ANAEMIA IN PREGNANCY IN MALAWI- A REVIEW

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1. Methods for this review

The literature search for this document was carried out by accessing PubMed, EmBase and PopLine using the search terms *'anaemia, pregnancy, developing countries, Malawi'*. In addition helpful documents were found and downloaded from the Royal College of Obstetricians and Gynaecologists (RCOG) website (rcog.org.uk). The WHO Reproductive Health Library and Cochrane Library web sites were also helpful especially for systematic reviews. Finally the personal collection of articles and books related to the topic from Dr. Nynke Van den Broek was very useful for successful completion of this review.
The Appendix to this document contains a table with an outline of all the referenced papers published in relation to anaemia in pregnancy in Malawi.

2. Definition of anaemia in pregnancy

As a result of normal physiological changes in pregnancy, plasma volume expands by 46-55%, whereas red cell volume expands by 18-25%. The resulting haemodilution has, perhaps wrongly, been termed 'physiological anaemia of pregnancy'.

2.1 Cut-off points for defining anaemia and severe anaemia in pregnancy

In most published studies, the mean minimum haemoglobin in healthy pregnant women living at sea level is 11-12g/dL[1]. The mean minimum acceptable haemoglobin level during pregnancy by WHO criteria is taken to be 11g/dL in the first half of pregnancy and 10.5 g/dL in the second half of pregnancy. The World Health Organization further divide anaemia in pregnancy into: mild anaemia (haemoglobin 10-10.9g/dL), moderate anaemia (Hb 7.0-9.9g/dL) and severe anaemia (haemoglobin < 7g/dL)[2]. This is an arbitrary classification. The definition of severe anaemia in the published literature, however, varies and this may also be defined as Hb < 8.0g/dL.

It must be noted that the cut-off points for severe and/or moderate anaemia are to a large degree arbitrarily chosen and are not indicative of specific increased risk of mortality or morbidity either to the mother or her baby. It is however very useful to have these internationally agreed cut-off points especially for the purpose of being able to compare outcomes for the various published studies.

2.2 Screening for anaemia in pregnant women

Due to lack of resources and lack of staff motivation, screening of anaemia is often done solely by clinical examination of the conjunctivae or is not carried out at all. A new colour scale for the estimation of haemoglobin concentration has been developed by the WHO. A study carried out in Malawi showed that the sensitivity using the colour scale was consistently better than for conjunctival inspection alone and intra observer agreement and agreement with coulter counter measurement was good. The haemoglobin colour scale is simple to use, well accepted, cheap and gives immediate results. It shows considerable potential for use in screening for anaemia in antenatal clinics in settings where resources are limited. [14] Further work is ongoing to assess the potential of this tool for wide spread use as a screening method in antenatal clinics.

Anaemia in pregnancy is defined as a haemoglobin concentration < 11.0 g/dl or <10.5 g/dl in the second half of pregnancy.

3. Distribution

3.1 Global estimates of prevalence of anaemia in pregnancy

Anaemia in pregnancy is thought to be one of the commonest problems affecting pregnant women in developing countries.
In 1993, the World Bank ranked anaemia as the 8th leading cause of disease in girls and women in the developing world. Data collected from all over the world indicate that a total of 2170 million people (men, women and children) are anaemic by WHO criteria. The most affected groups, in approximately descending order are pregnant women, the elderly, school children and adult men. In developing countries, prevalence rates in pregnant women are commonly estimated to be in the range of 40%-60%. Among non pregnant women this is 20%-40% and in school aged children and adult men the estimate is around 20%[3]
The following table shows the data for different continents of the world with a more detailed report for the African sub continent.

Table 1 Estimated prevalence of anaemia in women

	Pregnant women Hb below norm		**Non-pregnant women Hb below norm**		**All women Hb below norm**	
Region	**%**	**(000s)**	**%**	**(000s)**	**%**	**(000s)**
WORLD	51	58270	36	407780	37	466050
DEVELOPING COUNTRIES	56	55750	44	372320	45	428070
DEVELOPED COUNTRIES	18	2520	12	35460	13	37970
AFRICA	52	11450	42	47940	44	59400
Eastern	47	3380	41	13540	42	16920
Middle	54	1290	43	5330	45	6620
Northern	53	2240	43	11450	45	13690
Southern	35	380	30	2500	30	2880
Western	56	4170	47	15120	48	19290
ASIA	60	40140	44	294960	45	335100
LATIN AMERICA	39	4030	30	28640	31	32670
NOTHERN AMERICA	17	570	10	7050	11	7620
EUROPE	17	920	10	12100	11	13020

The data presented in Table 1 are based on data collated in 1988 by the World Health Organization. It was then estimated that up to 56% of all non pregnant women living in developing countries were anaemic by WHO standard (Hb < 12g/dl), compared with 18% in industrialised countries. The greatest burden of anaemia is borne by Asia and Africa where it is estimated that 60% and 52% of women, respectively, are anaemic, and between 1% and 5% are severely anaemic (Hb < 7g/dl).
Presented in Table 2 are some more recent prevalence rates for anaemia in pregnancy for individual countries in the same region as Malawi as published by various authors in their papers.

Table 2 Prevalence of anaemia in pregnancy in selected countries in South East Africa

Country	Year	Anaemia overall % (Hb < 11g/dL)	Anaemia severe % (Hb < 7g/dL)
Mozambique[5]	1996	58	1
Rural Zaire[6]	1991	76	3.7
Coastal Kenya[7]	1996	75.6	9.8
Tanzania[8,9]	1996 1972	74.5 86	7

The unacceptably high prevalence of anaemia in developing countries could be an underestimate; data from rural areas is still lacking, the actual prevalence rates for many individual countries are not known, and there are very few community based surveys.[4]

3.2 Prevalence of anaemia in pregnancy in Malawi

There are several studies related to anaemia in pregnancy that have been carried out in Malawi. Though the primary objective was not always to determine prevalence, most of these studies report prevalence as a co-output. A total of 19 research papers were identified during the literature search that covered research that had been carried out in various parts of Malawi. The information is summarised in Appendix 1.

One study specifically measured prevalence and risk factors for anaemia in pregnancy. This study is also the largest in the region. It showed that between July 1997 and June 1998, the prevalence of all anaemia (Hb < 11g/dl) in a population of urban women (n=4708) attending antenatal clinic at Queen Elizabeth Hospital in Blantyre was 57.1% and the prevalence of severe anaemia (Hb< 7g//dl) was 3.6%. In a rural area (Namitambo health centre in Chiradzulu district) prevalence of anaemia and severe anaemia in pregnant women (n=2293) was 72% and 4% respectively.[4]

A second study specifically measuring prevalence in an unselected group of women attending rural (Chiradzulu) and semi-urban (Mangochi) antenatal clinics reported a prevalence of 58% (n=729).[ref 14]

Other studies reporting prevalence of anaemia as a secondary outcome measure report prevalence rates ranging from 38.8% to 69%. All used Hb< 11g/dL as cut-off. (For details of studies see Appendix 1).

