ARTICLE

Evaluation of the effectiveness of iron and folate supplementation during pregnancy in a rural area of Limpopo province

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Objective. To evaluate the effectiveness of iron and folate supplementation in antenatal clinics.

Design. A cross-sectional analytical study.

Setting. Villages in the central region of Limpopo province, which are serviced by Mankweng Hospital.

Subjects. Third-trimester pregnant women ($N = 262$) attending antenatal clinics at eight local clinics in the villages.

Results. The prevalence of anaemia (haemoglobin < 10.5 g/dl) in this study population was 16.4%. The mean number of clinic visits during pregnancy was 4.1 (standard deviation (SD) 2.18). There were no differences in clinic attendance for the anaemic and non-anaemic participants. Maternal weight was found to be lower ($p = 0.051$) in the anaemic than the non-anaemic group. Iron, vitamin B_{12} and folate deficiencies are still common in this area, with 50.9%, 16.4% and 10.3% of the pregnant women respectively having biochemical evidence of these deficiencies. In the anaemic group iron deficiency was the most prevalent deficiency, with 62.8% of the women having iron deficiency (serum ferritin < 12 µg/ml). In the non-anaemic group there was a significant negative correlation between both serum and red cell folate and the time of first visit to the clinic, indicating that folate supplementation given by the clinics is effective in prevention of folate deficiency.

Conclusion. Although both iron and folate supplements are issued at antenatal clinics in the central area of Limpopo province, iron deficiency and iron deficiency anaemia are still prevalent in pregnant women. However there has been a marked improvement in the prevalence of folate deficiency.

Anaemia is highly prevalent in pregnant women, especially in low- to middle-income countries. Indeed, according to the World Health Organisation (WHO) it is estimated that more than half of pregnant women in these countries are anaemic.[1] In South African studies, anaemia has been found in 7 - 29% of pregnant women.[2-4] In other parts of sub-Saharan Africa the prevalence ranges from 22% in Kenya to 69% in Malawi.[5-7]

Factors causing anaemia include nutritional deficiencies (particularly iron, vitamin B_{12} and folate), genetic factors, and infections (e.g. malaria, schistosomiasis and hookworm).[6-8] Furthermore the effects of dietary deficiencies can be aggravated by external factors such as poor socio-economic status, which incorporates lack of education, unavailability of proper health care and poor infrastructure.[3,10]

Since the requirement for several nutrients increases during pregnancy to meet the increased demands imposed by the fetus, most nutritional deficiencies tend to lead to severe consequences either to the mother or to the unborn baby.[11] Several researchers have shown that these consequences include preterm delivery, intrauterine growth retardation, abnormal organ development, and increased fetal and maternal mortality.[12-15] Numerous studies have shown that iron and folate supplementation results in improved outcomes,[16-18] which has led to an increase in the daily recommendations for these nutrients during pregnancy.

In order to reduce and/or prevent anaemia in pregnant women, the Department of Health (DOH) introduced a policy some time ago making it compulsory for iron and folate supplements to be given routinely to all pregnant women attending antenatal clinics in South Africa. In view of this, the overall aim of the present study was to evaluate the effectiveness of the implementation of this programme by measuring the prevalence of anaemia and associated nutritional deficiencies in third-trimester pregnant women.

Methods

Subjects

The study was undertaken in villages situated in the central region of Limpopo province. The villages are predominantly rural with poor infrastructure, lack of electricity, inadequate sanitation, poor roads and poorly equipped schools.[19] Subjects were third-trimester pregnant women attending antenatal clinics at eight different local clinics situated within a 30 km radius around the referral hospital (Mankweng Hospital). The clinics routinely dispense iron (as ferrous sulphate, 175 mg/day) and folate (as folic acid, 5 mg/day) supplements to pregnant women.

All pregnant women in the third trimester (28 - 36 weeks) who attended antenatal clinics were recruited over a 2-year period: 152 women during the period May - August 1999 and 124 women during February - April 2000, comprising a total of 276 women. Written consent was obtained from each participant before commencement of any tests.

Ethical approval and permission to undertake the study were obtained from the Ethics Committee of the University of the North and the Limpopo Province Department of Health and Welfare's Research Committee.

Socio-demographic characteristics and medical histories

A questionnaire was administered to each participant during a personal interview conducted by a trained fieldworker to obtain data on the socio-demographic profile (age, educational level, marital status and occupation) and medical history of the participants (parity, previous miscarriages, time of first visit and number of visits to the clinics and/or private practitioners).

