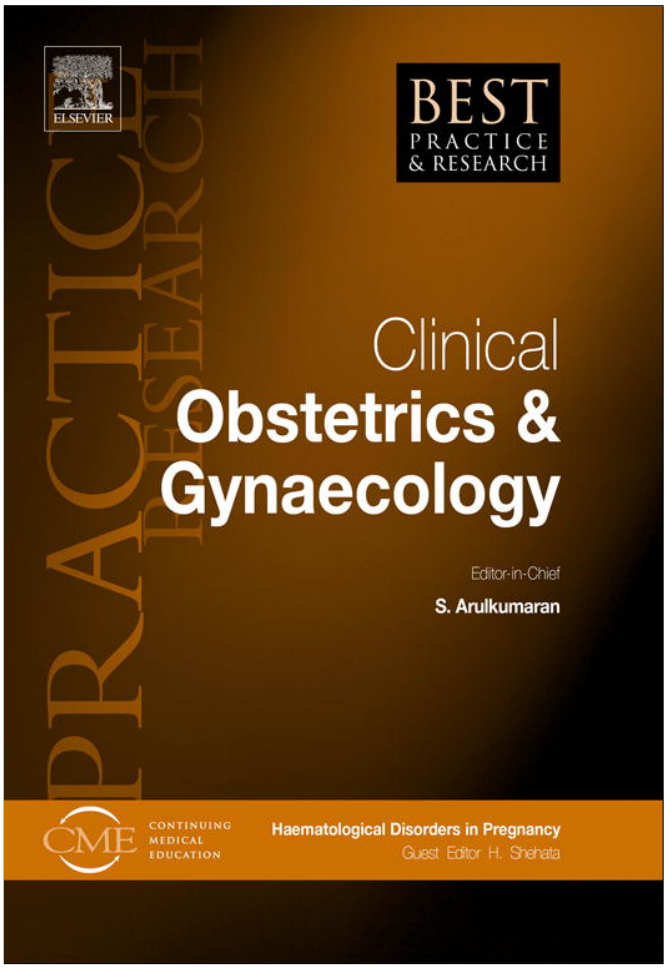
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Anaemia in pregnancy

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delayed cord clamping

Anaemia in pregnancy, defined as a haemoglobin concentration (Hb) < 110 g/L, affects more than 56 million women globally, two thirds of them being from Asia. Multiple factors lead to anaemia in pregnancy, nutritional iron deficiency anaemia (IDA) being the commonest. Underlying inflammatory conditions, physiological haemodilution and several factors affecting Hb and iron status in pregnancy lead to difficulties in establishing a definitive diagnosis. IDA is associated with increased maternal and perinatal morbidity and mortality, and long-term adverse effects in the new born. Strategies to prevent anaemia in pregnancy and its adverse effects include treatment of underlying conditions, iron and folate supplementation given weekly for all menstruating women including adolescents and daily for women during pregnancy and the post partum period, and delayed clamping of the umbilical cord at delivery. Oral iron is preferable to intravenous therapy for treatment of IDA. B12 and folate deficiencies in pregnancy are rare and may be due to inadequate dietary intake with the latter being more common. These vitamins play an important role in embryo genesis and hence any relative deficiencies may result in congenital abnormalities. Finding the underlying cause are crucial to the management of these deficiencies. Haemolytic anaemias rare also rare in pregnancy, but may have life-threatening complications if the diagnosis is not made in good time and acted upon appropriately.

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Introduction

In more than 80% of countries in the world, the prevalence of anaemia in pregnancy is >20%, and is a major public health problem. More than 56 million women are estimated to be affected globally, approx 32 million of them being from Asia. The global prevalence of anaemia in pregnancy is estimated to be approximately 41.8%, varying from a low of 5.7% in the USA to a high of 75% in Gambia (Table 1).[1] Some women are anaemic prior to the index pregnancy and others become progressively anaemic during pregnancy. In the USA, anaemia in pregnancy was estimated to increase from 1.8% to 8.2% and 27.4% in the first, second and third trimesters respectively.[2]

Table 1
Estimated Prevalence of Anaemia in Pregnant Women and Non-Pregnant Women (15 – 49 years).[1]

Region	Estimated Prevalence in Pregnant Women (%)	95% CI	Estimated Prevalence in Non-Pregnant Women (15–49 yrs)	95% CI
Africa	55.8	51.9–59.6	44.4	40.9–47.8
Asia	41.6	39.0–44.2	33.0	31.3–34.7
Europe	18.7	12.3–25.1	15.2	10.5–19.9
Latin America + Caribbean	31.1	31.8–40.4	23.5	15.9–31.0
North America	6.1	3.4–8.8	7.6	5.9–9.4
Oceania	30.4	17.0–43.9	20.2	9.5–30.9
Global	41.8	39.9–43.8	30.2	28.7–31.6

Definition

During pregnancy, anaemia is defined as a haemoglobin concentration (Hb) <110 g/L at sea level,[3,4] which is two standard deviations below the mean Hb expected. Consequent to the physiological haemodilution which is maximal during 20–24 weeks of gestation, the Hb varies with the period of gestation.[3–6] The Hb increases with high altitudes[4] and in those who smoke.[4,7] In those who smoke, the decrease in plasma volume and increase of Hb, both of which adversely affects fetal growth, are adaptations to increased carboxyhaemoglobin which has no oxygen carrying capacity.[7] Quitting smoking can reduce the Hb to its original levels within five years.[8] Although the Hb can increase in pregnant women who smoke,[9] a decrease of Hb with smoking[10,11] and the use of smokeless tobacco[12] have been shown. Genetic differences may also affect the Hb.[13,14] A haematocrit of <33% could also be considered for the diagnosis of anaemia in pregnancy.[3] Severe anaemia in pregnancy (Hb < 70 g/L)[3,4] requires urgent medical treatment and Hb <40 g/L is an emergency carrying a risk of congestive cardiac failure, sepsis and death.[3]

Pathophysiology

Nutritional Iron Deficiency (ID), accounting for more than half the cases in non-malarial areas, is the commonest cause of anaemia during pregnancy (Table 2).[3,4] A woman could progress from a healthy non-anaemic iron replete status, to a state of low iron stores (latent ID), to ID with no anaemia and finally to a clinical iron deficiency anaemia (IDA). Therefore the prevalence of ID is always greater and could be 2–2.5 times the prevalence of IDA.[3,15,16] ID per se (without IDA) can lead to functional impairment at tissue level.[3]

A 55 kg pregnant woman is estimated to need approximately an additional 1000 mg of iron over the whole pregnancy.[3,17] Iron requirements as well as iron absorption are very low in the first trimester, but progressively increase to reach a maximum in the third trimester.[3,16,17] It has been estimated that the daily iron requirements of a 55 kg pregnant woman increases from approximately 0.8 mg in the first trimester to 4–5 mg during the second trimester and >6 mg in the third trimester.[17]

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Table 2
Causes of anaemia in pregnancy.