Summary

- Anaemia in pregnancy in Malawi is common with prevalence rates reported between 38.8% and 72% for all anaemia (Hb < 11.0 g/dl) and 3.6% – 4% for severe anaemia (Hb<7.0g/dl).
- There are few population based data and a lot of studies report prevalence rates among women attending hospital antenatal clinics.
- Anaemia during pregnancy is possibly more common in adolescents but when corrected for gravidity and time of booking adolescence on its own does not seem to be a risk factor.
- Women in their first pregnancy may be more likely to have anaemia.
- Primigravid adolescents are more likely to be anaemic than multi gravid adolescents.
- There does not seem to be much seasonal variation in the prevalence of anaemia in pregnancy.

4. Determinants of anaemia in pregnancy

Few studies have comprehensively assessed the factors associated with anaemia in pregnancy in developing countries. This lack of research may be due to 3 main factors. [15]
1. Adequate diagnostic facilities are lacking in many health institutions in developing countries.
2. The aetiologic pattern is often complex such that, for example, infection and nutritional deficiencies co exist.
3. The contribution of each aetiologic factor is difficult to assess in pregnancy because maternal physiologic changes alter the indexes used to diagnose anaemia and nutritional deficiencies.

4.1 Biological Risk Factors

4.1.1 Physiological changes in pregnancy

The haemoglobin concentration, haematocrit and red cell count fall during pregnancy because the expansion of the plasma volume is greater than that of the red cell mass. However, there is a rise in total circulating haemoglobin directly related to the increase in red cell mass. This in turn depends partly on the iron status of the individual.[16]

Plasma volume rises progressively throughout pregnancy with a tendency to plateau in the last 8 weeks. Women with multiple pregnancies have proportionately higher increment of plasma volume and in contrast women with poorly growing foetuses have a correspondingly poor plasma volume. Red cell mass increases steadily between the end of first trimester and term. As with plasma volume the extent of the increase is related to the size of the foetus. [16] Thus women with multiple pregnancies are also at increased risk of anaemia.

4.1.2 Age

Although some studies have found that anaemia is more common among adolescents, this appears to be a result of the fact that adolescents are more often primigravidae and not from young age per se. Two studies from Malawi confirm this finding.
In a study carried out in Queen Elizabeth Central Hospital and Namitambo Health centre Malawi, univariate analysis showed an increased risk of anaemia for women under 20 years of age, but when corrected for gravidity and trimester at booking the increased risk with young age no longer existed.[4]
Verhoeff et al in the Shire valley area showed that adolescent primigravidae had the lowest mean haemoglobin concentration and the highest prevalence of anaemia (93.8%, n=495). Adolescent multigravidae, adult primigravidae and adult multigravidae had prevalences of 87.7% (n=144), 90.7% (n=322) and 88% (n=2614) respectively.[10] However, age was no longer associated with an increased risk of anaemia when adjusted for gravidity.

4.1.3 Gravidity

Only two published studies on prevalence of anaemia in pregnancy had information on how gravidity influences the degree of anaemia. The biological mechanism through which gravidity is associated with anaemia is unclear. The study carried out in Blantyre and Namitambo showed that, adjusted for age and trimester, primipara were at increased risk for anaemia (Hb<11g/dl) and severe anaemia (Hb<7g/dl) in both urban and rural settings, when compared to grand-multipara (≥ 5 pregnancies). The increase in risk for primipara when compared with the women in the second, third or fourth pregnancy was relatively small and not statistically significant except for anaemia (but not severe anaemia) in Blantyre.[4]
Analysis of the 4104 pregnant women attending antenatal care facilities at two hospitals in the Chikwawa area reported that mean haemoglobin concentration was significantly lower in

primigravidae (8.7g/dl) compared to secundigravidae (9.1g/dl) or multigravidae (@x.xg/dl). [10] A statistically significant difference in prevalence was observed only between primigravid and multigravid adolescents.

Another study on pregnant adolescents in Malawi found that 11.8% of primiparae and 8.3% of multiparae were severely anaemic, but the difference was not statistically significant. . But there was a significant difference in mean Hb concentration between the two groups (primiparae 8.7g/dl; multiparae 9.1g/dl; P<0.01).[13]

4.1.4 Nutritional Deficiencies

4.1.4.1 Iron deficiency

Despite the lack of stringent criteria, problems with definitions and lack of substantial supportive data, in sub Saharan Africa anaemia during pregnancy is most often believed to result from nutritional deficiencies, especially iron deficiency.
The definition and identification of iron deficiency is problematic especially in situations in which chronic inflammation is present. The gold standard for identifying iron deficiency anaemia is still the examination of suitably stained bone marrow aspirates for storage iron as haemosiderin. This method is invasive, and therefore not suitable for population screening. Serum ferritin has been shown to be a good measurement of storage iron.[21] However serum ferritin is also an acute phase protein which is raised in both acute and chronic infection. A study in from Malawi has evaluated all currently available iron parameters for their validity as markers of iron deficiency in pregnancy by comparing them against the 'gold standard' of bone marrow aspirates. This study showed that many parameters commonly used to 'identify' iron deficiency in pregnancy have a very low accuracy; these include parameters such as serum iron, zinc protoporphyrin and MCV (mean corpuscular volume) The single best measurement is serum ferritin using a cut off point of 30µg/L. This higher cut off point was found to more accurately reflect bone marrow iron stores (higher sensitivity and specificity) than the cut off point of 12 µg/L used in populations with no underlying infection. [21]

It is estimated that iron deficiency anaemia affects as many as 200 million people in the world probably making this the commonest nutritional deficiency in the world.[17]
Among pregnant women at least half of all anaemia cases have been attributed to iron deficiency.[18] The prevalence of iron deficiency may be 2-3 times that of anaemia, ranging from about 50% in some countries to nearly 100% in parts of others.[19] There is often evidence of iron deficiency before a drop in haemoglobin concentration is noted. As pregnancy proceeds, most women show haematological changes suggestive of iron deficiency especially if not receiving iron supplements. The additional demands placed on maternal iron stores by the growing foetus, placenta and the increased maternal red cell mass -though partially offset by cessation of menstruation and increased absorption of iron during pregnancy- lead to an increased demand of iron. Requirement during first trimester is low, 0.8mg per day, but this rises considerably during the second and third trimester to a high of 6.3mg/day. The total iron requirement over the whole pregnancy is about 1000mg.[2]

Iron deficiency is often nutritional in origin. One of the major contributory factors in less industrialised countries is consumption of plant based food containing in sufficient iron, especially insufficient available haem iron from meat. Iron is obtained in the form of non-heam iron from vegetables and as haem iron from meat. Heam iron is absorbed about two to three times better than non haem iron. A small amount of heam iron in the diet will improve absorption of non haem iron and thus the diet composition is an important determinants of the amount of iron actually absorbed. Iron is stored in the reticulo endothelial system as ferritin and haemosidderin[1].