Anthropometric and blood pressure measurements

Maternal weight was measured to the nearest 0.1 kg using a beam balance weighing scale. Subjects wore light clothes and were barefoot. Height was measured to the nearest 0.1 cm using a horizontal measuring tape mounted on a board with a flat sliding headpiece. The women stood on a flat surface against a wall. Body mass index was calculated as weight/height^2.[20] Blood pressure (BP) was determined using a mercury manometer, with participants in a sitting position. Patients rested quietly for 5 minutes before BP was measured. BP measurements were repeated twice (3 minutes apart) and the average was calculated and used in the analysis.

Blood collection and analysis

Fasting serum and plasma samples were collected from the women via venepuncture. After determination of the full blood count (FBC) from the plasma samples, the blood was centrifuged and stored at –70°C for further analyses. Analyses included ferritin, vitamin B_{12} and folate. Red cell folate (RCF) was determined from an aliquot of the whole blood, which was lysed and then stored at –70°C. Serum ferritin, vitamin B_{12}, folate, and RCF were determined using the Access ImmunoAssay (Beckman), whereas haemoglobin and red cell indices (FBC) were determined using a Coulter STKS analyser (Beckman). RCF was then calculated as haemolysed folate x 21/(haematocrit/100).

Anaemia was considered present in the pregnant women when their haemoglobin levels were below 10.5 g/dl. This cut-off point was set to account for the known haemodilution that occurs in pregnancy. Women with ferritin levels between 12 µg/ml and 20 µg/ml were said to be moderately iron depleted, while those with levels below 12 µg/ml were described as being severely iron depleted. Women were considered to have iron deficiency anaemia if they had low haemoglobin levels accompanied by severe iron depletion. Vitamin B_{12} deficiency was said to be present in the women when the levels were below 145 pg/ml and serum folate and RCF deficiencies were said to be present if the levels were below 3 ng/ml and 157 ng/ml respectively.[17,21-23] These cut-off values are shown in Table I.

Data analysis

Data were analysed using SPSS for Windows.[24] Statistical significance was set at a probability level of 0.05. Variables that were not normally distributed were log transformed before being analysed. Differences between categorical variables were analysed using χ^2 while for continuous variables both analysis of variance (ANOVA) and the independent *t*-test were used.

Pearson's correlations were done in order to investigate an association between blood variables and number of clinic visits. Odds ratios were computed to assess the effects of socio-demographic variables on the anaemic status of the women.

Results

Two hundred and seventy-six women were enrolled in the study. Fourteen failed to attend all sessions, giving a participation rate of 95%. Socio-demographic characteristics, and anthropometric and blood pressure measurements are given in Tables II and III.

There were no differences in maternal age, marital status, educational level, employment status, parity and number of previous miscarriages between the anaemic and non-anaemic participants. A large percentage of the study sample had at least a secondary level education (86.6%). It was also found that most of the women were single when they became pregnant. Although the anaemic group tended to have a higher parity than the non-anaemic women, this difference was not significant. On average the first visit to an antenatal clinic was during the second trimester. As indicated in Table II few women went to a private general practitioner (11.6%), and those who did attended once on average, usually in the first trimester. The mean (± standard deviation (SD)) number of clinic visits during pregnancy was 4.06 ± 2.18. There were no reported differences in clinic attendance between the anaemic and non-anaemic participants. However maternal weight was found to be lower ($p = 0.051$) in the anaemic than non-anaemic women.

Biochemical parameters (Table I)

Iron deficiency was present in 50.9% of the participants; 26.4% were severely iron depleted (ferritin levels < 12 µg/ml) and 24.5% were moderately depleted (ferritin levels between 12 and 20 µg/ml). Vitamin B_{12}

Table I. Iron, vitamin B_{12} and folate levels of anaemic and non-anaemic women and percentage of women below reference values

		Total		Anaemic (N = 43)		Non-anaemic (N = 219)		
Variable	**Cut-off values**	**% below cut-offs**	**Mean ± SD**	**% below cut-offs**	**Mean ± SD**	**% below cut-offs**	**Mean ± SD**	***P*-value**
Ferritin (µg/ml)	<12 12 - 20	26.4 24.5	25.77 ± 28.58	62.8 16.3	16.66 ± 19.00	21.1 27.5	27.63 ± 29.87	<0.0001
Haemoglobin (g/dl)	< 10.5	16.4	11.85 ± 1.66	–	9.25 ± 1.34	–	12.36 ± 1.17	–
Vitamin B_{12} (pg/ml)	<145	16.4	242.87 ± 115.84	18.6	258.47 ± 135.46	16.1	239.46 ± 111.82	0.921
Serum folate (ng/ml)	<3	10.3	8.34 ± 5.05	25.6	6.25 ± 4.22	7.3	8.78 ± 5.12	0.001
Red cell folate (ng/ml)*	<157	4.6	481.16 ± 283.19	4.7	456.11 ± 282.32	4.6	488.72 ± 284.68	0.595

*Red cell folate analysed in 175 subjects in the total study population, 36 in the anaemic group and 139 in the non-anaemic group.