Nutritional Deficiencies	Iron
	Folic Acid
	Vitamin B_{12}
	Vitamin C, Vitamin A
	Protein
Haemolysis and abnormal haemoglobin synthesis	Malaria
	Glucose 6 – phosphate dehydrogenase deficiency
	Thalassaemias
	Sickle cell disease
Blood loss, and defective iron absorption and metabolism	Helminthiasis, especially hookworm infestation
	Amoebiasis and Giadiasis
	Schistosomiasis
	Abnormal iron metabolism
	Bleeding haemorrhoids
	Antepartum haemorrhage
	Trauma
	High parity
Chronic conditions	Malignancies
	Tuberculosis
	Chronic renal disease including urinary tract infection
	Sexually transmitted infections including bacterial vaginosis
	Human Immune deficiency Virus infection
	Chronic rheumatic and rheumatoid disease

Pre-conceptual low iron stores are associated with an increased risk of anaemia during the latter half of pregnancy as well as adverse perinatal outcomes.[18] Ideally in order to meet the additional iron requirements of pregnancy a woman should have at least 300 mg of iron stores prior to conception.[19,20] Even a European type diet with high bio availability of iron, and the increased iron absorption during the latter half of pregnancy, may not be able to meet the additional requirements of iron for the pregnancy.[17,20] In the USA, >50% of women in reproductive age (WRA = 15–45 years) were found to have <300 mg of iron stores,[21] highlighting the need to ensure adequate iron nutrition prior to pregnancy even in well-resourced settings.

Diagnosis

Tiredness, weakness, lethargy, irritability and decreased work tolerance are the well known clinical presentations of anaemia.[3] However the majority of women with mild to moderate anaemia are asymptomatic. Rarely, a woman with severe anaemia may present with glossitis, angular stomatitis, ankle oedema and early evidence of congestive cardiac failure and may require emergency treatment. Multiple pregnancy,[22] teenage pregnancy[23] and high parity[24] are associated with increased risk of anaemia in pregnancy. Reduced inter-pregnancy non-lactating intervals may also be associated.[25]

Although Hb <110 g/L is the accepted criterion for the diagnosis of anaemia in pregnancy, considering physiological haemodilution, altitude, smoking and ethnicity, certain adjustments to this value have been suggested,[3–6,26] although adjustments based on altitude may not be justified.[27] Hb and the other haematological indices being defined for individual populations considering the above factors, their iron stores and patterns of diet intake,[6] and reducing the diagnostic level in a pregnant woman to Hb <100 g/L in the first and second trimesters, have also been suggested.[5,28, 29] However, a cut off level of 110 g/L is needed to compare prevalence and effects in different communities, regions and countries.

Confirmation of ID in pregnancy is difficult. Detection of stainable iron as haemosiderin in bone marrow aspirates excludes ID, but its absence is not proof of ID.[30] Serum ferritin (SF), transferrin saturation and bone marrow iron decrease over the course of pregnancy, even in women receiving high daily iron supplements.[31,32] Serum transferrin receptor (sTfR) levels of >8.5 mg/L indicate ID. A sTfR/log10SF ratio (sTfR-SF Index) >1.8 indicates ID in non-pregnant adults but is not as effective in

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pregnancy.[30,33] As sTfR assays are expensive and not freely available, a SF <12 μg/L is frequently used to diagnose ID.[3,18,32] SF increases in the presence of infection or inflammation.[3,32] Although assessment of C-reactive protein may not help, use of higher cut off levels of SF e.g. <30 μg/L or <50 μg/L may be indicated in the presence of chronic inflammation.[34,35] Although a combination of Hb, SF, sTfR and sTfR-SF Index would enable the diagnosis of IDA, this would not be possible in low resource settings.

In IDA, a Full Blood Count should characteristically show: Mean Corpuscular Volume (MCV) <80 fl, Mean Corpuscular Haemoglobin Concentration <30%, Mean Corpuscular Haemoglobin <30 μg/L and Red Cell Count <4.1 mil/mm^3. However haematological indices are altered during pregnancy.[34,36,37] A combination of iron deficient microcytes, folate or B_{12} deficient macrocytes and pregnancy and/or iron supplementation induced macrocytes would lead to a polymorphic blood picture. The haematological indices, which are average quantities, will not indicate this. Therefore in communities where nutritional anaemia is common, the examination of a peripheral blood film is important, and it supplements the Full Blood Count. If the anaemia is due to ID, an increase of 10 g/L of Hb and or 3% increase in haematocrit will be seen after six weeks of oral iron supplements.

If the MCV is >100 fl, and the peripheral blood picture suggests the possibility of folate or Vitamin B_{12} deficiency, with the assistance of a Haematologist, appropriate investigations should be carried out to establish the diagnosis. Measurement of serum and red cell folate levels, bone marrow examination, and a therapeutic trial with high dose folate (5 mg daily) and Vitamin B_{12} (1000 μg IM) may be tried.[38] The diagnosis will be confirmed by an increase of reticulocytes within seven days. The presence of plasma intrinsic factor antibodies will indicate pernicious anaemia. Although an undiagnosed pernicious anaemia with B_{12} deficiency is rare, the administrations of only folate in such a case may exacerbate the condition.[38] Other causes for a MCV >100 fl include liver disease, hypothyroidism, myelodysplasia and alcohol consumption.[39]

The cyanmethhaemoglobin method is used for assessment of Hb in a laboratory.[3] The Haemocue, a portable battery operated photometer with disposable cuvettes for collection of blood is useful at point-of-care.[40] The haematocrit complements and partially overcomes the limitations of a single measurement of Hb, but has no advantage over the Hb.[3] Adequate puncture of tissue and spontaneous blood flow from the wound is required when obtaining capillary blood from a finger prick, in order to reduce potential errors in the Hb and haematocrit estimations.[3] A new Colour Scale, similar to the original Talquist Scale, gives simple accurate assessments of Hb and is useful if there is no laboratory.[41]

If even an Hb assessment is not possible, clinical screening is required to detect moderate to severe anaemia in individual pregnant women. Mucosal pallor especially on the under surface of the tongue and the conjunctivae, and palmar and nail bed pallor are important signs. However clinical examination per se has low sensitivity in moderate anaemia and is unlikely to detect mild anaemia.[3,42]

