RESEARCH ARTICLE

Effect of intra-cellular trafficking on flow cytometric measurement of neutrophil's oxidative status in iron deficient pregnant females

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Background: Iron deficiency and iron deficiency anemia are prevalent among pregnant women particularly in developing countries. This study aimed to evaluate the iron status among Egyptian pregnant women and its impact on their neutrophil's count and antimicrobial functions.

Method: Ninety pregnant females underwent complete blood count, iron profile, flow cytometric studies for neutrophil myeloperoxidase expression & oxidative burst using dihydrorhodamine 123 (DHR) after phorbol-12-myristate-13-acetate (PMA) stimulation as well as neutrophil phagocytic and lytic indices.

Results: According to percent saturation 54/90 women (60%) were iron deficient (<15% saturation) (cases) and 36/90 (40%) were iron sufficient (controls). A higher proportion of iron deficient pregnant women were in their third trimester compared to controls. No significant difference was found between the iron deficient & sufficient groups as regards anemia despite a positive correlation between haemoglobin level and percent saturation ($P=.02$). Both the phagocytic and lytic indices were significantly lower among the cases compared to controls ($P=.014$ & $.002$ respectively). Cases and controls were comparable as regards flow cytometric studies of neutrophils' myeloperoxidase and oxidative burst ($P>.05$). No significant correlation was found between any of the iron profile parameters and the oxidative burst by flow cytometry.

Conclusion: Functional microphage assay (phagocytic and lytic indices) may be more relevant and cost effective than flow cytometry assays of myeloperoxidase and oxidative burst in reflecting either iron status or cellular immunity in pregnancy.

KEYWORDS

anemia, flow cytometry, immunity, iron deficiency, pregnancy

1 | INTRODUCTION

Anemia and iron deficiency during pregnancy have been associated with high maternal and prenatal mortality, higher risk of infection, muscle dysfunction, intrauterine growth retardation, increased labor time, premature birth, low birth weight and low Apgar scores.¹ Iron supplementation is widely used in pregnancy starting from second trimester.² However, the effectiveness of iron treatment to prevent anemia remains questionable.¹

Although haemoglobin (Hb) concentration is widely used to indicate iron deficiency, it is now considered as a pseudomarker, especially in pregnancy. Studies showed presence of a broad overlap between distribution of Hb in subjects with and without iron deficiency. As regards serum ferritin, a level <12 µg/L indicates iron deficiency anemia, a level of <15 µg/L indicates body iron depletion, while a value of 15-30 µg/L indicates small iron reserves. Therefore, serum ferritin concentration is still considered a good biomarker for iron status as it reflects the capacity of body iron

reserves. Meanwhile serum transferrin receptor (TfR) reflects iron deficiency on a cellular level.¹

Studies showed that iron is important for normal development of the immune system. It is necessary for proliferation of lymphocytes³ and regulates T-cell functions.⁴ Neutrophils are other key cells in the host defense. Earlier studies showed that iron is important for the bactericidal activity of neutrophil myeloperoxidase.⁵ Other studies reported that bactericidal activity of neutrophils and cell-mediated immunity are affected during iron deficiency.⁴ However, some studies found no difference between the iron sufficient and iron deficient groups as regards counts of granulocytes, lymphocytes, or monocytes.⁵ Clinicians are continuously re-evaluating 'classical concepts' on the immunology of pregnancy in different clinical scenarios.⁶ The effect of an additional burden such as iron deficiency is still an area of challenge in pregnancy.

This study therefore aimed to evaluate the iron status of our population of pregnant females in relation to their neutrophils' microphage functional assays and intra-cellular oxidative status.

2 | SUBJECTS AND METHODS

2.1 | Subjects

This study recruited 90 pregnant females during their antenatal care at the gynecology and obstetrics outpatient clinic at Ain Shams University Hospitals. Informed consent was obtained from each patient prior to enrollment. The study was conducted according to the stipulations of the local ethical committee. All the participants in this study were compliant to the antenatal care program and were being supplied with iron supplement. All subjects underwent full history taking, thorough clinical examination and laboratory investigations including complete blood count (CBC) using Coulter LH 750 cell counter (Coulter, Electronics, Hialeah, FL, USA), with examination of Leishman stained peripheral blood (PB) smears for differential leukocyte count, erythrocyte sedimentation rate (ESR), albumin, iron profile [serum iron, total iron binding capacity (TIBC) using humalyzer 3000 (Human) spectrophotometer, ferritin using Accubind ELISA microwells (Monobind Inc., USA) and transferrin saturation ($\text{iron/TIBC} \times 100^7$]. In addition, flow cytometric studies for neutrophil myeloperoxidase (MPO) and oxidative burst using dihydrorhodamine123 (DHR 123) as well as functional microphage assay (phagocytic and lytic index) were performed.