Infection can also contribute to iron deficiency in developing countries.. Infections causing chronic blood loss such as parasitic infestation with hookworm and to a lesser extent schistosoma, increase iron requirement. Viral and bacterial infections may also interfere with food intake, absorption, storage and use of many nutrients including iron. Repeated episodes of infection may thus contribute to the development of iron deficiency and anaemia. [2]

Studies that have tried to assess iron deficiency during pregnancy in Malawi include studies 3,10,12 and 13 in Appendix 1.

In the Shire valley, a cohort of women (n=4104) was screened at first antenatal visit and at delivery (n=1523) between March 1993 and June 1994. Estimation of iron level was by assessment of zinc protoporphyrin levels. Subjects found to have >3.1 g of zinc protoporphyrin per gram of haemoglobin were considered iron deficient. Iron deficiency was independently associated with moderately severe anaemia (defined as Hb <8gdl) in primigravidae (RR =4.2; 95% CI 3.0-6.0). Primi-gravidae were reported to have more evidence of iron deficiency than secundae- or multi-gravidae. But in primigravidae there was no age specific difference associated with iron deficiency (43.8% in adolescents and 41.5% in non-adolescents).[10] Peak prevalence of iron deficiency occurred between April and May. In the same study iron deficiency showed little increase with increasing gestational age. [10]

The impact of diet on anaemia was studied by Huddle *et al* [23] among 152 rural pregnant women in the Mangochi area. Iron deficiency anaemia (based on serum ferritin ≤50µg/l and Hb <110g/l) during pregnancy was attributed partly to inadequate dietary intake of iron and was partly found to be secondary to malarial parasitaemia. Most of the womens' dietary iron was non haem and was obtained via plant food (89%); meat intake was only 9%.[23]

A study in 150 anaemic women attending antenatal care at Queen Elizabeth Central Hospital in Blantyre evaluated all parameters of iron deficiency, including bone marrow iron. For 150 women for whom serum ferritin measurements were available, 55% were classified as iron deficient because their serum ferritin concentrations were < 30µg/L. With use of the conventional cut off of 12 µg/L, only 21% of the study participants would have been classified as iron deficient.[22]

For 93 anaemic pregnant women (Hb <11g/dl) bone marrow aspirates were available. In 43 (46.2%) cases there was no demonstrable iron present, in 15(16.2%) traces of iron only and in 35 (37.7%) of women there was sufficient or abundant iron in the form of haemosiderin. Therefore the study concluded that the percentage of iron deficiency among this cohort of women was between 46% and 55% and confirmed that iron deficiency was an important contributing factor to anaemia. [22]

4.1.4.2 Folate deficiency

Folates are a heat labile, light sensitive family of water soluble vitamins essential to red blood cell maturation. Folates are present in all foods but more plentiful in liver and dark green vegetable leaves. A diet that is rich in other B vitamins and in vitamin C is usually rich in folate too. Some important staples in the developing world such as rice, cassava, millet, sorghum and maize are poor sources of folate.

Folate requirements approximately double during pregnancy, especially in the last trimester and the puerperium. Since body stores of folate are limited and dietary folate is likely to be insufficient in developing countries, anaemia may develop as a consequence.[2] Many studies have demonstrated a steady fall in serum folate levels throughout pregnancy, especially in women from poor socio-economic groups, in multigravidae, in smokers, and in women with twin pregnancies.[24] The recommended intake of folate in pregnancy is at least 300µg/day. [25]

In the study on aetiological factors associated with anaemia in pregnancy involving 150 anaemic pregnant women in Blantyre, using a cut off of 9.1µmol/L for pregnancy, serum folate levels were found to be low in 34% of women. Of the 25 folate deficient women, 6 (24%) were not deficient in any of the other micronutrients studied, 10 (40%) were iron deficient, 4 (16%) were vitamin B-12 deficient, 4(16%) had low serum retinol concentrations and one was deficient in all 3 micronutrients. Whether folate deficiency in this population was primarily the result of dietary insufficiency, problems with absorption, or the result of malaria is difficult to establish.[22]

4.1.4.3 Vitamin B-12

Muscle, red cell and serum vitamin B12 concentrations fall during pregnancy. The megaloblastic anaemia which develops is due to long standing vitamin B-12 deficiency and folate deficiency. The recommended intake of vitamin B12 is 2.0µg per day in the non pregnant and 3.0 µg per day during pregnancy. This will be met by any diet which contains animal products but strict vegetarians who will not eat animal derived substances may have a deficient intake of vitamin B12 and their diet should be supplemented during pregnancy.[16]

In Blantyre, Malawi the study by van den Broek *et al* showed that one third of anaemic women (n=150) had serum vitamin B12 concentrations <148µmol/L, which is the accepted lower limit outside pregnancy. When deficiency was defined as serum vitamin B12 <52µmol/L, reflecting the observed decrease toward the end of pregnancy resulting from the active transplacental transfer from mother to foetus and the added effect of haemodilution, 16% of women were affected. Five (24%) of these 24 vitamin B12 deficient women were also folate deficient. An association was noted between vitamin B12 concentrations and megaloblastic changes observed in the bone marrow which was linear and highly significant.[22]

One other paper from the region looked carefully at Vitamin B12 deficiency and concluded that this was a previously unrecognised but important cause of anaemia in Zimbabwe in adults. [26]

4.1.4.4 Vitamin A

It has been known that vitamin A plays an important role in haematopoiesis [ref] and more recently it has been suggested that vitamin A supplementation, particularly in women with low or borderline serum retinol concentration, may improve mobilization of iron stores. Thus it has been proposed that for women in whom bone marrow iron was more than adequate but who had evidence of anaemia and of inflammation (study by van den Broek *et al*[22]), anaemia could have resulted from 'blockage' of the incorporation of iron into heam, which is described in association with inflammation in a number of studies. It has been proposed that Vitamin A which has been described as an anti inflammatory vitamin, may work by counteracting this 'block'. [22]

During pregnancy, serum retinol concentrations have been shown to drop below non pregnancy concentrations. Serum retinol concentrations of 1.05, 0.7, and 0.35µmol/L indicate inadequate, moderately inadequate, and very inadequate liver stores, respectively. In the study done in Blantyre, 39% of the 150 anaemic women were please leave in cut off points in umol as much confusion over cut off levels and defined differently in many papers…vitamin A deficient and 13% of women were very deficient. Of the women with serum retinol concentrations <0.7µmol/L, 52% (30 of 58) were also iron deficient. Vitamin A deficiency was the only micronutrient deficiency in 15% of all women possibly making this the second most frequent single micronutrient deficiency after iron deficiency in this group of anaemic women.[22]

Although serum retinol is commonly used as an indicator of Vitamin A status, it is under strict homeostatic control and more accurate ways of diagnosing Vitamin A deficiency in pregnancy include dose response tests. In a later study (Vitamin A trial) using dose response tests in a rural population, Vitamin A deficiency was not found to be a common problem in pregnant women (vdbroek unpublished information/in press).