Adverse effects

Employed women will need to continue to work and housewives will have to do their normal household chores during pregnancy. IDA leads to reduced work capacity, intellectual capacity and productivity,[3,43] and increased susceptibility to infection.[44,45] It also results in increased maternal mortality especially in non-industrialised countries with low resources, probably due to combination of anaemia with obstetric haemorrhage, infections and an impaired capacity to support vital functions.[46,47] Anaemia was the direct cause of maternal deaths in 3.7% of cases in Africa and 12.8% of cases in Asia.[47] Severe anaemia is associated with adverse perinatal outcomes such as preterm deliveries and small for gestational age babies.[3,28,29,48] Furthermore, babies born to anaemic mothers are iron deficient and have a higher risk of developing anaemia during infancy, although they are not anaemic at birth.[49,50] The adverse effects of ID at birth also have long term effects such as poor physical and mental growth and cognitive function leading to poor learning abilities continuing throughout childhood and adolescence, and even permanent neuro-physiological deficiencies.[50,51]

Prevention

Women should be advised to stop smoking prior to pregnancy and to immediately quit smoking or chewing tobacco if they become pregnant.[11,12,52]

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The anthelmintics mebandazole and albendazole which have been found to be safe and effective during pregnancy, should be administered to pregnant women as well as non-pregnant women in communities with a high prevalence of helminthiasis especially hookworm infestation.[53,54]

Teenage pregnancy[23] and high parity[24] should be avoided by counselling and the use of appropriate contraception. Temporary contraception to increase inter-pregnancy non-lactating intervals may also help.[25]

Intermittent preventive treatment of malaria using sulphadoxin pyrimethamine is indicated for women residing in malaria endemic areas.[55] All infections and chronic inflammatory conditions (vide Table 2) should ideally be controlled prior to pregnancy.

Although dietary advice has a limited impact, energy rich balanced food supplementation through sustainable community based programmes can lead to an increase in pregnant women's energy and protein intakes and thus improve fetal outcomes. However, high protein supplementation may be harmful to the fetus.[56]

"Raising the social and economic status of women in the non industrialised countries with limited resources, would be the best long-term solution to the under-nourished, pregnant woman".[57]

Weekly iron folate supplementation for all menstruating women, including school girls

Globally, more than 460 million non-pregnant women (15–49 years) are estimated to be anaemic and two thirds of them will be from Asia (Table 1).[1] Weekly iron (60 mg of ferrous sulphate) and folic acid (3 mg) supplementation (WIFS) for WRA, including adolescent girls between 10–19 years could be an effective strategy to achieve good iron stores before a woman becomes pregnant.[58] WIFS programmes for WRA have been effective in reducing the prevalence of anaemia in certain districts in Vietnam,[54,59] the Philippines,[60] and Cambodia.[61] Selecting a fixed day in the week as 'WIFS Day' or 'Iron Day' could be effective in addressing the problem of forgetfulness and to improve compliance.[58] The duration of supplementation should probably be for at least three months and it should be repeated preferably at six monthly intervals. Iron supplementation may lead to exacerbations of infections, and also malaria in communities with a high prevalence of malaria.[62,63] In malaria endemic areas, screening for anaemia and iron supplementation for the prevention and treatment of anaemia is recommended.[64] The implementation of a WIFS programme should be taken as an opportunity to establish or strengthen concurrent measures to prevent, control and treat malaria in malaria endemic areas.[65]

Any possible adverse perinatal outcomes for the baby are most likely to occur if IDA and ID are present during conception and embryogenesis, and correcting them prior to conception could result in significantly improved perinatal outcomes, while improvements may not be evident with supplements which are usually commenced only in the second trimester. Therefore a WIFS program is important to ensure adequate iron stores in non-pregnant women including adolescents prior to them becoming pregnant.

Daily oral iron and folic acid supplementation for pregnant women

A daily supplement of 60 mg of elemental iron and 400 μg of folic acid should be started as soon as possible in all pregnant women including adolescents, given throughout pregnancy and continued for six months postpartum to ensure adequate iron stores in the woman, in communities where anaemia in pregnancy is considered to be a significant problem.[3] This regimen increases maternal haemoglobin levels and the birth weight of the new borns, and reduces maternal anaemia and ID at term, and the delivery of low birth weight babies. However there is no significant reduction in preterm deliveries and neonatal deaths.[66] Interactions between multiple micro nutrient deficiencies including iron and folate deficiencies, and underlying concurrent chronic inflammatory conditions will contribute to the adverse sequelae of anaemia in pregnancy. Therefore all the adverse effects of anaemia in pregnancy would not be expected to be reduced by iron and folate supplementation.

The iron requirements of the fetus as well as maternal iron absorption are very low in the first trimester.[3,17,20] Furthermore 'morning sickness' and the possible gastrointestinal side-effects of iron supplements will usually reduce compliance.

In well-resourced settings, selective oral iron supplementation based on SF levels at the booking visit, is feasible (Table 3).[28]

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Table 3
Serum Ferritin levels at booking visit (<12 weeks gestation) for selective oral iron supplementation.[28]

Serum Ferritin μg/L	Action
>60	No supplementation
20–60	Iron supplement from 20 weeks gestation
15–19	Iron supplement from 12 weeks gestation
<15	Considered as a patient and treated

If ID or latent ID manifests as clinical anaemia during pregnancy, correcting it and ensuring adequate Hb levels at partus is important especially in low resource settings to reduce the risk of maternal morbidity and mortality due to a combination of anaemia with obstetric haemorrhage and sepsis,[46,47] as well as possible long term adverse effects in the newborn.[49–51]

Oral iron supplements may interfere with absorption of other trace materials such as Zinc[67] and affect fetal bone growth.[68] Concurrent Zinc supplementation has been suggested to address this issue but the combined preparations could be less effective than individual supplements.

Vitamin A may be required for the mobilization and utilization of iron from haemoglobin synthesis, and thus contribute to haemopoesis.[69] In Indonesia, antenatal iron and vitamin A supplements were shown to be better than iron alone.[70] However studies in Malawi did not show a similar association.[71]

Multiple micronutrient supplementation during pregnancy have no added benefit compared to supplementation with iron and folic acid alone.[72]

The high prevalence of anaemia in non industrialised countries[1] is in spite of antenatal daily oral iron and folic acid supplementation programmes. Globally, the reasons for failure of these programmes are poor compliance mainly due to side-effects, poor motivation and a lack of awareness of their value.[73,74]

Gastrointestinal side-effects of oral iron supplements are dose related, and are increased if taken on an empty stomach.[3,66] Therefore iron supplements are commonly taken after a meal in order to reduce these side-effects.[54,74] However this reduction in side-effects is probably due to poor absorption due to phytates and polyphenolic compounds in a full stomach.[75] This would decrease the effectiveness of the supplements.