2.2 | Methods

2.2.1 | Sample collection

Samples were collected from each patient under complete aseptic conditions (sterile vacutainers). Two mL venous blood were obtained on ethylenediamine tetra-acetic acid, dipotassium salt ($\text{K}_2\text{-EDTA}$) tubes (final concentration of 1.5 mg/mL) for CBC, preparation of Leishman-stained peripheral blood smears, ESR and flow cytometric assays. Also, 1.8 mL of venous blood were collected on a sodium

citrate tube (32 gm/L) to be used for phagocytic lytic index. Two mL of venous blood were collected and used for iron profile and albumin.

2.2.2 | Flow cytometric assay for neutrophil myeloperoxidase expression

Peripheral blood samples were processed on the same day of sample collection. They were counted using Coulter Cell Counter and the total leukocytic count (TLC) was adjusted to be around $5.0 \times 10^9/\text{L}$ using phosphate buffered saline (PBS) (120 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer) pH 7.4 (Commercially available from Sigma, St. Louis, MO, USA).

Cytoplasmic staining for neutrophil MPO was performed on neutrophils gated using PC5 conjugated CD45 monoclonal antibody and phycoerythrin (PE) conjugated CD13 monoclonal antibody. This was followed using intraprep cytoplasmic staining kit for intracellular permeabilisation, 5% formaldehyde for fixation and finally addition of FITC labeled MPO monoclonal antibody for identifying MPO expression (materials supplied by Beckman Coulter, Electronics, Hialeah, FL, USA). Percent and mean fluorescence intensity (MFI) of myeloperoxidase expression on CD45/CD13 gated neutrophils were measured using EPICS XL, SYSTEM II software.

2.2.3 | Flow cytometric assay of oxidative burst using DHR 123

The procedure used in this study is basically that described by O'Gorma and Corrochano in 1995⁸ with modifications. Stock solutions of DHR 123 and phorbol-12-myristate-13-acetate (PMA) were made in dimethyl sulfoxide (DMSO) and stored at -20°C then further dilutions were made with PBS, 0.2% Bovine serum albumin and 0.2% sodium azide. Three preparations were used per sample of patient or control; one with blood only, the second had blood and DHR 123 and the third had blood, DHR 123 and PMA (supplied by Sigma-Aldrich, St Louis, MO). The procedure involved pre-dilution of blood with PBA (PBS with azide) and pre-incubation with the DHR 123 for 15 minutes prior to addition of PMA. All steps of incubation were at 37°C . Results were expressed as percent of neutrophils showing oxidative burst after stimulation (Normal range was 90%-100%) and MFI of stimulation peak.

2.2.4 | Phagocytic lytic index

Peripheral blood samples were processed on the same day of sample collection.

Separation of neutrophils

One milliliter of anticoagulated fresh whole venous blood was carefully layered on the upper gradient of a tube after 500 uL of Histopaque-1077 was layered over 500 uL of Histopaque-1119. Tubes were centrifuged at 700 g for 30 minutes at room temperature. Careful aspiration of each of upper mononuclear layer and lower polymorphonuclear (PMN/neutrophil) layer was done. The granulocytic layer was washed by isotonic PBS then re-suspended in PBS and used for phagocytic/lytic index.

Phagocytic lytic index

The neutrophils were applied on a clean dry glass slide with edges sealed and the slide was incubated for 30 minutes in a humid atmosphere at 37°C. Then, the slide was flooded with opsonized candida suspension and then incubated for 20 minutes in a humid atmosphere at 37°C. This was followed by gently washing the slide with PBS to remove extracellular candida and leaving it to air dry. Slides were stained by Leishman's stain and then examined.⁹

Interpretation

$$\text{Phagocytic index} = \frac{\text{Number of PMN that have engulfed candida} \times 100}{\text{Number of PMN counted}}$$

$$\text{Lytic index} = \frac{\text{Number of PMN that have lysed candida} \times 100}{\text{Number of PMN counted}}$$

2.3 | Statistical analysis

The statistical analysis of all data was performed using SPSS version 20. All descriptive qualitative data was presented as number & percentage. All descriptive quantitative data were presented as average (mean or median) & variation (SD or IQR). Comparisons for quantitative data were performed using unpaired student *t*-test or Mann-Whitney test. Comparisons for qualitative data were performed using Pearson chi-square and likelihood-ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2×2 tables. Correlations for quantitative parameters were performed using linear correlation coefficient. Cut-off values were

established using the receiver operating characteristic curve analysis (ROC curve). Statistical difference was considered significant if $P < .05$ & highly significant if $P < .001$.