4.1.5 Infections

4.1.5.1 Malaria

Malaria due to *Plasmodium falciparum* may cause severe anaemia in pregnancy. It is estimated that in sub Saharan Africa 23 million pregnant women are exposed to malarial infection annually. Women in their first and second pregnancies living in an endemic area are at a higher risk of acquiring malaria than non pregnant women or multi gravidae, due to reduction of an appropriate immune response to the malaria parasite. [2]

Anaemia associated with malaria is caused by haemolysis of the red blood cells. Hypersplenism, a condition characterised by exaggeration of the inhibitory or destructive functions of the spleen, contributes to the anaemia in up to 25% of women who suffer from malaria in pregnancy. Several studies have shown that protection against malaria contributes to the prevention of anaemia in pregnancy [27,28,29] thus highlighting the importance of chemoprophylaxis and other methods of malaria control. The adverse effects of malaria on maternal and foetal well being are thought to be for the most part due to the associated severe anaemia. [2]

There is evidence that malaria can induce iron deficiency by several mechanisms: possibly through immobilising iron in haemazoin complexes and loss of urinary iron, as well as reducing intestinal iron absorption during the acute illness period[30]. However these effects exerted by malaria on body iron status are still poorly understood, in part because biochemical and haematological indices of iron status are confounded by the malaria infection.

In the study by Huddle et al [23] 152 rural Malawian pregnant women were recruited to study the impact of malarial infection and diet on anaemia status. Women were divided in to two groups in the analysis; women with and without malarial parasitaemia. A greater proportion of women (83%) positive for malaria on the test day had anaemia (compared to 63% without malaria).[23]

In the study examining aetiological factors associated with anaemia in pregnancy in Blantyre Malawi, among 150 anaemic pregnant women, *falciparum* malaria was identified in only 8% of women. But one of the study limitations was the absence of a non anaemic control group. Malaria infection may have been underestimated because diagnosis was by examination of one thick blood film only taken at the time of recruitment. In addition, the study was conducted in the season of low malaria transmission. [22]

In the study by Rogerson *et al* [31] screening for malaria and anaemia was performed over a 12 month period in Blantyre, Malawi. During that time 4764 of 6765 women attending the ante natal clinic for the first time were screened. Mean (standard deviation) haemoglobin levels were lower in women with malaria (10.3 (1.8)g/dl) than in aparasitaemic women (10.8 (1.8g/dl) t=-4.4; P< 0.0001). There was evidence of correlation between decreased haemoglobin and increased parasite count. Also women with malaria were more likely to be moderately or severely anaemic, and this relationship was seen in both primigravidae and multigravidae. A total of 25.4% of primigravidae with malaria were moderately or severely anaemic, whereas only 14.7% of parasite free primigravidae were moderately or severely anaemic (OR = 1.97, 95%CI = 1.52-2.56, P <0.0001). In multi gravidae, 20.4% of

parasitaemic and 14.8% of aparasitaemic women had moderate or severe anaemia (OR = 1.45, 95% CI = 1.2-1.76, P < 0.0001). Similarly, moderate or severe anaemia was more common in parasitaemic women, both in younger (24.8% vs 12.4%, OR = 2.31, 95% CI = 1.67-3.22, P< 0.0001) and older women (21.4% VS 15.5%, OR = 1.48, 95% CI = 1.24-1.76, P < 0.0001)[31].

The effect of malaria on maternal anaemia was evaluated in 2040 pregnant women attending routine antenatal care services at 2 rural health centres in Blantyre district. The prevalence of maternal anaemia was 64% at enrolment. Those who had a positive peripheral blood film for malaria at enrolment had a significantly lower mean haemoglobin compared to those with a negative film [9.1 g/dL vs 10.4 g/dL; P<0.0001]. Likewise the prevalence of anaemia was significantly higher among those with a positive malarial film compared with a negative film at enrolment (76.5% vs 61.7% ; P< 0.0001). [32]

4.1.5.2 Hookworm Infection

Hook worm infection is described to be one of the principal causes of iron deficiency anaemia in developing countries especially in children. It is prevalent throughout the tropics and subtropics wherever there is faecal contamination of the environment and is acquired mainly by skin contact with contaminated soil or vegetation.[2]

Adult hook worms live in duodenum and jejunum of humans attached to the intestinal mucosa and suck blood. Once they leave the attached site this causes chronic blood loss from the mucosa. In people whose dietary intake of iron is low and whose blood iron stores are already depleted, hookworm infection can presumably give rise to iron deficiency anaemia in just a few weeks, especially during pregnancy, when iron requirements are increased.[2]

Only one study looked at hook worm infestation among the group of 150 anaemic women in Blantyre. Out of the total, 6% had hook worm infestation and none had a high density infestation. A maximum worm load of ++ was found in 2 women only. Therefore intestinal parasites are unlikely to have contributed significantly to the presence of anaemia in this population. [22]

No other study on the prevalence of hookworm infestation among pregnant women was found in the literature.

4.1.5.3 Human Immuno Deficiency Virus infection

HIV infection must now be included in the differential diagnosis of anaemia in pregnancy. Where anaemia is associated with leucopaenia and thrombocytopaenia, the antenatal health worker should be alerted to the possibility of AIDS,

Transmission of HIV infection by blood transfusion is possible in developing countries, where there is a high prevalence of HIV positivity among donors and where the ability to screen for HIV is sub optimal. This further highlights the importance of the antenatal clinic in the prevention of anaemia early in pregnancy, which may avoid the need for a blood transfusion later in the pregnancy.

In the study in Chickwawa (4104 pregnant women attending the antenatal care facilities of two hospitals), it was observed that in primigravidae, anaemia prevalence was lower with HIV infection, whereas in multigravidae the reverse was observed.[10]

In the study examining in detail aetiological factors associated with anaemia in the 150 women in Blantyre, one of the factors studied was HIV seropositivity. The observed prevalence of HIV seropositivity in the group of women with anaemia was 47.1% (95% CI

39.2-55.0). This is significantly higher than the HIV prevalence in the whole antenatal population (30.1%: P < 0.001).[33] The mean haemoglobin concentration for HIV seropositive participants was 8.1g/dl which was significantly lower than the 8.8g/dl for seronegative participants (P<0.001). The median C- reactive protein (CRP) concentration was significantly higher in the HIV seropositive group compared to the HIV seronegative group (167 compared with 29nmol/L; p < 0.0001).The difference in iron concentration between HIV seropositive seronegative women were not statistically significant. Similarly no significant difference was observed in either bone marrow megaloblastic change or iron content between seropositive and seronegative women for whom bone marrow aspirates were available.[22]

4.2 Behavioural determinants

Surprisingly, the behavioural and social risk factors associated with anaemia in pregnancy have not been reported widely and it is difficult to find published literature on this subject. Behavioural risk factors mainly involve dietary pattern, and health seeking behaviour. Pregnancy is recognised as a special event in a woman's life by most societies and often dietary restrictions are imposed on women. These food taboos are culturally defined (see paragrap 4.3.1) and can adversely affect the health of women during pregnancy.

