DOI: https://doi.org/10.53350/pjmhs22163539

ORIGINAL ARTICLE

Haematological Parameters and Oxidative stress Changes in Apparently Healthy Pregnant Women in Pakistan

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ABSTRACT
Pregnancy is associated with alterations in haematological and oxidative stress parameters as physiological adjustments are made to accommodate the increasing demand from the fetus and the maintenance of maternal wellbeing. In this cross-sectional study, baseline values for haematological and oxidative stress changes were evaluated in pregnant women attending an ante-natal clinic at selected private clinics in Lahore. A total of 100 subjects (80 pregnant women and 20 non-pregnant women) were recruited for the study. Haematological and oxidative stress parameters were determined following standard protocols. When comparing pregnant and non-pregnant women's white blood cell, neutrophil, monocytes, and catalase mean values (p 0.05), it was observed that the pregnant women's eosinophil mean values were lower than those of the non-pregnant control (p 0.05). During the third trimester, neutrophils and catalase levels were significantly higher in the pregnant group than they were in the non-pregnant group (p 0.05) Compared to catalase, superoxide dismutase was shown to be adversely associated with both mean cell volume and haemoglobin concentration. Other variables studied were mean haemoglobin, mean haemoglobin concentration, total white blood cell and neutrophil counts, and mean cell volume.s There was a negative correlation between glutathione and neutrophils and monocytes whereas a positive correlation was found between malondialdehyde and the mean cell haemoglobin content, total white blood cell, monocyte, eosinophil, and basophil count. According to the findings of this study, pregnant women's haematological parameters may be affected by oxidative stress.
Keywords: Haematology, pregnancy, oxidative stress, antioxidant enzymes

INTRODUCTION
Pregnancy describes the period of the conception and development of a fetus inside a woman's uterus usually lasting for about 40 weeks. This period is divided into 3 segments called trimesters of approximately 12 weeks or 3 months each[1,2]. During this period, maternal physiology adapts considerably to accommodate the growing fetus. Hence, pregnancy is an anabolic state requiring several metabolic adjustments to support fetal growth demands and development while maintaining maternal homeostasis[3-5]. These changes include a considerable weight gain which puts the pregnant woman at a risk of dislocation[3,6], changes in secretion of estrogen, progesterone, cortisol and growth hormone and a slight reduction in blood pressure[3,7-9], increase in respiratory rate and tidal volume and a reduction in functional residual capacity and peak expiratory flow rate[3,10,11].

Haematological changes occur in pregnancy as an adaptive tool in preparation for full fetal hematopoiesis and a cushion for expected blood loss in the course of delivery. Some of these haematological adaptations could appear pathological in non-pregnant states. For example, there is a general increase in plasma volume, red cell mass and adaptive immunological changes triggered by leukocytosis as white blood cells increase significantly with more increase in neutrophils stimulated by estrogen[3,12-14]. These parameters under stable conditions reflect the general health of pregnant women.

Living cells create reactive oxygen and nitrogen species (ROS/RNS) during metabolism (RNS). The radicals hydroxyl (OH), superoxide anions (O2-), and hydrogen peroxide are all examples of reactive oxygen species (H2O2)[15,16]. On the other hand, Nitric oxide (NO) is an abundant intracellular messenger who regulates cardiovascular and neural physiology. Higher than normal levels of NO become deleterious under pathological conditions due to its high reactivity with other free radicals like the superoxide anions (O_2^-) to form peroxynitrite ($ONOO^-$), a strong oxidant capable of reacting and damaging biological molecules[17,18]. These reactive species (ROS and RNS) have very high chemical reactivity. Their reactions result in peroxidation of lipids, enzymatic oxidation and extensive protein oxidation and degradation[19-21]. Antioxidants are molecules capable of limiting or inhibiting the oxidation of other molecules by scavenging ROS or inhibiting its production, hence preventing oxidative damage[22-24]. Oxidative stress results when there is a distorted balance between the generation of the reactive species and the scavenging ability of antioxidants[25,26]. Oxidative stress results in varying effects on female reproductive function like ovarian steroid genesis, ovulation, implantation, oocyte maturation and luteal maintenance in pregnancy[25,27,28]

Objectives: The main objective of the study is to find the haematological parameters and oxidative stress changes in apparently healthy pregnant women in Pakistan.

MATERIALS AND METHODS
Study Population: This study was carried out in Akhtar Saeed Hospital, Lahore during 2020 to 2021.