3 | RESULTS

On classifying the subjects according to the percent of iron saturation (% saturation), 54/90 (60%), were iron deficient (cases) and 36/90 (40%) were iron sufficient (control) [iron deficient (cases): if % saturation is <15% and iron sufficient (controls): if % saturation is ≥15%].¹⁰ However, on utilizing both % saturation and serum ferritin level, 74/90 (82.2%) were considered iron deficient and 16/90 (17.8%) were considered iron sufficient [iron deficient (cases): if % saturation is <15% and serum ferritin <30 ng/mL and iron sufficient (controls): if % saturation is ≥15% and serum ferritin ≥30 ng/mL].

A significant higher proportion of females in their third trimester were among iron deficient cases compared to control ($P=.051$).

3.1 | Comparison between cases and controls as regards to routine haematological data

Upon utilizing the first method of classification (% saturation alone), our study revealed cases to have a significantly higher absolute lymphocyte count and higher ESR compared to controls ($P=.047$ and 0.036 respectively). Interestingly cases did not have significantly lower Hb level compared to controls ($P=.100$) (Table 1). However, a significant positive correlation was found between Hb and % saturation ($P=.023$).

TABLE 1 Comparison between cases and controls as regards to routine haematological data

Parameter	Case (n=54) $\bar{x} \pm \text{SD}$ (Med., IQR)*	Control (n=36) $\bar{x} \pm \text{SD}$ (Med., IQR)*	t/z*	P
TLC ($\times 10^9/\text{L}$)	9.42±3.04	8.63±2.69	1.267	0.208
DLC ($\times 10^9/\text{L}$)				
Neutrophils	5.96±2.73	5.57±2.26	0.711	0.479
Lymphocytes	3.04±0.96	2.6±1.09	2.019	0.047
Monocytes	0.28 (0-1)*	0.32 (0-1)*	1.069	0.285
Eosinophils	0.00 (0-0.56)*	0.00 (0-0.23)*	0.481	0.630
Basophils	0.00 (0-0.29)*	0.00 (0-0.10)*	1.465	0.143
RBCs ($\times 10^{12}/\text{L}$)	4.11±0.39	4.13±0.51	0.227	0.821
Hb (g/dL)	10.71±1.31	11.16±1.11	1.662	0.100
Hct (L/L)	33.99±3.63	34.85±3.57	1.111	0.270
MCV (fL)	82.74±7.23	84.74±6.33	1.349	0.181
MCH (pg)	26.08±2.77	27.12±2.57	1.793	0.076
MCHC (g/dL)	31.49±1.31	31.92±1.31	1.517	0.133
RDW (%)	15.54±2.38	15.12±2.37	0.833	0.407
PLT ($\times 10^9/\text{L}$)	250.74±63.27	226.25±63.94	1.791	0.077
ESR (mm)	40 (5-90)*	25 (5-95)*	2.101	0.036

TLC, total leukocytic count; DLC, differential leucocytic count; RBCs, red blood cells; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; PLT, platelets; ESR, erythrocyte sedimentation rate.

Parameter	Case (n=54) $\bar{x} \pm SD$ (Med., IQR)*	Control (n=36) $\bar{x} \pm SD$ (Med., IQR)*	t/z*	P
Phagocytic index (%)	73.73±19.91	83.76±14.55	2.503	.014*
Lytic index (%)	30.78±10.91	40.45±17.59	3.153	.002*
Resting MPO				
MPO %	90.78±21.22	90.40±22.00	0.081	.936
MPO abs. ($\times 10^3/\mu L$)	5.35±2.82	4.95±2.28	0.698	.487
Mdx	1.91 (0.45-5.13)*	1.62 (0.66-10.91)*	0.531*	.595
MPO PS				
10 min %	90.72±14.73	92.73±10.71	0.658	.512
40 min %	83.26±22.95	86.07±21.93	0.547	.586
Δ MPO				
Δ MPO ₁	0.90 (0-49.2)*	0.80 (0-24.8)*	0.226*	.821
Δ MPO ₂	2.57 (0-49.7)*	0.60 (-4.65-45.3)*	1.249*	.212
DHR	84.56±23.95	91.06±15.69	1.345	.183

MPO, myeloperoxidase; MPO abs., absolute myeloperoxidase; Mdx, median intensity; PS, post stimulation; DHR, dihydrorhodamine; Δ MPO₁, Difference in percentage of MPO of positive cells at 10 min post stimulation from resting values; Δ MPO₂, Difference in percentage of MPO of positive cells at 40 min post stimulation from 10 min values.