Table II. Characteristics of third-trimester pregnant women in the study (mean ± SD)

Characteristic	Total (N = 262)	Anaemic (Hb < 10.5 g/dl) (N = 43)	Non-anaemic (Hb > 10.5 g/dl) (N = 219)	p-value
Age (years)	25.67 ± 7.02	26.91 ± 8.31	25.42 ± 6.74	0.279
Height (m)	1.58 ± 0.64	1.58± 0.61	1.59 ± 0.64	0.372
Weight (kg)	68.23 ± 11.75	64.96 ± 11.19	68.78 ± 11.80	0.051
BMI (kg/m^2)	27.13 ± 4.37	26.05 ± 3.78	27.33 ± 4.46	0.078
Systolic blood pressure (mmHg)	113.59 ± 12.05	112.21 ± 11.89	113.86 ± 12.12	0.441
Diastolic blood pressure (mmHg)	70.86 ± 10.07	68.47 ± 10.54	71.29 ± 9.97	0.133
Parity	1.51 ± 1.87	2.00 ± 2.10	1.41 ± 1.82	0.092
First visit (months)*	4.67 ± 1.84	5.00± 2.08	4.59 ± 1.78	0.242
First clinic visit	5.25 ± 1.58	5.51± 1.74	5.19 ± 1.54	0.276
First doctor visit	1.56 ± 2.24	1.09 ± 2.06	1.67 ± 2.27	0.111
Appointments†	4.95 ± 2.61	4.58 ± 2.57	5.03 ± 2.62	0.303
Visits to clinic	4.06 ± 2.18	3.88 ± 1.95	4.09 ± 2.22	0.536
Visits to doctor	0.95 ± 1.69	0.77 ± 1.46	0.99 ± 1.73	0.387

*Time at which the clinic or private doctor was first consulted.
†Total number of visits to both clinic and private doctor.
SD = standard deviation; BMI = body mass index.

Table III. Percentage distribution of subjects by selected demographic characteristics

Variable	Percentage: Total (N = 262)	Anaemic (N = 43)	Non-anaemic (N = 219)	χ^2
Maternal age				
Less than 20 years	30.5	32.6	30.3	
21 - 30 years	45.8	34.9	47.4	0.136
Above 30 years	23.7	32.6	22.0	
Marital status				
Single	67.2	60.5	68.5	0.124
Married	32.8	39.5	31.5	
Parity				
0	44.7	39.5	45.9	0.230
1 - 3	40.8	37.2	41.3	
4	14.5	23.3	12.8	
Miscarriages				
Yes	12.2	11.6	12.4	0.700
No	87.8	88.4	87.6	
Educational level				
Primary	13.4	18.6	12.4	0.447
Secondary or further	86.6	81.4	87.6	
Occupation				
Working	11.1	11.6	11.0	0.969
Currently unemployed	36.3	37.2	29.8	0.120
Housewife	21.4	11.6	23.4	0.147
Student	36.3	39.5	35.8	0.935

and both serum and RCF deficiencies were less common (16.4%, 10.3% and 4.6% respectively).

The prevalence of anaemia (haemoglobin < 10.5 g/dl) was 16.4%. Iron deficiency (ferritin < 20 µg/ml) was the most common deficiency in the anaemic subjects (79.1%) followed by folate and vitamin B_{12} deficiencies (25.6% and 18.6% respectively). In the non-anaemic group 48.6% of the women presented with iron deficiency, while vitamin B_{12} deficiency was found in 16.1% and folate deficiency in 7.3%.

The anaemic group had significantly lower serum folate (p = 0.001) and ferritin levels (p < 0.0001) than the non-anaemic group, but RCF levels were not different between the two groups (Table I).

In these women the means (SD) for mean cell/volume (MCV), mean corpuscular haemoglobin (MCH) and haematocrit (Hct) levels in the total group were 89.46 fl (8.23 fl); 30.18 pg (6.20 pg) and 36.09% (5.28%) respectively. There were significant differences in all three parameters, with lower levels observed in the anaemic group. The mean levels in the anaemic v. non-anaemic women were: MCV 84.93 fl (11.55 fl) v. 90.40 fl (7.08 fl), p = 0.004; MCH 27.17 pg (4.96 pg) v. 30.76 pg (6.27 pg), p = 0.0001; and Hct 30.09% (4.86%) v. 37.30% (4.50%).