4.2.1. Health seeking behaviour

A woman's own perceptions of pregnancy and her health status are major factors that will influence her health decision making. These health perceptions, not only reflect the cultural back ground but also a woman's role and status within the family and community. In many traditional societies one of the most important roles of women is to produce children. Pregnant women are expected to carry on with their daily activities the same way as they would in a non pregnant state and often the side effects of pregnancy are de-emphasized. In addition the community may look unfavourably on women who complain about symptoms associated with pregnancy. Thus health problems associated with nutritional anaemia may not be recognised or may be ignored. Cultural idioms may play an important role in a pregnant woman's decision to seek health care and to comply with a prescribed treatment regime.[35]

In Blantyre, Malawi 80.8% of women booked in the first or second trimester, similar to the group in Namitambo. In Namitambo (rural area) trimester at booking was not a risk factor for anaemia, but in Blantyre (urban area) the risk of anaemia overall and the risk of severe anaemia was increased in women who booked late in pregnancy. [4] Trimester was estimated by palpation only and ultrasonography to date the pregnancy more accurately was not available.

4.3 Sociocultural and environmental determinants

Social risk factors may include the perceptions of the society of the problem of anaemia during pregnancy, cultural food taboos, socio economic status and literacy level.

One study in Nigeria showed that many foods that are avoided during pregnancy provide all the key nutrients (calcium, iron, vitamin A, folic acid, vitamin C) needed by pregnant women.[37] Nigerian women complaining of dizziness attributed this to insufficient blood and used traditional medicine for this condition.[38] A study from Sierra Leone reported that certain illnesses, including worm infestations are believed to drain the blood or 'dirty' it.[39] Many pregnant women in India and Thailand believed that taking iron and vitamin tablets will cause them to have big babies, resulting in difficult deliveries.[36]

Extensive literature search revealed no studies related to social and behavioural risk factors carried out in Malawi. For this review however, we were able to obtain some of the food taboos associated with pregnancy in Malawi by personal communication with a doctor practicing in Malawi and a team of research midwives.[34] They reported that most people seem to believe that the following food items should not be eaten by a pregnant woman for reasons as given:

1. Pepper
   It is prohibited because babies are born with sores all over the body and red eyes.
2. Eggs
   The head of the child is born without hair.
3. Sweet potato leaves
   It causes delay during birth of the child. Causes delay in labour progress and post maturity.
4. Pig meat
   The baby is born with skin rashes.
5. Mudfish
   The mudfish because of its slippery body, you know it has no scales causes abortions.
6. Sugar cane
   It causes the baby to be born with grey skin.

4.3.1 Seasonality

In the Shire valley, the seasonal variation in anaemia in pregnancy among 539 pregnant adolescents at their first antenatal visit was examined; the highest prevalence occurred in the month of June (38.2% of cases). Whether this was significantly higher than for other months was not reported.[10]

In the Blantyre / Namitambo study [4] prevalence rates for the dry season (May to October) and wet season (November to April) were calculated. The prevalence of all anaemia was reduced during the dry season, both in rural areas (OR=0.83; 95% CI 0.69-0.99) and urban areas (OR=0.64; 95% CI 0.57-0.73). The reduction in prevalence of severe anaemia in the dry season did not reach statistical significance in rural areas but did in urban areas (OR=0.71; 95% CI 0.51-0.99).[4]

5 Impact of maternal anaemia

Although the prevention of anaemia is an important priority for mothers and children to what extent is it correct to say that anaemia prevention will reduce maternal and child mortality? The relationship of anaemia as a risk factor for maternal or child mortality have recently been reviewed.[40] One of the major problems with previous studies is that no randomised controlled trials have been conducted to establish the effect of interventions on cause specific mortality. As anaemia is usually multi-factorial in developing countries it becomes difficult to establish attributable risk for specific causes. The definitions of anaemia across different studies vary, as do the methods for estimation of haemoglobin (Hb) and the type of study sample, which may be either hospital or community. This variation between studies complicates comparative analysis.[41]

5.1 Maternal Mortality and Morbidity

Each year more than 500,000 women die from pregnancy related causes, 99% of these in developing countries[42]. Anaemia is probably a chronic rather than acute condition in many cases. There is a resulting compensatory shift of the oxygen dissociation curve to the right. Thus, women may be seen with very low haemoglobin in their early antenatal period without any overt symptoms of cardiac failure. They will, however, easily become tired and may decompensate, e.g. as a result of labour. Should any adverse event such as bleeding occur, their risk of death is high. The available data relating maternal and neonatal mortality to pregnancy anaemia is limited and in most of the cases causality is difficult to establish. Some times the pregnancy outcome is related to the underlying cause of anaemia, e.g. HIV, ante-partum haemorrhage, etc. rather than to the anaemia itself.

It must also be noted that maternal mortality and morbidity data are most often based on hospital data. Hospital based studies do not represent the actual situation in developing countries and more community based studies are needed. These must include appropriate methodology to establish a clear causal relationship. Finally it must be noted that there are currently no agreed international standards or sets of criteria for attributing death to anaemia.

Rush (2000) [50] considered it a reasonable working assumption that maternal mortality is greatly increased with severe anaemia, and the strength of this relationship makes it appropriate to assume a causal association with severe anaemia, but he noted that the association with moderate anaemia is less clear.
A United Nations expert panel considered severe anaemia (<7g/dL) an associated cause in up to half of the maternal deaths worldwide[13]. Estimates of maternal mortality resulting from anaemia range from 34/100,000 live births in Nigeria to as high as 194/100,000 in Pakistan.[2,3]

In combination with obstetric haemorrhage, anaemia is estimated to be responsible for 17-46% of cases of maternal death[43-45] The proportion of maternal deaths due to anaemia has been estimated for some countries: India (16%), Kenya (11%), Nigeria (9%) and Malawi (8%).[4]

Maternal case fatality rates, mainly from hospital studies vary from <1% to >50%. These large differences in risk were related primarily to differences in available obstetric care for women living in areas with inadequate antenatal and delivery care facilities and in particular availability of blood transfusion. The relative risk of mortality associated with moderate anaemia (Hb 40-80g/L) was 1.35 (95% CI 0.92 – 2) and for severe anaemia (Hb <40 g /L) was 3.51 (95% CI 2.05 – 6).

