient department of Obstetrics and Gynaecology and India Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India. It was a cross sectional study entitled “Evaluation of haematological indices, neutrophils and platelets of pregnant women attending tertiary care centre.
Study was done in 24 months starting from 1 oct. 2013 to 30 Sep 2015.
Department of Pathology, Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, India.
Sample Size: 400 consecutive blood samples of pregnant women’s as study group and 400 consecutive blood samples of non-pregnant women’s as control group.
Study Design: It was a cross sectional study& was carried out after being approved by the ethical review committees of index medical college hospital and Research Centre, Indore.

Procedures
Sample collection: A blood sample (4.5 mL) was withdrawn from each participant with minimal stasis from the antecubital vein using a dry, sterile disposable syringe and needle. The blood was dispensed into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The specimens were labelled with the subject’s age, and identification number. The EDTA samples were kept at room temperature until processing, which occurred within 4 hours of collection. Each blood sample was mixed well and then approximately 20 μL was aspirated by allowing the analyzer’s sampling probe into the blood sample and depressing the start button. Results of the analysis were displayed after about 30 seconds, after which the analyzer generated a paper copy of the results on thermal printing paper. In citrate tube for coagulation studies such as PT & APTT done in fully automated coagulation analyser.CA-1500.

Laboratory analysis
Analysis was done with fully automated 5 part differential haematology analyzer TRANSASIA
Model-XS-800i, able to test 18 parameters per sample including Hb concentration, PCV, RBC concentration, MCH, MCV, MCHC, WBC count, and PLT count. Standardization, calibration of the instrument, and processing of the samples were done according to the manufacturer’s instructions. Along with smear study.

**Investigations done:** Complete blood count with reticulocyte count and peripheral blood smear
Blood grouping and typing
Platelet count, Mean Platelet Volume (MPV)

**Statistical Analysis**
Data were analysed using SPSS (v 16; IBM, Armonk, NY, USA). The descriptive data are presented herein as means±standard deviation (SD). Pearson’s Chi-square test and one-way analysis of variance (ANOVA) were used for analytic assessment and the differences were considered statistically significant when the P value obtained was<0.05.

**Observations and Results**
The present study is a cross sectional design. The study population comprised 400 normal pregnant women and 400 healthy non pregnant women (control group).

Table 1 summarizes the age classification which showed that 63 (19.75%) controls and 95 (23.75%) pregnant women were ≤20 years old. Age group 21-30 years comprised 266 (66.13%) controls, and 263 (65.75%) pregnant women. Controls and pregnant women aged >30 years old were 71 (14.13%) and 42 (10.50%). The difference between pregnant and non-pregnant women in term of age distribution was not significant (P=0.999). The mean ages of controls and pregnant women were 27.4±6.3, 27.6±6.5, 27.3±6.8 and 27.7±6.6 years old, respectively. The independent sample t- test also showed no significant difference between mean ages of pregnant and non-pregnant women (P=0.991).

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Case Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>&lt;= 20 years</td>
<td>95</td>
<td>63</td>
</tr>
<tr>
<td>21-30 years</td>
<td>263</td>
<td>266</td>
</tr>
<tr>
<td>31-40 years</td>
<td>42</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>400</td>
</tr>
</tbody>
</table>

**Blood Indices:** Primary blood indices (RBC, hemoglobin and hematocrit) of the study population.
Primary blood indices were provided in table 2. Hemoglobin content and hematocrit value were significantly lower in pregnant women compared to non-pregnant women. And RBC count is also lower in pregnant women as compared to non-pregnant women. Secondary blood indices (MCV, MCH and MCHC) of the study population Table 2 shows secondary blood indices of the study population. There was no significant change in MCV among pregnant and non-pregnant women. On the other hand, MCHC and MHC showed significant decrease in pregnant women. Platelets of the study population Platelet count of the study population is indicated in Table 2. There was a significant decrease in platelet count in pregnant women compared to non-pregnant women.