Preparations containing 30 mg or less of iron have less side-effects[66,76] and they could be used for preventing and treating anaemia in pregnancy but they carry a higher risk of ID.[76,77]

Most oral iron supplements contain ferrous sulphate. To reduce the unpleasant gastrointestinal side-effects and improve compliance, supplements containing ferrous fumarate, gluconate and glycine and various gastric delivery systems have been tried out, without much success.[17,78–80] The non ionic ferric polymaltose complex, given with meals for better absorption, has been shown to be in-effective in treating IDA.[81] Calcium and iron supplements should not be taken together because calcium inhibits iron absorption.[3,82]

Antenatal oral supplementation with iron 65 mg and folic acid 1 mg, daily throughout pregnancy, has been found to be safe in multigravidae in a high malarial transmission area.[83]

Weekly oral iron and folic acid supplementation for pregnant women

It has been suggested that daily oral iron supplementation results in high iron levels in the intestinal mucosal cells and lumen, leading to an increased severity and frequency of undesirable gastrointestinal side-effects, and progressively reduces further iron absorption. Weekly supplements, based on a mucosal cell turnover of five to six days, which may improve iron absorption and patient compliance, has been suggested as a better option which is equally effective as daily supplements.[66] However a radio-isotopic iron absorption study found no evidence of any progressive mucosal block following daily oral iron. Iron supplements given twice weekly with food were found to provide <10% of the iron requirements during the third trimester of pregnancy.[84]

The association of Hb levels to perinatal outcome has been shown to be U shaped with both high and low Hb levels being associated with adverse perinatal outcome such as low birth weight, increased still births and increased perinatal mortality rates.[5,29,48,85,86] High Hb levels especially >130 g/L at term

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have been found after daily oral iron supplementation and therefore it has been suggested that intermittent weekly iron supplementation should be considered as a better alternative.[66] While weekly iron supplements should have some effect, the marked reduction in the total dose is unlikely to be as effective as a seven day dose. Weekly or even twice weekly iron supplements could be grossly inadequate to meet the iron requirements of pregnancy especially in communities where ID is high and the normal diet of pregnant women not only lack iron rich food sources but also contain substances which reduce iron absorption.[84,87–90]

Intrapartum management

Prior to admission to the labour ward, maternal Hb should be >10 g/dl. If the Hb is <7 g/dl, there is a high risk of maternal death due postpartum haemorrhage (PPH), cardiac failure or sepsis. During labour women should be propped-up and in a semi-recumbent position, have peri partum intravenous antibiotics, adequate analgesia and intermittent oxygen inhalation if necessary. Undue straining and a prolonged second stage of labour should be avoided. Active management of third stage of labour[91] is indicated to reduce the risk of even a mild PPH. Delaying the clamping of the umbilical cord for 1–2 minutes improves the iron stores of the neonate.[92] This simple intervention can reduce the risk of the adverse sequelae of ID during infancy and childhood, in communities where women have ID.

Postpartum management

If the postpartum Hb is <8 g/dl after delivery, a blood transfusion should be considered. Appropriate contraceptive counselling and services are required to either prevent future childbearing or delay the next pregnancy for at least one year, depending on the woman's needs.

Treatment

The treatment of anaemia in pregnancy needs identification of the underlying cause (vide Table 2) and its appropriate treatment. Expert advice should be sort where relevant.

It has been suggested that all pregnant women with severe anaemia in a malaria endemic area should be treated with antimalarials because malaria contributing for their anaemia cannot be excluded as more than half of them will not have positive peripheral blood films or fever.[93]

The threshold for a blood transfusion is often considered to be <6 g/dl in general clinical practice, and <7 g/dl in pregnancy.[94] Considering the very high risk of maternal mortality due to PPH and sepsis especially in low resource settings,[94] it would be prudent to use packed cell transfusions if the Hb is <9 g/dl at term.

Dietary advice

Pregnant women should be advised to have a realistic, easily digestible and affordable, balanced diet rich in iron and protein. Although red meat and liver are excellent sources of haem iron which promotes erythropoiesis, many women especially in Asia, are vegetarians. Vegetarians should be advised to increase the intake of iron rich food sources such as lentils, green peas, green vegetables, figs etc. and also to include an iron absorption enhancer such as a vitamin C rich food source e.g. orange juice. They should also be advised to avoid coffee and especially tea (which inhibits iron absorption) soon after a meal.[3,74,95]

Iron therapy

Current available evidence does not clarify the best method of treating IDA of pregnancy.[96] However oral iron therapy is the most feasible.[97] Sensitivity reactions and increased risk of venous thrombosis, and pain and skin discoloration at site of injection, have been associated with intravenous and intramuscular iron therapy respectively.[96,97] In comparison to iron dextran and iron sorbitol which have been used frequently in the past, intravenous iron sucrose complex and iron polymaltose complex have

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been found to be effective in special situations such as unresponsiveness, intolerance or non-compliance with oral iron supplements or in the presence of severe anaemia which requires urgent correction while avoiding the risks of blood transfusion[98,99] and it could be combined with recombinant human erythropoietin in well resourced settings (Table 4).[99]

IDA summary

IDA in pregnancy is a major public health problem, especially in Asia, and is associated with increased maternal and perinatal morbidity and mortality, and adverse effect in the newborn which could persist throughout infancy, childhood and adolescence. Because of physiological haemodilution, underlying inflammatory conditions and several other factors which have an effect on Hb and the other haematological indices during pregnancy, the diagnosis of anaemia in pregnancy and the adoption of preventive and therapeutic strategies to manage it are difficult.

Ideally a woman should have adequate iron stores when she conceives, in order to meet the additional requirements of pregnancy. Weekly supplementation of all menstruating women including adolescents periodically with iron and folic acid, may achieve this. However, most pregnant women will need additional iron supplements, which are best given daily to meet this additional need (Table 4). Delayed cord clamping can increase the iron stores of the newborn.

The optimum dose, frequency and formulation of oral iron supplements, methods of improving compliance in women, the feasibility and cost effectiveness of universal iron supplementation or targeted iron supplementation of pregnant women, and their safety in malaria endemic areas, women who are iron replete and women who could have excess iron stores, needs to be established. The possible improved clinical outcomes of iron supplementation in pregnant women with latent ID, ID or IDA, also needs to be established.

Table 4
Possible oral iron and folic acid supplementation programmes to prevent anaemia in pregnancy.