Research Design, Data and Sample Collection: This was a cross-sectional descriptive study. The study included 100 people (80 pregnant women and 20 non-pregnant women). A simple questionnaire was used to obtain information such as the age and gestational age of the participants. About 3ml of blood was collected from the participants and dispensed into EDTA and plain sample bottles for the determination of haematological and oxidative stress parameters respectively.

Laboratory Analysis: Haematological parameters were assayed using an automated haematology analyzer while oxidative stress parameters were analysed using colorimetric procedures with standard kits.

Statistical Analysis: Data were analysed using SPSS version 25. Social demographics were expressed in frequency and percentages. Contrary to this, pregnant women were compared with non-pregnant pregnant women using the student's t-test, whilst non-pregnant pregnant women were compared with pregnant women using the ANOVA followed by the LDS posthoc analysis. Haematological and oxidative stress indicators were correlated using Pearson correlations. At p 0.05, all the differences were declared statistically significant.

RESULTS
Table 1: Demographic variables of the study population

Parameters		Non-Pregnant (n=20)	Pregnant (n=80)
Age Groups	<21	11 (55%)	10 (12.5%)
	21-30	6 (30%)	52 (65%)
	31-40	3 (15%)	18 (22.5%)
Marital Status	Single	18 (90%)	6 (7.5%)
	Married	2(1 0%)	74 (92.5%)

Table 2: Hematological profile of pregnant and non-pregnant subjects

Parameters	Non-Pregnant Control n=20	Pregnant Subjects n=80	t-test (p-value)
RBC ($x10^{12}$/L)	4.80±0.70	4.51±0.711	0.79
HB (g/dL)	13.10±1.86	12.24±2.22	0.22
PCV (%)	41.95±3.75	40.83±4.44	0.18
MCV (fL)	88.06±7.10	90.93±9.36	0.27
MCH (Pg)	27.66±4.79	27.22±4.40	0.17
MCHC (g/dL)	31.35±4.20	29.97±4.13	0.79
WBC($x10^9$/L)	2.90±2.31	4.11*±3.20	0.04
Neutrophils ($x10^9$/L)	1.30±0.92	2.24*±1.85	0.01
Lymphocytes ($x10^9$/L)	1.20±1.01	1.25±0.96	1.00
Monocytes ($x10^9$/L)	0.25±0.33	0.28*±0.27	0.01
Mosinophils ($x10^9$/L)	0.45±0.45	0.22*±0.34	0.01
Basophils ($x10^9$/L)	0.02±0.03	0.03±0.08	0.86
Platelets ($x10^9$/L)	158.00±60.77	137.28±52.58	0.39

Data are expressed as mean±standard deviation
*Significantly different compared to non-pregnant control (p<0.05)

Table 1 above shows the age and marital status of the study population. The result indicates that younger adults (<21years) participated in the study for the non-pregnant women while older adults (21-30years) took part in the study for the pregnant females. The non-pregnant women were predominantly single (90%) while most of the pregnant women were married (92.5%).

Table 2 shows the mean values of some haematological variables Blood counts of pregnant and non-pregnant women in Pakistan. The results show that the mean values for WBC, neutrophils, and monocytes in pregnant women were substantially greater than in non-pregnant controls ($p < 0.05$). In addition, the mean eosinophil count was found to be considerably lower in pregnant women than in non-pregnant women ($p < 0.05$)

Table shows participants' haematological characteristics during the three trimesters. Each of the three blood types grew gradually from the first to the third trimester. P>0.05) compared to the non-pregnant control group. The statistics show that the third trimester mean neutrophil value was significantly higher than the non-pregnant control (p0.05).

Table 3: Haematological profile of non-pregnant and pregnant subjects in different trimesters

Parameters	Non-Pregnant (n=20)	First Trimester (n=8)	Second Trimester (n=40)	Third Trimester (n=32)	ANOVA (p-value)
RBC ($x10^{12}$/L)	4.80±0.70	4.25±0.89	4.48±0.72	4.63±0.66	0.21
HB (g/dL)	13.10±1.86	11.88±1.25	12.10±2.63	12.50±1.85	0.34
PCV (%)	41.95±3.75	39.75±4.74	40.50±4.83	41.50±3.87	0.47
MCV (fL)	88.01±7.10	93.16±10.05	90.83±9.48	90.51±9.27	0.54
MCH (Pg)	27.66±4.79	28.38±3.21	27.06±5.29	27.13±3.37	0.86
MCHC (g/dL)	31.35±4.20	30.53±1.99	29.78±5.08	30.06±3.17	0.58
WBC($x10^9$/L)	2.90±2.31	2.50±1.93	4.08±3.12	4.56±3.49	0.15
Neutrophils, ($x10^9$/L)	1.30±0.92	1.25±0.04	2.18±1.58	2.56*±2.23	0.04
Lymphocytes ($x10^9$/L)	1.20±1.01	1.00±0.93	1.33±1.02	1.22±0.91	0.84
Monocytes, ($x10^9$/L)	0.25±0.33	0.17±0.08	0.28±0.28	0.30±0.29	0.70
Eosinophils, ($x10^9$/L)	0.45±0.45	0.12±0.09	0.23±0.33	0.24±0.40	0.08
Basophils, ($x10^9$/L)	0.02±0.03	0.01±0.01	0.03±0.09	0.04±0.10	0.68
Platelets, ($x10^9$/L)	158.00±60.77	128.88±22.45	131.40±41.09	146.72±12.06	0.27