Upon reclassifying subjects (using % saturation and serum ferritin), no significant difference was found as regards routine haematological data (data not presented).

3.2 | Comparison between cases and controls as regards functional microphage assay and flow cytometric studies

Upon comparing cases and controls according to % saturation, our study revealed that cases had a significantly lower phagocytic and lytic indices compared to controls ($P=.014$ and $.002$, respectively). However, there was no significant difference found regarding flow cytometric studies of neutrophils' functions (MPO expression; resting, post-stimulation or DHR 123) (Table 2). According to both % saturation and serum ferritin level we found that, along with the lytic index ($P=.006$), only the absolute count of resting MPO positive cells (MPO abs.) ($P=.006$) among the flow cytometric studies was significantly higher in the cases compared to the controls (Figure 1).

3.3 | Correlations of iron profile with functional microphage & flow cytometric studies of neutrophil myeloperoxidase & oxidative burst

When we compared cases and control according to % saturation alone, Serum TIBC showed a significant positive correlation with TLC ($P=.042$) and negative correlations with each of % MPO positive resting cells ($P<.001$), % MPO positive cells post stimulation (at 10 & at 40 minutes) ($P=.003$ & $.010$ respectively) and phagocytic index ($P=.019$). The serum ferritin showed significant positive correlations with each of TLC ($P=.017$), absolute neutrophil count ($P=.005$) and absolute count of MPO positive resting cells ($P=.006$). There were significant negative correlations between % saturation & each of TLC

TABLE 2 Comparison between cases and controls as regards functional microphage assay and flow cytometric studies

FIGURE 1 Comparison between patients and control regarding absolute count of resting MPO positive cells and lytic index. (A) Significantly higher MPO positive cells in the patients compared to the controls. (B) Significantly lower lytic index in the patients compared to the controls. (MPO abs.: absolute myeloperoxidase)

($P=.009$) & absolute neutrophil count ($P=.010$). However, there was no significant correlation between any of the iron profile parameters and DHR123 studies.

When we compared cases and control according to both % saturation and serum ferritin level, the % saturation showed a significant positive correlation with lytic index ($P=.049$) (Figure 2A) and negative correlations with each of lymphocyte count ($P=.027$) & ESR ($P=.046$). The lytic index showed a significant negative correlation with each of

FIGURE 2 (A) Positive correlation between lytic index and % saturation; (B) Positive correlation between phagocytic index and MPO% (C) Positive correlation between phagocytic index and DHR

TABLE 3 Performance characteristics of absolute MPO positive cells and lytic index in relation to iron status

Variables	Cut-off point	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Lytic index	$\leq 41.6\%$	0.683	77.22	71.43	93.8	35.7	74.32
MPO absolute	$> 3.6 \times 10^3/\mu\text{L}$	0.727	73.17	75.00	93.7	35.3	74.00

AUC, Area under the receiver operating characteristic curve; PPV, Positive predictive value; NPV, Negative predictive value; MPO, myeloperoxidase.

MCH ($P=.036$) & MCHC ($P=.031$). Meanwhile the phagocytic index showed a significant positive correlation with each of % MPO ($P=.002$) (Figure 2B) & DHR ($P=.048$) (Figure 2C) as opposed to significant negative correlations with each of TIBC ($P=.001$) & eosinophil count ($P=.007$).

3.4 | Performance characteristics of flow cytometric studies (MPO abs.) and lytic index in relation to iron status (using both % saturation and serum ferritin level)

The cut-off values of absolute count of MPO positive resting cells and lytic index were determined using ROC analysis. Recommended cut-off value for each parameter were determined for optimum sensitivity and specificity. The diagnostic value and area under the curve are presented in Table 3 & Figure 3. Albeit statistically non-significant ($P>.05$), the phagocytic index cut-off value was below or equal to 80.6% with sensitivity, specificity, PPV, NPV and diagnostic accuracy of 60.4%, 72.7%, 78.00%, 53.3%, and 66.55%, respectively.