Target Group	Programme	Duration
All pregnant women including adolescents in communities where IDA is considered a problem	Daily Iron 60 mg + Folic Acid 0.4 mg	Throughout pregnancy starting as early as possible
All pregnant women including adolescents in communities where IDA is not considered a problem	Daily Iron 30 mg + Folic Acid 0.4 mg	Throughout pregnancy starting as early as possible
Postpartum women including adolescents	Continue antenatal supplementation	Six months
All menstruating women including adolescents	Weekly Iron 60 mg + Folic Acid 2.8 mg	Three months, and repeated at six monthly intervals

Vitamin B12 deficiency in pregnancy

Introduction

During pregnancy, there is a gradual decline in serum vitamin B12 levels, however this does not represent body stores or deficiency, and may be independent of dietary intake[100] Due to increased metabolic needs, the FAO/WHO recommends a 40 percent increase in dietary vitamin B12 intake.[101] In pregnancy, deficiency of vitamin B12 is rare, however megalobastic anaemia due to vitamin B12 deficiency was reported in pregnant women in Zimbabwe and India.[102,103]

Pathophysiology

Vitamin B12, otherwise known as cobalamin, is a water-soluble vitamin, which is a vital growth factor.[104,105] It is essential for proper red blood cell formation, neurological function and DNA synthesis. It is naturally present in most foods of animal origin, eggs, milk and milk products. The normal daily requirement for an adult ranges from 2 to 3 ug per day. The absorption of vitamin B12 requires binding to intrinsic factor, a glycoprotein secreted by the stomach's parietal cells, and then uptake into the ileum.

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Deficiency in vitamin B12 may be attributed to a diet deficient in the vitamin, particularly women following a vegan diet, or absence of intrinsic factor, in which case the deficiency becomes known as pernicious anaemia.[106]

Diagnosis

In pregnancy, vitamin B12 deficiency presents with the symptoms and signs of anaemia, as well as that of spinal and peripheral nerve involvement. These include mental slowness, memory defects, hallucinations, and numbness or tingling in the extremities. Rapid diagnosis of vitamin B12 deficiency is essential if the treatment is to be effective, as the reversibility of the neurological symptoms relies heavily on this. Thus, the following investigations should be carried out if vitamin B12 deficiency is suspected:

- Full Blood Count – macrocytic anaemia may be present
- Estimation of serum vitamin B12
- Gastric biopsy – if the blood and bone marrow results appear normal.[106]

Adverse effects on pregnancy

Throughout pregnancy, there is a steady decrease in the vitamin B12 levels, reaching its lowest at term. Days after delivery, the levels steadily rise until they reach pre-pregnancy levels within three to five weeks postpartum. An explanation for the decreasing levels in pregnancy is due to the shunting of Vitamin B12 to the placenta, as studies showed that the cord-blood level is considerably higher than that of the mother's.[107,108]

In terms of obstetric health, low levels of vitamin B12 increase the risk of birth defects such as neural tube defects (NTDs). In a study carried out by the National Institute of Health, Trinity College Dublin, and the Health Research Board of Ireland, it was found that women low levels of B12 were 2.5 to three times more likely to have a child with a neural tube defect. Implications have also been made that it may be a cause of infertility, recurrent spontaneous abortion, as well as pre-term delivery, although this needs further evaluation.[109]

As well as having adverse effects on pregnancy, if an adequate vitamin B12 status is not achieved during pregnancy or available in the breast-milk, it may lead to frank deficiency in the infant.[110]

Prevention & treatment

A diet rich in vitamin B12 is essentially the best prevention. Supplementation is rarely needed in pregnant women as the large maternal vitamin B12 stores make it virtually impossible for the events of pregnancy to have that great of an impact on the vitamin B12 levels. That being said, pregnant women following a vegan diet may benefit from vitamin B12 supplements. The levels of vitamin B12 should also be measured before giving folate supplements in pregnancy, as this may mask the effects of vitamin B12 deficiency.[105,111,112]

Folate deficiency in pregnancy

Aetiology

Hibbard first described folate deficiency in pregnancy in 1964, and its correlation to anaemia, placental abruption and pregnancy losses.[113] In 1965, Hibbard and Smithells suggested a link between folate deficiency and malformations in the developing central nervous system of an embryo.[114] In the early 1990s, large, randomized trials confirmed that periconceptional folate supplementation prevents the occurrence and recurrence of NTDs.[115,116] The use of multivitamin preparations including folic acid may also have a protecting effect on adverse outcomes and complications in pregnancy.[117–122]

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The recommended daily requirement for folate is 100–200 μg, however this requirement may increase during pregnancy due to increased cell turnover and possibly, an inadequate dietary intake. Body stores are adequate for four months only, after which signs and symptoms of folate deficiency develop. Macrocytosis is defined as MCV above the normal range of 80–95 fl. It may be due to vitamin B12 and folate deficiency, in which there is megaloblastic bone marrow, or other causes such as alcohol, liver disease and hypothyroidism, which present with normoblastic bone marrow.

Folate deficiency may be due to diet, intestinal causes such as gluten-induced enteropathy and tropical sprue, increased cell turnover such as pregnancy, inflammation, or drugs such as anti-convulsants.[123,124]

Definition

Folate deficiency results in a type of anaemia in which the low levels of folic acid result in macrocytosis and the development of megaloblastic bone marrow. Serum folate below the normal range of 2.0–15 μg/L, and red cell folate concentration below the normal range of 160–640 μg/L is diagnostic of folate deficiency.[125]

Pathophysiology

The naturally occurring chemical structure of folic acid consists of a pterin ring combined with p-aminobenzoic acid (PABA) and conjugated with one or more glutamate units. This is known as the polyglutamate form of folic acid, which is the only form capable of remaining within the cell. However, this form is not readily absorbed, and this is where the role of enzymes in the small intestine comes into play. These enzymes convert the polyglutamate form to monoglutamate form, which is absorbed in the proximal jejenum to reach the plasma, where folate is present in the 5-methyltetrahydrofolate (5-methyl THFA) form. This form enters the cell, via various transport mechanisms, and is demethylated to THFA, which is the active form that takes part in folate-dependent enzymatic reactions. The conversion of 5-methyl THFA to THFA requires vitamin B12, hence its absence keeps folate in its inactive form, resulting in megaloblastic anaemia.

THFA plays an integral role in the synthesis of DNA, RNA, and proteins, and so its absence may result in impairment of cellular division and the accumulation of homocysteine as a by-product of a decrease in the methylation cycle. Elevated homocysteine levels have been implicated in the increased risk of arteriosclerosis, premature coronary artery disease, stroke, transient ischaemic attacks (TIAs) and decreased left ventricular systolic function.[126–130]

Diagnosis

Clinical features of folate deficiency include symptoms of anaemia, hyperpigmentation and a low-grade fever, which begins to fall within 24–48 hours of vitamin therapy and returns to normal within a few days. Neuropsychiatric symptoms such as depression, dementia and peripheral neuropathy are usually seen in conjunction with vitamin B12 deficiency.

Laboratory investigations include serum folate, red cell folate assay, serum B12 assay, serum homocysteine and serum methylmalonic acid. Bone marrow aspiration may be considered for megaloblastic changes suggestive of vitamin B12 or folate deficiency. Liver and thyroid function tests may be done to find the cause of macrocytosis.