Results are given as mean±S.D (Range)
*Significantly different compared to non-pregnant control p<0.05.

DISCUSSION

Pregnancy is associated with haematological adaptations and disturbance in the antioxidant status of the mother due to increased metabolic demand from the developing fetus[35-38]. The present cross-sectional study evaluated the haematological and oxidative stress parameters among pregnant (80) and non-pregnant (20) women in Pakistan.

Pregnant women showed greater mean white blood cell, neutrophil, and monocyte counts than non-pregnant women, but lower mean eosinophil counts (p0.05). Increases in haemoglobin and white blood cell counts were seen from the first to the third trimester of pregnancy. Comparing the third trimester to a non-pregnant control, researchers discovered that the neutrophil mean value was substantially greater. Assessment of haematological profile has remained a simple, easy and reliable means of evaluating the general health status and in the case of pregnancy, it becomes more important in understanding physiological changes to interpret any need for therapeutic interventions during pregnancy[14,39]. The slight decrease in the mean value of RBC and HB has been attributed to the increased demand for iron and oxygen with advancing gestation as more iron and oxygen are required to meet the needs of the developing foetus[40,41]. However, the slight gradual rise in the mean RBC and HB with gestational age could be because of estrogen and progesterone which are released by the placenta and causes the release of renin from the kidneys which in turn enhances erythropoiesis[40,42]. These PCV and HB changes in pregnancy have been observed elsewhere[39-41,43].

It was also observed that the mean values of the WBC, neutrophil and monocyte count among the pregnant women were increased compared to non-pregnant control (p<0.05). There was a gradual rise observed in these parameters from the first to the third trimesters. Pregnancy has long been associated with leukocytosis due to increased inflammatory response with the increase in the circulation of neutrophils observed in the second month of pregnancy[12,37] and remaining elevated till term. In the context of robust antimicrobial immunity, the body builds the foetus' immunity through immunomodulation, immunological tolerance, and immunosuppression[38,44]. Also, the shift of white blood cells between the marginal and circulating pools may produce an increase in neutrophils. It has also been suggested that there is a decrease in neutrophil apoptosis, chemotaxis and phagocytic activity thereby increasing their number in circulation[45,46]. Anxiety, pain, nausea, and vomiting can cause leukocytosis without infection[42]. Similar reports showing increased WBC and neutrophil among pregnant women has been reported by previous studies[13,14,38-42]. The large decline in eosinophils might be due to parasite infection[47,48].

CONCLUSION

Pregnancy is associated with alterations in haematological and oxidative stress parameters as physiological adjustments are made to accommodate the increasing demand from the fetus and the maintenance of maternal wellbeing. While haematopoiesis is enhanced to fulfil increased tissue demand, there is a constant adjustment between pro-oxidant and antioxidant agents to avoid materno-fetal complications. The present study has demonstrated there is a possible link between oxidative stress and haematological parameters among pregnant women and hence antioxidants could be used routinely to reduce or prevent haematological complications during pregnancy.

REFERENCES

1. NICHD. Pregnancy USA: Eunice Kennedy Shriver National Institute of Child Health and Human Development; 2017 [cited 2021 06/11/2021]. Available from: https://www.nichd.nih.gov/health/topics/pregnancy.