4 | DISCUSSION

Iron deficiency anemia is still a major and global public health problem. It affects women especially at childbearing age particularly pregnant women.¹¹ The impact of iron insufficiency is not limited to developing anemia but also affects the immune system.³ Our study aimed to assess the iron status in pregnancy and investigate its relation to neutrophils' microphage functional assays and intra-cellular oxidative status.

The present study revealed a preponderance of iron deficient women among those in their third trimester of pregnancy despite the standard routine administration of iron supplements. We attributed this

finding to progressive increase in iron requirements during pregnancy. This is supported by Milman's report in 2006¹² which demonstrated that iron requirements increase with pregnancy from 0.8 mg/day in the first trimester to reach 7.5 mg/day in the third trimester.

Our study revealed cases to have a lower Hb level compared to controls but the difference was not statistically significant ($P=.100$). The lack of statistical significance may be attributed to a lag time,

FIGURE 3 The ROC curve of MPO positive resting cells and lytic index in relation to iron status in cases. The area under ROC curve: 0.727 for MPO positive resting cells and 0.683 for lytic index

where iron deficiency is still compensated before manifesting clinically as anemia,¹⁰ hence, the previously mentioned statement that Hb is a pseudomarker for iron status. Albeit a significant positive correlation was established between Hb & % saturation ($P=.023$). Similar, significant positive correlations were found by Lee & colleagues in 2006¹ between Hb level and each of serum iron and TIBC. In our study, this correlation was found only with respect to % saturation because our populations were those of iron deficiency even without anemia while their study incorporated only anemic pregnant females.

Since all the recruited pregnant female in our study were already on iron therapy we further reclassified our study population according to both % saturation and serum ferritin level. This was based on reports recommending that in cases with normal serum iron and high TIBC or normal serum iron and high normal TIBC, serum ferritin level should be used in association with % saturation to indicate iron deficiency.¹³

Although the primary focus of our study was neutrophils, we found cases to have a significantly higher absolute lymphocyte count compared to controls ($P=.047$). This agrees with Ichipi- Ifukor & colleagues in 2013¹⁴ who reported that there is a significant increase in lymphocyte count in pregnant compared to non pregnant. Also this finding agrees with what Luppi in 2003¹⁵ reported about increase in lymphocyte count in early pregnancy that remains elevated throughout pregnancy. On the other hand, a significant positive correlation was found between serum ferritin level & each of TLC ($P=.017$) & absolute neutrophil count ($P=.005$). The correlation was attributed to the ferritin being an acute phase protein.¹⁶ From the above, it is demonstrated that the negative balance in iron status in pregnancy aggravates the already physiological elevation observed in the neutrophils of pregnant females.¹⁷

Our study revealed cases to have a significantly lower phagocytic & lytic indices compared to controls ($P=.014$, $.002$, respectively). This agrees with what Ward & colleagues in 2011¹⁸ reported about impairment of intracellular bactericidal activity of neutrophils in iron deficiency. These findings coincide with those by Paino & colleagues in 2009¹⁹ who demonstrated that there is a decrease in phagocytic activity of neutrophils in patients (particularly elderly patients) with iron deficiency anemia.

Our work revealed significant negative correlations between serum TIBC level & each of % MPO positive resting cells ($P<.001$) and cells post-stimulation (at 10 minutes & at 40 minutes) ($P=.003$ & $P=.010$). Also, there was a significant positive correlation between serum ferritin level & absolute count of MPO positive resting cells ($P=.006$). All such correlations, as well as others mentioned in the results, indicate a significant effect of iron sufficiency on the neutrophil MPO enzyme whether at resting state or post stimulation. These findings coincide with those by Ahluwalia & colleagues in 2004⁵ who stressed on the importance of iron for MPO activity.