A prospective assessment of mortality among cohort of pregnant women (4053 monitored pregnant mothers, 27 deaths) done in rural Malawi between 1987 and 1989 highlighted that that for rural Malawian women, pregnancy and delivery are risk periods, that the death of the mother adversely affects the survival of her children, and that HIV and anaemia are important contributors to non maternal mortality (3-10 months after delivery) in reproductive age women.[49]

This report by Rush was one of the best ones written to date (not speculative like manyothers…) I think should keep it in and delete some of the other stuff….

The relative risk for severe (<4.7g/dL) and moderate anaemia (4.0-8.0g/dl) have been calculated for five Nigerian studies with adequate data[51,52,53,54,40]. The sample sizes for some of these studies are again small. The pooled data estimates a RR of 1.35 (95% confidence Interval 0.92-2.00) for moderately anaemic women and RR = 3.51 (95% Confidence Interval 2.05-6.00) for severely anaemic women.

Maternal morbidity resulting from anaemia is similarly difficult to establish. Diminished work capacity and physical performance have been reported as a result of anaemia[47,48] Iron deficiency anaemia leads to abnormalities in host defence and neurological dysfunction[49,50].

5.2 Infant mortality and morbidity

Data from industrialised countries suggest that maternal anaemia and iron deficiency increases low birth weight and pre term birth risk[55]. Low birth weight (either IUGR or pre-maturity) is the most important risk factor for infant mortality and significant detriment of childhood morbidity.

Information collected from 1423 live born singletons and from their mothers prior to delivery attending in two hospitals in Chickwawa district, Malawi suggested that IUGR of the baby is significantly associated with moderately severe anaemia at booking (Hb <8.9g/dl) at first antenatal clinic visit (RR 1.6; 95% CI 1.2-2.2). The study concludes that prevention of IUGR requires reduction of anaemia during pregnancy[55]. Furthermore, the prevention of IUGR requires efforts to reduce anaemia prevalence prior to pregnancy considering the high prevalence of anaemia at first antenatal visit and late attendance in pregnancy to antenatal clinics of many women[55]. The findings depend on the definitions used in the study. Asymmetric growth retardation was defined as a ponderal index {calculated as 100 times the birth weight (in grams) divided by the cube of birth length (cubic centimeters)} <10% of reference value. Serial scanning of the foetus, which is very useful in accurately diagnosing IUGR, was not used in this study.

Because of the poor reduction observed in child mortality rates in the past decade in sub Saharan Africa and the noted increase in post-neonatal infant mortality (PNIM defined as mortality occurring after 28days and before 1 year of age) in Malawi, a cohort study of mothers and their infants was carried out to analyse the risk factors for PNIM. Information was collected on women attending the antenatal services of two hospitals in a rural area of Malawi and 561 of their babies were enrolled in a follow up study. There were 128 with low birth weight (LBW, <2500g), 138 with foetal anaemia (FA, haemoglobin <12.5g/dl), 42 with both and 228 with a normal birth weight and no foetal anaemia. The study showed there was no significant association between post neonatal infant mortality and foetal anaemia (RR 1.60, 95% CI 0.78-3.27)[56].

6. Interventions

Interventions to combat anaemia in pregnancy have most recently focussed on antenatal supplementation with iron and Vitamin A and on prevention of malaria during pregnancy.

6.1 Iron supplementation (with folic acid)

The available data from controlled trials provide clear evidence of an improvement in haematological indices in women receiving iron supplementation.[46] At present therefore there is no evidence to advice against a policy of routine iron supplementation in pregnancy. Therefore routine supplementation is warranted in populations in which iron deficiency is common.[20]

Studies from developing countries[58,59] suggest that 120mg elemental iron and 1mg folic acid are the optimum daily dosages needed to prevent anaemia in pregnant women. But WHO recommends, for all pregnant women, if there are no high risk factors a combination tablet containing 60mg of elemental iron and 250µg of folate to be taken twice a day. Where this is not available, tablets such as ferrous sulphate, containing 60mg of elemental iron, should be given twice a day together with one folic acid tablet (1 mg)[1]

Recently a weekly iron/folate supplement was compared with a standard daily iron/folate supplement in pregnant women living in rural Malawi. Women enrolled as they attended the local antenatal clinic, then were stratified by grade of anaemia and then randomly received either 60mg iron/ 0.25 mg folic acid per day or 120 mg iron/ 0.5 mg folic acid once a week. The results indicated that the haemoglobin values increased by similar levels in both groups with the anaemic women increasing by an average of 6.3g/L in the daily group and 5.9g/L in the weekly group for all women. This study concluded that a weekly iron supplement given to pregnant women in rural Malawi had similar haematological effects, and an improved side effect profile, in comparison with the standard daily supplement when administered through an existing primary health care programme, although both regimens were unsuccessful in the reduction of anaemia prevalence during pregnancy[60].

The side effects of oral iron administration or women's dislike of the tablets owing to smell or taste have often being blamed for treatment failure, although a literature review[61] found out that side effects accounted for only 10% of non compliance. Rather, in most cases, women did not take their tablets because they never received them or received inadequate quantities. The impact of iron supplementation could be improved by counselling on why, how and when to take iron tablets and by supplying the tablets[46].

In Malawi, iron, but not folate, supplementation is part of routine antenatal care. The Malawi Demographic and Health Survey (2000)[63] indicated that iron supplements were provided to 70% of mothers with a recent birth. But the supply had a great variation over the region (Northern 83.8%; Central 64.4%; Southern 71.1%) and level of education (no education 63.7% and secondary education 79.8%).

The effectiveness of iron / folate supplementation to reduce iron deficiency and anaemia in women of reproductive age was assessed in Thyolo district.. A base line survey on iron status and haemoglobin level was conducted in June 1996. A second survey was carried out after 2 years following a 15 month intervention with iron/folate supplementation. Two hundred and ten pregnant women, 210 non pregnant women (who had delivered within last 6 months) and 315 men were recruited for the survey. After the intervention, anaemia was present in 60% (baseline, 67%) of pregnant women and 51% (baseline, 61%) of women who had delivered in the last 6 months. Moreover, severe anaemia (haemoglobin <7g/dl) was found in 1.9% (baseline, 3.3%) of pregnant women, 2.4% (baseline, 4.3%) of recently delivered women, and

1.3% (baseline, 0.3%) of men. The intervention programme was successful in increasing haemoglobin levels and decreasing the prevalence of anaemia, particularly among women who had delivered in the last 6 months. [62]

6.2 Blood Transfusion

Although it is difficult to give absolute recommended haemoglobin level at which to transfuse, it should be considered in women at or above 34 weeks gestation who have haemoglobin less than 7g/dl, as it is important that severe anaemia is corrected prior to delivery. There is no role for treatment of severe iron deficiency anaemia at community level. The community health workers must immediately refer pregnant women with severe anaemia to appropriate health care level[46].
There are no studies done in Malawi in relation to blood transfusion as an intervention to anaemia in pregnant women.