**WBC of the study population:** WBC count of the study population is indicated in Table 2. There was a significant increase in WBC count in pregnant women compared to non-pregnant women. Neutrophils are significantly increased in pregnant women compared to non-pregnant women.
Table 2: Comparison of Haematological value between the two Groups (N=800)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case Group (n=400)</th>
<th>Control Group (n=400)</th>
<th>'t' Value</th>
<th>df</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>9881 ± 3759</td>
<td>8729 ± 21.89</td>
<td>5.30</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>HGB</td>
<td>10.27 ± 1.90</td>
<td>12.64 ± 0.78</td>
<td>-23.02</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>RBC</td>
<td>4.23 ± 0.62</td>
<td>4.29 ± 0.55</td>
<td>-1.50</td>
<td>df=798</td>
<td>0.133</td>
</tr>
<tr>
<td>HCT</td>
<td>32.52 ± 5.08</td>
<td>34.6 ± 15.3</td>
<td>-3.57</td>
<td>df=798</td>
<td>0.016*</td>
</tr>
<tr>
<td>MCV</td>
<td>77.33 ± 9.53</td>
<td>76.78 ± 7.59</td>
<td>0.91</td>
<td>df=798</td>
<td>0.356</td>
</tr>
<tr>
<td>MCH</td>
<td>25.26 ± 4.03</td>
<td>26.99 ± 3.82</td>
<td>-6.25</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.56 ± 2.47</td>
<td>34.24 ± 2.20</td>
<td>-10.22</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>Platelet</td>
<td>1.99 ± 0.70</td>
<td>2.93 ± 0.58</td>
<td>-20.75</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>73.06 ± 7.98</td>
<td>64.86 ± 5.10</td>
<td>16.83</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>21.62 ± 7.07</td>
<td>27.54 ± 6.19</td>
<td>-12.61</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.41 ± 1.38</td>
<td>4.73 ± 1.26</td>
<td>-14.73</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>1.94 ± 2.06</td>
<td>3.00 ± 1.19</td>
<td>-8.96</td>
<td>df=798</td>
<td>0.000*</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>-</td>
<td>df=798</td>
<td></td>
</tr>
</tbody>
</table>

Unpaired ‘t’ test. * - Significant difference

Comparison of Mean Neutrophil between the groups
One-Way ANOVA test was used. F value = 16.09, P value = 0.000, Significant
The F value obtained was 16.09 with a P value of < 0.05, which is statistically significant. Thus, neutrophil values in all the three groups are statistically different.

Post-hoc Tukey Test was applied to see the difference between the pairs

<table>
<thead>
<tr>
<th>Pair</th>
<th>Mean Difference</th>
<th>‘t’ Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester – Second Trimester</td>
<td>4.89</td>
<td>5.61</td>
<td>0.000*</td>
</tr>
<tr>
<td>First Trimester – Third Trimester</td>
<td>1.65</td>
<td>1.62</td>
<td>0.238</td>
</tr>
<tr>
<td>Second Trimester – Third Trimester</td>
<td>3.24</td>
<td>3.17</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

* - Significant difference

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Comparison of Mean Platelet between the groups
One-Way ANOVA test was used. F value = 1.27, P value = 0.282, Not Significant

The F value obtained was 1.27 with a P value of > 0.05, which is statistically not significant. Thus, platelet values in all the three groups is comparable. As the ANOVA value was found to be non-significant, Post-hoc Tukey test has not been applied.

Table 3: Comparison of Mean Platelet between the groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First Trimester (n=156)</th>
<th>Second Trimester (n=155)</th>
<th>Third Trimester (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
</tr>
<tr>
<td>Platelet</td>
<td>2.02±0.69</td>
<td>1.92±0.70</td>
<td>2.05±0.69</td>
</tr>
</tbody>
</table>

Discussion
Pregnancy causes significant changes in metabolism, fluid balance, organ function and blood circulation which are driven by estrogen and the presence of the feto-placental unit. These dramatic changes influence a wide variety of hematological parameters. Acknowledge of these changes is essential when interpreting the result of hematological investigation to diagnose or monitor illness pregnant woman. The mortality rate during pregnancy and 6 weeks after delivery as well as the infant mortality rate are expected to be increased in the next few years, more likely as a result of pregnancy complications. This will no doubt impose a potential burden on the health sector and on the family members. Prognostic assessment of complications accompanied pregnancy in terms of blood testing may be of diagnostic and therapeutic values in the course of pregnancy complications. Such strategy may enable us to alleviate and/or to prevent such complications in order to protect women to have a successful pregnancy.
**Hematological profile of the study population**