2. Davis PD. Medical Definition of Pregnancy: MedicineNet, Inc. ; 2021 [cited 2021 04/06/2021]. Available from: https://www.medicinenet.com/pregnant_fetus_food_first_trimester_pregnancy/article.htm.
3. Talbot L, Maclennan K. Physiology Of Pregnancy. Anaesthesia & Intensive Care Medicine. 2016;17(7):341-5.
4. Longo LD. Maternal Physiology of Pregnancy. The Rise of Fetal and Neonatal Physiology: Springer; 2018. p. 217-80.
5. King JC. Physiology Of Pregnancy And Nutrient Metabolism. The American journal of clinical nutrition. 2000;71(5):1218S-25S.
6. Ireland ML, Ott SM. The Effects Of Pregnancy On The Musculoskeletal System. Clinical Orthopaedics and Related Research®. 2000;372:169-79.
7. Kodogo V, Azibani F, Sliwa K. Role Of Pregnancy Hormones And Hormonal Interaction On The Maternal Cardiovascular System: A Literature Review. Clinical Research in Cardiology. 2019:1-16.
8. Ngene NC, Moodley J. Physiology of blood pressure relevant to managing hypertension in pregnancy. The Journal of Maternal-Fetal & Neonatal Medicine. 2019;32(8):1368-77.
9. Amadi CN, Chinko BC, Green KI. Changes in Serum Growth and Cortisol among Pregnant Women in Port Harcourt, Nigeria. Sch Int J Obstet Gynec. 2022;5(1):21-5.
10. LoMauro A, Aliverti A. Respiratory Physiology Of Pregnancy: Physiology Masterclass. Breathe. 2015;11(4):297-301.
11. Chinko BC, Green KI. Peak Expiratory Flow Rate Of Pregnant Women In Port Harcourt. International Research Journal of Medical Sciences. 2014;2(6):1-5.
12. Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological Changes In Hematological Parameters During Pregnancy. Indian journal of hematology and blood transfusion. 2012;28(3):144-6.
13. Azab E, Albasha MO, Elhemady SY. Haematological parameters in pregnant women attended antenatal care at sabratha teaching hospital in Northwest, Libya. American Journal of Laboratory Medicine. 2017;2(4):60.
14. Dhariwal SK, Narang S, Singh A, Nema S. Evaluation Of Haematological Indices, Neutrophils And Platelets In Pregnant Women Attending Tertiary Care Centre. Indian Journal of Pathology and Oncology. 2016;3(2):297-304.
15. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis. 2000;21(3):361-70.
16. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free Radicals And Antioxidants In Normal Physiological Functions And Human Disease. The international journal of biochemistry & cell biology. 2007;39(1):44-84.
17. Martínez MC, Andriantsitohaina R. Reactive Nitrogen Species: Molecular Mechanisms And Potential Significance In Health And Disease. Antioxidants & redox signaling. 2009;11(3):669-702.
18. Calabrese V, Sultana R, Scapagnini G, Guagliano E, Sapienza M, Bella R, et al. Nitrosative Stress, Cellular Stress Response, And Thiol Homeostasis In Patients With Alzheimer's Disease. Antioxidants & redox signaling. 2006;8(11-12):1975-86.
19. Altomare A, Baron G, Gianazza E, Banfi C, Carini M, Aldini G. Lipid Peroxidation Derived Reactive Carbonyl Species In Free And Conjugated Forms As An Index Of Lipid Peroxidation: Limits And Perspectives. Redox Biology. 2021;42:101899.
20. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nature reviews Molecular cell biology. 2020;21(7):363-83.
21. Demirci-Çekiç S, Özkan G, Avan AN, Uzunboy S, Çapanoğlu E, Apak R. Biomarkers of Oxidative Stress and Antioxidant Defense. Journal of pharmaceutical and biomedical analysis. 2022;209:114477.
22. Gulcin İ. Antioxidants And Antioxidant Methods: An Updated Overview. Archives of Toxicology. 2020;94(3):651-715.
23. Neha K, Haider MR, Pathak A, Yar MS. Medicinal Prospects Of Antioxidants: A Review. European Journal Of Medicinal Chemistry. 2019;178:687-704.
24. Bano A, Gupta A, Rai S, Fatima T, Sharma S, Pathak N. Mechanistic Role of Reactive Oxygen Species and Its Regulation Via the Antioxidant System under Environmental Stress. In: Hasanuzzaman M, Nahar MK, editors. Plant Stress Physiology - Perspectives in Agriculture: IntechOpen. https://doi.org/10.5772/intechopen.101045; 2021.