In our results, there was no significant correlation established between any of the iron profile parameters and DHR, but the absolute count of MPO positive resting cells was significantly higher in cases compared to controls ($P=.006$, Figure 1). Meanwhile there was no significant difference between cases and controls post-stimulation (Table 2). The for mentioned lack of correlation as well as the paradox

in response of pregnancy neutrophils post-stimulation despite higher resting levels may be explained by previous work that addressed the discrepancy observed in neutrophils from pregnant women with respect to their response to stimulation. These studies found that production of reactive oxygen metabolites (ROM) is least in non-stimulated neutrophils of non-pregnant females then slightly more in non-stimulated neutrophils of pregnancy; however, this baseline level of ROM in unstimulated neutrophils from pregnant women is still lower than that produced by stimulation of neutrophils in non-pregnant women. Upon stimulation of neutrophils in pregnant women, these cells cannot become fully activated for ROM release as their counter part from non-pregnant females.²⁰

Normally stimulated neutrophils produce oxidant molecules, superoxides, ROM & reactive nitrogen intermediates (RNI). Superoxide is produced under influence of NADPH oxidase. A key source of NADPH is the hexose monophosphate shunt (HMS). However, molecules known to stimulate the HMS (e.g., N-formyl-Met-Leu-Phe [fMLP] and LPS) are unable to increase ROM release from pregnancy neutrophils. Studies revealed that in non-pregnant women HMS enzymes are found at the cell periphery but in pregnant women these enzymes undergo retrograde transport to the cell microtubule organizing center.²¹ In this location, HMS is under effect of glycolytic enzymes at cell periphery and can no longer metabolize glucose-6-phosphate (G-6-P) and produce NADPH. Thus, researchers concluded that intracellular trafficking of HMS enzymes complex regulates ROM production and explains its reduction in stimulated pregnancy neutrophils.²²

The other pathway that explains higher basal oxidant release in unstimulated pregnancy neutrophils involves the trafficking of MPO enzyme. The MPO (with their substrates and modifiers) and other intracellular chemical concentration oscillations (as NAD(P)H and superoxide) combine with each other to yield high-amplitude oscillations and increased ROM release. Chemical coupling of MPO and NADPH oxidase occurs when deposited in the same compartment; this is normally prevented by their residence in separate membrane-bound compartments. Accumulating MPO at cell surface of pregnancy neutrophils, but not in non-pregnancy cells, allows its coupling with other intracellular elements that yield high amplitude oscillations and heightened ROM release even in unstimulated state.²⁰

Resting neutrophils from pregnant women not only demonstrate surface MPO but also release reactive oxygen metabolites (ROM) and reactive nitrogen intermediates (RNI) while non-pregnancy cells exhibit none of the above. Therefore, researchers hypothesized that "MPO trafficking affects basal oxidant release by maternal neutrophils".²⁰

From the above facts, we can explain our results with respect to heightened basal level vs. blunted stimulation response in accordance with previous reports that stated "Neutrophils may therefore regulate the extent of their activation by altering MPO enzyme trafficking, just as they can regulate the HMS by translocation of enzyme complexes".²⁰

Concurrent physiological changes of pregnancy impeded the establishment of strong clinically relevant cut-off values. However, using ROC analysis, the sensitivity, specificity, PPV, NPP, and diagnostic accuracy were, 77.22%, 71.43%, 93.8%, 35.7%, and 74.32% for lytic

index and 73.17%, 75.00%, 93.7%, 35.3%, and 74.00%, respectively, for absolute count of MPO positive resting cells. The previous results in relation to the lytic index in particular hold some importance with regard to effect of iron insufficiency on cellular immunity.¹⁹

Our study revealed no significant difference between cases and controls as regards previous infections and obstetric complications. However, we could not reliably dismiss association of such clinical conditions with iron status since previous studies reported associations between asymptomatic bacteriuria and anemia, often refractory to treatment. They also proposed that immunosuppression in anemic women makes them more susceptible to infection.²³ From the previous data, we therefore recommend performing routine screening for silent bacteriuria in iron deficient pregnant women even in the absence of symptoms and signs. The latter recommendation could be further guided by use of the abovementioned cut-offs as indicators of subclinical iron insufficiency that could potentially affect cellular immunity.

In conclusion, functional microphage assays are good parameters for evaluating bactericidal activity of neutrophils. However, the utility of flow cytometric studies for neutrophil myeloperoxidase & oxidative burst are still uncertain especially during pregnancy. Accumulating MPO at cell surface of pregnancy neutrophils, but not in non-pregnancy cells, may account for some functional differences that hindered utility of these tests.

In view of the present study, we recommend the following: (i) performing routine screening for silent bacteriuria in asymptomatic iron deficient pregnant women, (ii) increasing iron supplementations throughout pregnancy particularly during the third trimester, (iii) further search for alternative flow cytometric parameters that are relevant to function of neutrophils in pregnancy.

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