White blood cells and neutrophil were progressively increased whereas lymphocyte count was decreased in pregnant women compared to non-pregnant women as pregnancy proceeded. Such findings are in concurrent with that obtained by Kuhunert et al. (1998); James et al. (2008) and Osonuga et al. (2011). Also findings are consistent with previous study which reported that the decreases in hemoglobin and red cell indices concentration are common findings during pregnancy and results from increased plasma volume combined poor iron intake (Bashiri, et al. 2003), (Ruchi et al. 2013).12,13

We found significant increased WBC count significant higher compared to that of the controls. The finding in agreement with previous study reported that total leucocyte count rising in early pregnancy and remained elevated through pregnancy. This may be as a result of the body building the immunity of the fetus and it is achieved by a state of selective immune tolerance, in the presence of a strong antimicrobial immunity. Similarly to the previous study reported that pregnancy leucocytosis, primarily related to increased circulation of neutrophils in the second month of pregnancy (Rouse et al., 1998).14

Leukocytosis occurring during pregnancy may be due to the physiologic stress induced by the pregnant state. In this context, Osonuga et al. (2011) explained this change as a result of the body building the immunity of the fetus and it is achieved by a state of selective immune tolerance, immunosuppression and immunomodulation in the presence of a strong antimicrobial immunity.64 There is also down-regulation of potentially dangerous T-cell-mediated immune responses, while activating certain components of the innate immune system, such as neutrophils which are the major type of leukocyte on differential count (Guyton and Hall, 2011). Neutrophilia is likely due to impaired neutrophil apoptosis in pregnancy (Gatti et al., 1994).15 Neutrophil chemotaxis and phagocytic activity are depressed, specially due to inhibitory factors present in the serum of a pregnant female (Jessica et al., 2007).16 The lymphopenia observed during pregnancy in the present study may be attributed to monocytes which help in preventing fetal allograft rejection by infiltrating the decidual tissue (7th – 20th week of gestation) possibly, through Prostaglandin E2 mediated immunosuppression (Kline et al., 2005).17

The above unique dysregulation between different components of the immune system plays a central role in the maternal adaptation to pregnancy.

Red blood cell count, hemoglobin content and hematocrit value were significantly lower in pregnant women compared to non-pregnant women. Mean corpuscular volume and MCH were not different whereas MCHC and platelet were decreased in pregnant women compared to non-pregnant women (Papadopol et al., 2001 and James et al., 2008).18 Platelet count was progressively decreased in pregnant women compared to non-pregnant women as the pregnancy advanced. Significant decreases in platelet count of pregnant women compared to non-pregnant women (P. value<0.05) in agreement with study reported that: although platelet counts remain in the normal pregnant range in most women during uncomplicated pregnancies (Matthews et al., 1990),18 mean platelet counts of pregnant women may be slightly lower than in healthy non pregnant women (Verdy et al., 1997).19

Large cross sectional studies done in pregnancy of the women (specifically excluding any with hypertension) shown that is termed gestational thrombocytopenia (Shehata et al., 1999 and Yerushalmi, 2007).20,21 It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance (Shehata et al., 1999 and McCrae, 2010).20,22 Gestational thrombocytopenia does not have complication related to thrombocytopenia and babies do not have severe thrombocytopenia (Margaret, 2010).23

**Conclusions and Recommendations**

1. White blood cells and neutrophils were progressively increased whereas lymphocyte count, RBC count, hemoglobin, hematocrit, MCHC and platelet were decreased in pregnant women compared to non-pregnant women as pregnancy advanced.

2. More direct dependence on hemoglobin for pregnant women in their second and third trimesters, along with a more aggressive approach to the level of iron stores at which iron supplementation should be prescribed.

3. The estimation of anemia prevalence is an important step for health policy makers, as hemoglobin estimation is most important parameter to start supplementation of iron or removing other cause of anemia to prevent unfavorable outcome.

4. The references provided in this study should prove useful for diagnostic and research purposes.

5. In order to get better pregnancy care more accurate and reliable figures, uniform and
standard methods and study designs are also recommended for primary studies at the national level.

References