25. Zahra KF, Lefter R, Ali A, Abdellah E-C, Trus C, Ciobica A, et al. The Involvement of the Oxidative Stress Status in Cancer Pathology: A Double View on the Role of the Antioxidants. Oxidative Medicine and Cellular Longevity. 2021;2021.
26. Bano A, Gupta A, Rai S, Fatima T, Sharma S, Pathak N. Mechanistic Role of Reactive Oxygen Species and Its Regulation Via the Antioxidant System under Environmental Stress. 2021.
27. Lu J, Wang Z, Cao J, Chen Y, Dong Y. A Novel And Compact Review On The Role Of Oxidative Stress In Female Reproduction. Reproductive Biology and Endocrinology. 2018;16(1):1-18.
28. Sharma RK, Agarwal A. Role Of Reactive Oxygen Species In Gynecologic Diseases. Reproductive medicine and Biology. 2004;3(4):177-99.
29. Burton GJ, Jauniaux E. Placental Oxidative Stress: From Miscarriage To Preeclampsia. The Journal of the Society for Gynecologic Investigation: JSGI. 2004;11(6):342-52.
30. Chiarello DI, Abad C, Rojas D, Toledo F, Vázquez CM, Mate A, et al. Oxidative Stress: Normal Pregnancy Versus Preeclampsia. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2020;1866(2):165354.
31. Lunghi L, Ferretti ME, Medici S, Biondi C, Vesce F. Control Of Human Trophoblast Function. Reproductive Biology and Endocrinology. 2007;5(1):1-14.
32. Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. The international journal of biochemistry & cell biology. 2010;42(10):1634-50.
33. Igbara PI. The Emergence of Bori town : The Ogoni heartland. ort Harcourt, Nigeria: Onyoma Research Publications; 2013.
34. WMA. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects Fortaleza, Brazil.: The World Medical Association, Inc; 2013 [cited 2020 26/08/2020]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.
35. Toboła-Wróbel K, Pietryga M, Dydowicz P, Napierała M, Brązert J, Florek E. Association Of Oxidative Stress On Pregnancy. Oxidative medicine and cellular longevity. 2020;2020:6398520.
36. Herrera E, Ortega-Senovilla H. Maternal Lipid Metabolism During Normal Pregnancy And Its Implications To Fetal Development. Clinical Lipidology. 2010;5(6):899-911.
37. Kaur S, Khan S, Nigam A. Hematological Profile And Pregnancy: A Review. Int J Adv Med. 2014;1(2):68-70.
38. Ichipi-Ifukor PC, Jacobs J, Ichipi-Ifukor RN, Ewrhe OL. Changes In Haematological Indices In Normal Pregnancy. Physiology Journal. 2013;2013.
39. Azab AE, Albasha MO, Jbireal J, El Hemady SY. Haematological Changes during Pregnancy: Insight into Anaemia, Leukocytosis, and Thrombocytopenia. East African Scholars Journal of Medical Sciences. 2020;3(5):185-92.
40. Akinbami AA, Ajibola SO, Rabiu KA, Adewunmi AA, Dosunmu AO, Adediran A, et al. Hematological Profile Of Normal Pregnant Women In Lagos, Nigeria. International Journal Of Women's Health. 2013;5:227.
41. Amah-Tariah F, Ojeka S, Dapper D. Haematological Values In Pregnant Women In Port Harcourt, Nigeria II: Serum Iron And Transferrin, Total And Unsaturated Iron Binding Capacity And Some Red Cell And Platelet Indices. Niger J Physiol Sci. 2011;26(2):173-8.
42. Akingbola TS, Adewole IF, Adesina OA, Afolabi KA, Fehintola FA, Bamgboye EA, et al. Haematological Profile Of Healthy Pregnant Women In Ibadan, South-Western Nigeria. Journal Of Obstetrics And Gynaecology. 2006;26(8):763-9.
43. Ifeanyi OE, Ndubuisi OT, Leticia EOB, Uche EC. Haematological Profile Of Pregnant Women In Umuahia, Abia State, Nigeria. Int J Curr Microbiol App Sci. 2014;3(1):713-8.
44. Chaudhari SJ, Bodat RK. Tracking Of Haematological Parameters In First And Second Trimester Of Pregnancy. Age (Yrs). 2015;5(3):0.69.
45. Gatti L, Tenconi PM, Guarneri D, Bertulessi C, Ossola MW, Bosco P, et al. Hemostatic Parameters And Platelet Activation By Flow-Cytometry In Normal Pregnancy: A Longitudinal Study. International Journal of Clinical and Laboratory Research. 1994;24(4):217-9.
46. Crocker IP, Baker PN, Fletcher J. Neutrophil Function In Pregnancy And Rheumatoid Arthritis. Annals Of The Rheumatic Diseases. 2000;59(7):555-64.
47. Kim Y-J, Nutman TB. Eosinophilia. In: Walker PF, Barnett ED, editors. Immigrant Medicine. Edinburgh: W.B. Saunders; 2007. p. 309-19.
48. Dawson D. Eosinophils and Pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology. 1953;60(5):727-31.