It is important to rule out vitamin B12 deficiency as a cause of neurpsychiatric symptoms, because they will not improve with folic acid therapy. It is important to establish an underlying cause in both folate and vitamin B12 deficiency. Testing for autoantibodies present in pernicious anaemia and Schilling test can be done. Low serum folate is found in folic acid deficiency, while high serum folate is indicative of severe vitamin B12 deficiency. Low red cell folate concentration indicates profound depletion of body stores. It may occur in megaloblastic anaemia due to both folate and vitamin B12 deficiency, however if the latter has been ruled out, it is used as an indicator of severity of folate deficiency.[125]

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Serum homocysteine levels are increased in both folate and vitamin B12 deficiency, while serum methylmalonic acid levels are increased in vitamin B12 deficiency only. If folate deficiency in confirmed, it is vital to assess dietary folate intake and to rule out gluten-induced enteropathy by performing an endoscopy and duodenal biopsy.

Differential diagnosis

The only clinical condition, which presents with similar symptoms and signs as folate deficiency, is pernicious anaemia.

Prevention

A prophylactic daily dose of 400 μg is recommended for all women, in the preconception period and throughout the pregnancy. Food fortification with folic acid is possible, and should be set up in areas where the prevalence of folate deficiency is high, such as in developing countries.[131] A diet rich in foods containing high folate levels such as green, leafy vegetables, lentils and beans as well as fortified breads and cereals is advised for pregnant women. Patients with severe haemolytic anaemia, such as sickle cell anaemia, may receive a prophylactic dose of 5 mg folic acid once weekly.

Treatment

In pregnancy, folate deficiency is treated with folic acid, usually 5 mg daily orally for four months, however this may continue throughout the pregnancy if the underlying cause may not be corrected. The exclusion of vitamin B12 deficiency in all patients starting folic acid treatment is mandatory, as high doses of folic acid may correct the anaemia present in vitamin B12 deficiency but precipitate neuropsychiatric symptoms. In patients who develop folate deficiency secondary to the use of medication such as anticonvulsants, or who have previous history of a pregnancy affected by NTDs, a dose of 5 mg is administered. Multivitamin and folic acid supplementation has been shown to reduce placental abruption[132] and recurrent early pregnancy losses.[133]

Haemolytic anaemias

Thrombotic thrombocytopenic purpura in pregnancy

Aetiology

Thrombotic thrombocytopenic purpura (TTP) is an uncommon haematological disorder; its annual incidence in the United States is 4 to 11 cases per million people.[134] However, it is a severe and life-threatening condition. It is more common in women than men.[135,136] It can be either chronic relapsing or acute. Acute TTP can be further categorized into idiopathic or secondary to several predisposing factors, of which pregnancy is one. Recently, idiopathic cases of TTP have been found to be due to an acquired autoimmune deficiency of the enzyme ADAMTS13, a plasma metalloprotease.[137] Secondary TTP has several predisposing factors; cancer, bone marrow transplantation, HIV-1, and medications including ticlopidine and clopidogrel.[138,139] Pregnancy-associated TTP and the postpartum state accounts for 10–25% of patients who have TTP. It usually presents before 24 weeks of gestation.[140]

Definition

TTP is characterized by thrombocytopenia, microangiopathic hemolysis and thrombosis, and organ dysfunction. Due to its life-threatening nature, it requires prompt intervention when suspected.

Pathophysiology

The pathophysiology of TTP is still unclear, however endothelial cell damage and the release of von Willebrand factor multimers have been described to play a role. These multimeres are of large molecular

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weight and contribute to increasing platelets' clumping, resulting in thrombosis and arterial ischaemia to organs. Normally, these multimeres would undergo proteolysis by ADAMTS13. This enzyme's presence in chronic TTP is deficient. However, in acute idiopathic TTP, ADAMTS13 is present but IgG antibodies inhibit its activity, rendering TTP an autoimmune disorder. The involvement of platelets in clot formation produces an overall decrease in platelet count, with resultant thrombocytopenia.[141]

Diagnosis

Classically, the following pentad of features diagnoses TTP,[140] however in most cases, not all are present.[142]

- Microangiopathic haemolytic anaemia
- Thrombocytopenia leading to bleeding, bruising and purpura
- Neurological symptoms, such as changes in level of consciousness and headaches.
- Renal involvement
- Fever

Other symptoms include fatigue, shortness of breath, and focal neurological deficits. Laboratory investigations include complete blood count, platelet count, bilirubin, creatinine, blood film, urinalysis, LDH and ADAMTS13 activity level. There will be low haemoglobin count, low platelet count, high bilirubin, high creatinine, blood film will show fragmented red cells and schistocytes, urinalysis may show protein and red cells. LDH will be elevated due to haemolysis. ADAMTS13 level may be $<5\%$, however some patients with TTP may have normal levels of ADAMTS13.

Differential diagnosis

The co-existence of thrombocytopenia and haemolytic anaemia in pregnancy poses a difficult clinical situation. Due to the similarity of the clinical symptoms, the four main differential diagnoses would be TTP, idiopathic thrombocytopenic purpura (ITP), haemolytic uremic syndrome (HUS) and HELLP syndrome (haemolysis, elevated liver enzymes, low platelets). Due to the life-threatening nature of TTP as well as the possibility of HELLP syndrome progressing into eclampsia, it is necessary to make an accurate diagnosis in any patient presenting with any neurological abnormalities, anaemia and unexplained thrombocytopenia.[143] The relevant differential diagnoses are:

- Idiopathic thrombocytopenic purpura
- Haemolytic uremic syndrome
- HELLP syndrome
- Disseminated intravascular coagulation
- Paroxysmal nocturnal haemoglobinuria
- Evan's syndrome

Prevention

Prevention of TTP is not possible due to the fact that the cause is not known.