6.3 Vitamin A supplementation

The influence of vitamin A and iron supplementation was studied in anaemic pregnant women in West Java, in a randomised, double masked placebo controlled field trial. 251 women aged 17-35 years, parity 0-4, gestation 16-24 weeks and haemoglobin between 80 and 109g/L were randomly allocated to four groups: vitamin A (2.4mg retinol) and placebo iron tablets; iron (60 mg elemental iron) and placebo vitamin A ; vitamin A and iron; or both placebos, all daily for 8 weeks.[64]

Maximum haemoglobin was achieved with both vitamin A and iron supplementation (12.78g/L, 95% CI 10.86-14.70), with one third of the response attributable to vitamin A (3.68g/L, 95%CI 2.03-5.33) and two thirds to iron (7.71g/L, 95%CI 5.97-9.45). After supplementation the proportion of women who became non anaemic was 35% in the vitamin A supplemented group, 68% in the iron supplemented group, 97% in the group supplemented with both, and 16% in the placebo group. Therefore this study concluded that improvement in vitamin A status may contribute to the control of anaemia in pregnant women.[64]
Two further studies were conducted in Malawi to test this.

A randomised, double masked, controlled clinical trial was conducted in Blantyre to test the hypothesis that vitamin A supplementation[65] increases haemoglobin concentration,[66] increases plasma erythropoietin concentration and[67] changes the plasma erythropoietin and haemoglobin concentration among pregnant women with a high prevalence of anaemia. HIV Negative women were randomised to receive either a daily supplement containing iron (30 mg elemental iron), folate (400 micro grams), and vitamin A (3000 micro gm retinol equivalent), or iron (30mg) and folate (400 micro gm) from enrolment until delivery. All the tablets were identical in appearance and tablet counts were conducted every 4 weeks to assess the compliance[57]. Mean change in haemoglobin from enrolment to 38 weeks was 4.7 ± 1.6g/L (p=0.005) in vitamin A group (n=63) and 7.3 ± 2.3g/L (p=0.003) in control group (n=52), by paired *t*-test. At 38 weeks gestation, the prevalence of anaemia (haemoglobin < 110 g/dL) was 28.9% in the vitamin A group and 35.2% in the control group (p=0.46 in the chi square). At 38 weeks of follow up there was no significant difference in mean plasma erythropoietin concentrations between the vitamin A and the control groups. Therefore this study concludes that vitamin A supplementation did not appear to modulate the relationship between haemoglobin and plasma erythropoietin concentration in this group of pregnant women with a high prevalence of anaemia. One of the limitations identified in this study was that malaria parasitaemia was not measured initially[57].

The second study (van den Broek in press) carried out in a pregnant population in rural Malawi tested the hypothesis that if vitamin A does protect against death from diverse pregnancy associated causes (as some studies in Nepal have suggested), it may do so by decreasing anaemia, and improving iron stores and infective status. Seven hundred (700) women with singleton pregnancies at 12-24 weeks by ultrasound, and with screening haemoglobin <11 g/dl, were randomly allocated to oral supplementation with 5000IU or 10,000 IU vitamin A or placebo. The study findings showed that vitamin A in rural population was less common than predicted. Vitamin A supplementation had no meaningful impact on anaemia, severe anaemia, iron status or indices of infectivity. Vitamin A stores were less likely to be depleted at the end of pregnancy in supplemented group.[69]

6.4 Intermittent anti malaria therapy

In 1993 Malawi adopted intermittent presumptive therapy with sulfadoxine- pyrimethamine (SP) as malaria prophylaxis for all pregnant women.

Malaria in pregnancy is a significant cause of maternal and infant mortality and morbidity but accurate estimates of the contribution of malaria to maternal or infant deaths are difficult to make without extensive resources[70] There is adequate evidence to show that malaria during pregnancy could give rise to severe anaemia. Therefore trials were undertaken to examine if anti malarial drugs used during the antenatal period could reduce the incidence of severe anaemia in pregnant women.

Women attending the maternity unit of the Queen Elizabeth Central Hospital, Blantyre, Malawi for delivery over a 21 month period, from July 1997 to April 1999 were studied to evaluate the effectiveness of intermittent presumptive therapy with Sulphadoxine Pyrimethamine during the antenatal period to the presence of malaria infection and indicators of morbidity due to malaria in pregnancy. The reduction in anaemia prevalence associated with SP prescription was maximal in Primigravidae and secundigravidae. Anaemia prevalence was 40.6% for primi and secundigravidae not receiving SP and 28.5% for women receiving 2 doses (χ^2= 8.70, df=2, P=0.013). For multigravidae the prevalence was 34.9% and 35.4% respectively. Moderate to severe anaemia (Haemoglobin < 9g/dL) was also less common in primigravidae and secundigravidae prescribed 2 doses of SP (6.3% vs 12% for women not receiving SP, P= 0.04), but not in multigravidae. Therefore improved implementation of IPT with SP is recommended as one of the effective methods in preventing anaemia and malaria among pregnant Malawian women.[71]

The prevalence of infection with malarial parasites and the incidence of anaemia and delivery of infants with low birth weight babies was investigated in 575 Malawian women who attended Chikwawa district hospital, Malawi. Women received either one, two or three doses of Sulphadoxine-Pyrimethamine during pregnancy. The results showed that the mean haemoglobin concentration at delivery in two dose multigravidae (10.4 (1.4) g/dL) was slightly and significantly higher than in those who had just one dose (10.0 (1.6) g/dL; P = 0.009), but that difference was not seen in primigravidae (10.1 (1.5) vs 10.2 (1.7) g/dl; P = 0.92). The number of times haematinics had been supplied did not affect haemoglobin concentration at delivery in primigravidae. However, when linear regression analysis was used to adjust for the effect of haematinic supplementation, the contribution of SP to haemoglobin concentration at delivery in multigravidae was no longer found to be significant (P = 0.70).[72]

The Malawi Demographic and Health Survey (2000)[63] showed intermittent anti malaria therapy was provided to 72% of mothers who gave birth to a child within 5 years preceding the survey. It also showed that prophylaxis availability varied among geographically and socio-economically defined groups. For instance, about 86% of expectant mothers in Mulanje

district received intermittent treatment against malaria parasite, compared with just 59% in Machinga district. Urban areas had coverage of 83.3% whereas rural areas had only 70.1% of coverage for the same.