Treatment

The mainstay of treatment is plasmapharesis.[144] It is the most effective therapy. It involves the exchange fresh frozen plasma one to two times the estimated plasma volume or 40–80 ml/kg, up to 4.0 L. This treatment is repeated on a daily basis until improvement is noted on repeat blood tests, which may be in the range of 1–8 weeks. Plasmapharesis is superior to plasma infusion, which may only be used when a patient is transferred to a unit, which facilitates the use of plasmapharesis. There is

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a higher rate of response with plasmapharesis in the immediate recovery period as well as six months after treatment.[145]

The standard replacement fluid used is fresh frozen plasma; however, cryosupernatant has been reported as a successful susbtitute. Cryosupernatant is the remaining fraction of plasma after separation of cryoprecipitate.[146,147]

People who do not respond to plasmapharesis or who experience frequent relapses may require a splenectomy. Due to the fact that the spleen is a main site of autoantibody production, some patients profit from a splenectomy.[148]

Some patients with refractory or relapsing TTP may receive additional immunosuppressive therapy with prednisolone, vincristine, cyclophosphamide, rituximab or a combination of the above. In patients with history of TTP developing in previous pregnancies, and those who have had recurrences, rituximab can be used in the third trimester of pregnancy with no maternal or neonatal toxicity.[149]

Prognosis

Mortality rate is 95% in untreated cases, however in patients diagnosed early and treated, survival rates are 80–90%. In the long-term, morbidity and mortality are common, despite early treatment with plasmapharesis. There is risk of recurrence with subsequent future pregnancies.[149,150]

Haemolytic uremic syndrome in pregnancy

Aetiology

Haemolytic-uremic syndrome (HUS) was first described in 1955 by Gasser et al. In 1988, Wardle described HUS and TTP as separate entities, but in 1987, Remuzzi suggested that these two states are different manifests of one entity. With the detection of ADAMTS-13, HUS and TTP are now two different conditions in spite of their clinical similarities.[151–156]

HUS can be classified into two main categories; shiga-like toxin (Stx)–associated HUS (Stx-HUS), which is the classic form of HUS, and non–Stx-associated HUS (non–Stx-HUS), which is the atypical form of HUS. Stx-HUS is mainly a disease of children and is often accompanied with diarrhoea. Non-Stx-HUS can be sporadic or familial, and infection by Stx-producing bacteria is not the cause. Patients with non–Stx-HUS have a poor outcome, and as many as 50% may progress to end-stage renal disease (ESRD) or permanent brain damage. In the acute phase, up to 25% of patients may die.[157,158]

The atypical, non-Stx form of HUS may occur due to pregnancy, which is known as pregnancy-associated HUS. This type infrequently occurs as a complication of preeclampsia. Patients' condition may advance to HELLP syndrome. Postpartum HUS usually occurs within the first 3 months after delivery. The prognosis is poor, with a 50–60% mortality rate, and residual renal dysfunction and hypertension occur in most patients.

Data suggest that atypical HUS is associated with pregnancy in 20% of HUS patients, and, in 79 percent, this occurs postpartum. Complement abnormalities were found in a considerable number of patients. Pregnancies of patients with complement abnormalities were complicated by fetal loss and preeclampsia.[165] Data also suggests that the risk of developing atypical HUS is highest in the second pregnancy.[165]

Definition

HUS is a clinical syndrome, which is characterized by progressive renal failure that is associated with microangiopathic haemolytic anemia and thrombocytopenia. It is the most common cause of acute renal failure in children, but is gradually more recognized in adults and in pregnancy.

Pathophysiology

There are several concepts regarding the pathogenesis of Stx-associated HUS, however, because non–Stx–associated HUS is the type that is more commonly found in adults and in

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pregnancy, its pathogenesis is discussed in this chapter. Non–Stx-HUS, or atypical HUS is less common than Stx-HUS. It accounts for 5–10% of all HUS cases. It may occur at any age, but it is most commonly occurring in adults and without diarrhoea. It can occur sporadically or in families.

In sporadic cases, various conditions have been found to set it off: non-enteric infections, viruses, medication, cancer, transplantation and pregnancy. Idiopathic HUS accounts for 50% of all sporadic cases. Familial cases account for less than 3% of all HUS cases. Both autosomal dominant and autosomal recessive forms of inheritance are found. Autosomal recessive HUS often occurs early in childhood. Autosomal dominant HUS often occurs in adults. This carries a poor prognosis and the risk of death or ESRD is 50–90%.[157,158] Aberrations in both complement regulators (factor H, factor I, and membrane co-factor protein) and activators (C3 and factor B) have been expressed in familial and sporadic cases. Moreover, mutations in the gene encoding thrombomodulin and factor H auto-antibodies, which hinder the activity of factor H, have now been identified.[159–162,164]

Diagnosis

Patients present with symptoms similar to TTP, however renal function is severely affected in HUS, with patients presenting with anuria and acute renal failure. Oedema occurs quite frequently in HUS. Seizures may also occur.

Laboratory investigations include complete blood count, platelet count, bilirubin, creatinine, blood film, urinalysis, LDH and stool culture. Tests will show low haemoglobin count, low platelet count, high bilirubin, high creatinine, blood film will show schistocytes, urinalysis may show protein and red cells. LDH will be elevated due to haemolysis. This denotes a similar picture to TTP. Stool culture is assessed for *E. coli* 0157:H7 and Shigella bacteria. Renal ultrasonography can be undertaken in patients with renal failure to rule out any obstruction. Renal biopsy is rarely required, however may be done, depending on each case.

Differential diagnosis

Due to the similarity in presentation, TTP is one of the main differential diagnoses. In addition to it, HELLP syndrome and preeclampsia are also differential diagnoses to keep in mind.

Prevention

Pregnancy-associated HUS and non-Stx HUS cannot be prevented, however cooking meat and avoiding unclean water can prevent Stx-HUS, by preventing infection with *E. coli*.

Treatment

Plasmapharesis is the first-line treatment of HUS. It has improved survival rates from 10% to 75–92%.[163] Renal transplantation is usually not an option for atypical, non–Stx HUS because this type of HUS has a high recurrence rate and a high possibility of graft failure in patients with recurrence. Supportive therapy is given to maintain fluid and electrolyte balance, control blood pressure, azotemia and provide optimum nutrition involving protein restriction for patients with renal insufficiency. Consultation of subspecialists such as nephrologists, haematologists, and neurologists in case of neurological involvement, may be required.

Prognosis

Data suggests that 76% of patients develop ESRD despite plasmapharesis. The prognosis for renal transplantation in these patients is poor, especially in those who have a factor H mutation,

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80% of whom will experience graft rejection due to recurrent disease within 2 years of transplantation.[164] Pregnancy-associated HUS has high maternal mortality and long-term morbidity. Preterm delivery, fetal loss and preeclampsia are frequent complications of these pregnancies.[165,166]

Paroxysmal nocturnal Haemoglobinuria in pregnancy

Aetiology

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired yet rare condition, which arises due to a genetic mutation of the PIG-A gene in a pluripotent hematopoietic stem cell. The gene encodes a protein that is vital for the synthesis of glycosylphosphatidylinositol (GPI), which is a lipid that is embedded in the plasma membrane of cells such as erythrocytes and platelets. Its main function is to fix a range of proteins to the cell surface. Its absence results in the deficiency of proteins that are usually attached to the red cell surface, and protect the cell from complement-mediated attack.[167,168] This results in haemolysis, haemoglobinuria and venous thromboembolism.[169]

PNH was first described in 1882[170] and in the late 1930s; Ham's test was established as its main diagnostic test.[171] It is founded on the fact that PNH-affected red cells have an amplified sensitivity to be broken down by complement, however it is no longer used as a diagnostic assay because it is less sensitive and quantitative than flow cytometry, which is the preferred investigation.

PNH takes a chronic course, of which the median survival is approximately 10 years. Men and women are equally affected, and it does not run in families. PNH may occur at any age, however it is commonly found in adults, with the median age at the time of diagnosis being 42 years.[169]

PNH in pregnancy has a serious morbidity and increased mortality.[172] In a retrospective study, the diagnosis of PNH was first made during pregnancy in 25% of cases. It also accounted a high mortality rate, with an all-cause mortality of 20.8%. In women with PNH, the prevalence of venous thromboembolism during pregnancy was found to be 10%. The sites involved in thromboembolism were the cerebal and hepatic veins, which is parallel to those affected in the non-pregnant population.[173]

Definition

PNH is a haematopoietic disorder that is characterized by intravascular haemolysis, haemoglobinuria and venous thromboembolism.[169]

Pathophysiology

Due to the absence of GPI, several proteins that protect the plasma membrane from complement-mediated attack are unable to attach to the plasma membrane, two of which are CD55 and CD59.[174–176] The function of CD55 is the regulation of early complement activation by inhibiting C3 convertases.[177] CD59 serves to reduce the gathering of the complex C5b–C9, which attacks the plasma membrane, by interacting with C8 and C9.[174,175] The increased sensitivity of PNH-affected red cells and platelets to complement is due to the lack of CD59,[174,178–183] which is the basis of Ham's test.

Diagnosis

Clinical features include symptoms of anaemia, dark urine in the morning as a manifestation of haemoglobinuria, haemorrhagic symptoms, aplastic anaemia, gastro-intestinal symptoms such as episodic abdominal pain, cerebral and hepatic vein thromboses, infections and uncommonly, neurological symptoms.

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A diagnosis of PNH is made based on flow cytometric analysis[184–186] which assesses the absence of CD 55 and CD59 on PNH-affected red cells, and is considered the most sensitive assay available. Yearly analysis of peripheral blood GPI-AP is recommended for patients with established and stable PNH. Changes in clinical parameters, such as frequent haemolysis or the occurrence of a thromboembolic episode, merit prompt re-evaluation.[187]

Other investigations include complete blood count, platelet count, LDH levels and urinalysis. Iron deficiency or haemolytic anaemia may be present. Pancytopenia may be present. Platelet count will show thrombocytopenia, LDH levels may be increased due to haemolysis and urinalysis will show the presence of haemoglobin.

Differential diagnosis

- Paroxysmal cold haemoglobinuria
- Mesenteric artery thrombosis
- Budd-Chiari syndrome
- Renal vein thrombosis

Treatment

In pregnancy, red cell or platelet transfusion is the mainstay of treatment of PNH, because maternal and fetal exposure to potentially toxic treatment is a concern. There is no evidence to support the safe use of complement inhibitors, such as eculizumab, in pregnancy.[188]

Prophylactic anticoagulation using low-molecular weight heparin (LMWH) is recommended once pregnancy is confirmed and continued for 6 weeks after delivery. It is also recommended to counsel the patient on potential risks and the possible need for intervention. The involvement of an experienced haematologist and an obstetrician who specializes in high-risk pregnancies is advised.[172] Treatment is mainly supportive and is used to improve symptoms of anaemia and haemolysis.

Prognosis

Pregnancy in patients with PNH poses a high risk of complications and should not be recommended. In 6% of PNH cases, pregnancy precipitated fatal complications, and 30% of pregnancies ended in stillbirth.[189] In pregnancies complicated by PNH, maternal and foetal mortality is remarkably high, being 11.6% and 7.2% respectively. The major cause of maternal mortality is thromboembolism.[190] The rate of prematurity in pregnancies affected by PNH is high.[191] Major maternal complications are more frequent in the post-partum state, therefore accurate monitoring of pregnancy and the puerperium is considered crucial.[190]

Practice points 1

- Hb $<$110 g/L at sea level, is defined as anaemia.
- Multiple pregnancy, teenage pregnancy and high parity carry a high risk of anaemia in pregnancy.
- Physiological haemodilution results in lowered Hb in second trimester.
- Nutritional IDA is common, but other causes of anaemia should be considered and excluded.
- Examination of a peripheral blood film is useful for differential diagnosis of nutritional anaemias of pregnancy.
- Haematological indices are altered during pregnancy.
- If MCV $>$ 100 fl, consider folate (and rarely Vitamin B_{12} deficiency)
- SF $<$ 12 μg/L indicates ID. SF is increased in inflammatory conditions.

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Practice points 2

- Maternal IDA periconceptionally and at term can adversely affect the fetus and newborn leading to long term complications
- Even a moderate haemorrhage in an anaemic pregnant woman can be fatal
- Weekly iron (60 mg) and folic acid (2.8 mg) should be given to all menstruating women including adolescents, periodically, in communities where IDA is considered a problem
- Appropriate dietary advice especially in vegetarians, and anthelmintic therapy if required, should be given to pregnant women, especially in communities where IDA is considered a problem
- Daily oral iron (60 mg) and folic acid (400 μg) should be commenced as soon as possible together with behaviour changing communications when a woman becomes pregnant, and continued up to six months postpartum. The dose of iron could be reduced to 30 mg in women who have no ID
- Clamping the umbilical cord should be delayed for 1 -–2 mins in communities where IDA is considered a problem.

Research agenda

- Identify the best methods for diagnosis of IDA, ID, latent ID and normal iron replete state.
- Identify the different underlying conditions in different communities, which contribute to anaemia in pregnancy.
- Evaluate the possible adverse maternal, perinatal, and long term outcomes in the new borns in women with IDA, ID and latent ID in the first trimester.
- Evaluate possible improvements of adverse maternal, perinatal, and long term outcomes in the new borns, if iron, folic acid or other micronutrient supplements are administered from the second trimester in women with IDA, ID and latent ID.
- Identify the optimum frequency and cost effective dose, formulation and delivery system of oral iron supplements to achieve good compliance and optimum iron status at term in women with IDA, ID and latent ID.
- Evaluate the effect of Hb $>$130 g/L, after antenatal oral iron supplementation, on perinatal outcomes, after adjusting for other pathological conditions which result in Hb $>$130 g/L.
- Evaluate the effectiveness, safety and feasibility of providing pregnant women and all menstruating women including adolescents, iron fortified food
- Evaluate the safety of providing iron supplements and iron fortified food supplements to iron replete women, women in malaria endemic areas and women having excess iron stores due to conditions such as haemolytic anaemia.

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