Another study carried out in Malawi found both malaria infection and anaemia to be frequent in occurrence in pregnant women. Both malaria and anaemia were more common in first pregnancies than in subsequent ones, but age appeared to be a greater determinant of malaria risk than gravidity in this study population. Malaria infection was found to be associated with anaemia and was most prevalent and intense in women with moderate anaemia (Haemoglobin =7-8.9g/dl). Significant proportions of low grade or high grade malarial infection occurred in second or subsequent pregnancies, and authors caution against targeting only primigravidae for anti-malarial interventions in pregnancy because this strategy would miss half the women with high grade parasitaemia. Women in third or later pregnancies accounted for approximately 35% of parasitaemic women. The authors also suggest that the population of pregnant women at risk for malaria related morbidity in urban centres may be larger than that in rural areas.[31]

There are currently no reports on bed net studies in pregnant women from Malawi.

6.5 Control of other parasitic infections

Because ant-helmintic treatment has been proven to slow the decrease of hameoglobin levels during pregnancy, WHO recommends that anti helminthic treatment should be included in strategies to control maternal anaemia in most of the developing countries. [79]

6.6 Dietary modification

Dietary modification could be an important way of decreasing the incidence of nutritional deficiency, though this may be difficult due to the limited purchasing power of a household and problems in persuading people to change long established dietary habits. Increased consumption of normal foods so that energy needs are met can however increase iron consumption. Such a strategy was used in rural India and increased total iron consumption by 25-30%.[80,81] Enhancing the bio availability of ingested iron rather than increasing the total amount in the diet is another possibility. This can best be achieved by promoting iron absorption enhancers such as haem iron (e.g. meat) and vitamin C. Any campaign to promote the intake of haem iron could run into the obstacles of unavailability and high cost, as well as religious cultural and philosophical objections to the consumption of meat. In contrast, efforts to increase the content of non heam iron, which is found in food such as pulses and in green vegetables in conjunction with an increased vitamin C intake, have perhaps a greater chance of success. In many communities vegetables and fruits are eaten only infrequently and in small amounts. Persuading families to add these foods to their diets can have a considerable impact on iron absorption. Pregnant women should also be encouraged to increase their intake of pulses and green vegetables. Prolonged cooking of vegetables should also be discouraged as this destroys much of the vitamin C and folic acid content: nutrition education should stress this and teach ways of avoiding it. Health workers should also stress the importance of reducing tea and coffee consumption, particularly during meals. This alone can have a considerable impact on bioavailability of iron[35].

No reports are currently available for Malawi.

Appendix 1

Studies from Malawi with a focus on anaemia in pregnancy.

Study Number	Authors	Year of publication	Number of women screened	Study area (Study period)	Mean Hb (SD)	Percentage of women Hb<11g/dL	Main scope of the study
1	Rogerson SJ, Van den Broek NR *et al.,*	2000	4764	Queen Elizabeth Central Hospital Blantyre Malawi (August 1997-July 1998)	10.5g/dL (1.8)	57.2	To determine the relation ship between anaemia and malaria in this population
2	Van den Broek NR, Rogerson SJ *et al.,*	2000	6939	QECH (4646) Namitambo Health Centre (2293) (July 1997-June 1998)	10.5g/dL 10.1g/dL	57.1 71.7	To find out prevalence and risk factors for anaemia in pregnancy
3	Brabin BJ, Verhoeff FH *et al.*	2004	4104	Rural district hospitals in Chickwawa and Montfort (March 1993-June 1994)	Adolescent8.9g/dL Adult 9.1g/dL	Adolescent 90.7 Adult 89	To reveal the haematological profile of people from rural Southern Malawi
4	Van den Broek NR, Ntonya C *et al.*	1999	729	Rural ANC in Malawi (3 rural hospitals and 2 health centres)	More than 50% ranged 10-11.9g/dL	58.1(Hb<11g/dl) 32 (Hb≤ 10.9g/dl) 4 (Hb≤ 8.9g/dl)	To assess the potential of the haemoglobin colour scale in diagnosing anaemia in pregnancy
5	Young MW, Lupafya E *et al.,*	2000	413	Ekwendeni or one of the mobile MCH clinic in rural Malawi	10.5g/dL	62 (Hb7-10.9g/dl) 34 (Hb 7-9.9g/dl)	To ensure the effectiveness of weekly iron supplementation in

				(April-August 1997)			pregnant women
6	Semba RD, Kumwenda M *et al.,*	2001	203	ANC of QECH Blantyre Malawi (November 1995-December 1996)	10.7	50	To show the impact of vitamin A supplementation on anaemia
7	Abraham ET, Milner Jr DA *et al*	2004	567	Labour ward, QECH Blantyre, Malawi (Dec 2000 to July 2002)	-	52.9	To assess risk factors and mechanism of pre term delivery
8	Brabin L, Verhoeff FH *et al.,*	1998	615 adolescent pregnant women	Two hospitals in Chickwawa district Shire valley (March 1993 to Sept 1994)	Nullipara 8.7g/dl Multipara 9.1g/dl	92.6 (Hb<11g/dl) 11.2 (Hb<7g/dl)	Why the adolescent antenatal care programme need to be improved
9	Van den Broek NR, Letsky AE	2000	265	ANC at QECH Blantyre Malawi	8.5 g/dl (coulter) 8.2g/dl (haemocu)	58.5 (Hb<10.5g/dl) 16 (Hb<7g/dl)	To find out the aetiology of anaemia in a cohort of pregnant women
10	Huddle JM, Gibson RS *et al.,*	1999	152	Jalasi HC, Mangochi District, Southern Malawi (Nov1993 to Feb 1994)	10.4 (1.3)g/dl	69	To show the impact of malarial infection and diet on the anaemia status of rural pregnant women
11	Rogerson SJ, Chaluluka E *et al.,*	2000	1623	Maternity Unit, QECH, Blantyre, Malawi (July 1997 to Apr 1999)	11.4g/dl	38.8	To study the effectiveness of intermittent SP therapy on malaria morbidity in pregnancy
12	Van den Broek NR, Letsky AE	1998					To find out which measurements are valid in iron deficiency

13	Van den Broek NR, White SA et al.,	1998	155	Maternity clinic Queen Elezabeth Central Hospital, Blantyre, Malawi		60.0	Relationship between asymptomatic HIV infection and maternal anaemia
14	Verhoeff FH, Brabin BJ et al.,	2001					To analyse Intra Uterine Growth Retardation in rural Malawi
15	Verhoeff FH, Saskia LC et al.,	2004					To determine the causes for post neo natal infant mortality
16	Brabin BJ, Gearlings et al.,	2003					Reducing childhood mortality in poor
17	Steketee RW Jack J et al.,	1996					To study malaria treatment and its prevention in pregnancy
18	Holtz TH Kachur SP et al.,	2004					To enumerate ante-natal care services and intermittent preventive treatment for malaria among pregnant women
19	Semba RD, Taha ET etal.,	2001	483				To show the iron status and indicators of HIV disease severity among pregnant women

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Nb I think the references were correct !!! but it seems in working on the document you now have 1 2 3 and again 1 2 3….. I am reluctant to change again as this will be very confusing. Please finalise the text and I will then proof read and check over all the references…

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