Correlations

In the anaemic group positive significant correlations were observed between vitamin B_{12} and first visit to a health facility (either the clinic or a private doctor) (r = 0.325, p = 0.033) and also between serum folate and both maternal age (r = 0.416, p = 0.005) and parity (r = 0.481, p = 0.001). In the non-anaemic group negative correlations were observed between serum folate and both age (r = –0.183, p = 0.007) and time of first visit to the clinic (r = –0.145, p = 0.034) and also between RCF and the time of first visit to the clinic (r = –0.261, p = 0.002), suggesting that folate supplementation as given at the antenatal clinics is effective especially in those women who go there early in pregnancy.

The association between anaemic status and socio-demographic characteristics was investigated by looking at relative risk for anaemia (Table IV). From the

Table IV. Association between anaemic status and socio-demographic variables (unadjusted and adjusted for age) (*N* = 262)

			Unadjusted			Adjusted for age		
Exposure variable	**Level**	***N***	**Odds ratios**	**95% CI**	**Chi-square *p*-value**	**Odds ratios**	**95% CI**	**Chi-square *p*-value**
Age (yrs)	< 20	80	1					
	20 - 30	120	0.516	0.140 - 1.910	0.174			
	> 30	62	0.705	0.138 - 3.619	0.675			
Marital status	Married	86	2.108	0.876 - 5.073	0.095	2.177	1.844 - 2.570	< 0.0001
	Single	175	1					
Parity	0	117	1			1		
	1 - 3	107	2.389	0.612 - 9.318	0.209	1.712	1.385 - 2.117	< 0.0001
	4	38	5.250	0.959 - 28.74	0.056	5.461	4.278 - 6.973	< 0.0001
Miscarriage	Yes	32	0.989	0.346 - 2.822	0.407	0.905	0.749 - 1.092	0.297
	No	230	1					
Education code	Primary	35	0.694	0.256 - 1.880	0.472	0.925	0.763 - 1.122	0.429
	Secondary	227	1					
Electricity	Yes	73	1.424	0.707 - 2.868	0.323	1.383	1.207 - 1.586	< 0.0001
	No	189	1					
Working	Yes	29	4.52	0.551 - 37.047	0.160	5.332	3.524 - 8.068	< 0.0001
	No	233	1					
Currently unemployed	Yes	85	8.428	1.213 - 58.565	0.031	14.061	9.650 - 20.489	< 0.0001
	No	177	1					
Housewife	Yes	56	0.080	0.338 - 12.455	0.434	3.370	2.375 - 4.783	< 0.0001
	No	206	1					
Student	Yes	93	11.43	1.227 - 106.517	0.032	20.247	13.070 - 31.367	< 0.0001
	No	169	1					

table it can be seen that mothers who were students had an 11.43 higher risk of being anaemic than those who were not students (CI: 1.23 - 106.52), while for those who were unemployed the risk was 8.43 compared with those who were employed (CI: 1.21-58.56). After adjusting for age it did not matter what the occupational level of the mother was, as all variables became significant. Being married, with an increased number of children and presence of electricity in the household presented as risk factors for anaemia.

Discussion

The results of this study imply that the DOH policy of supplementation may be reaping some rewards. The prevalence of anaemia (16.4%) is generally far lower than reported in most studies on pregnant women undertaken in South Africa, particularly in a poor province such as Limpopo.[2-4, 25] The prevalence is also lower than figures from other sub-Saharan countries (22 - 69%). It should be kept in mind that in many African countries malaria contributes significantly to the higher prevalences, whereas malaria is not common in the study area. Additionally, many studies used a higher cut-off value of 11.0 g/dl. Application of this reference value (Hb < 11 g/dl) increases the prevalence of anaemia in the study area to 23.2%. However as inflammations and infections can increase ferritin levels in the body,[26,27] the prevalence of iron deficiency and iron deficiency anaemia may be underestimated in this study.

Similar to several studies of pregnant women worldwide,[3,4,28-31] the most common deficiency found in anaemic women in this study was iron deficiency, with 62.8% of anaemic women in this study having severe iron depletion (ferritin levels below 12 µg/ml), which according to the Centers for Disease Control criteria signifies iron deficiency anaemia.[32] This value converts to approximately 10% of all the pregnant women studied. The high prevalence of iron deficiency in anaemic pregnant women is in accord with the findings of earlier South African studies where the prevalence of iron deficiency in anaemic women was 60%.[4,33] However these values are far higher than the 25% found by Kruger and colleagues[3] in their study conducted in Cape Town. Since we could not get information on the duration of supplementation in these women it can be assumed that on average supplementation started towards the end of the second trimester, as shown by the mean first visit to the clinic (Table II). This assumption is based on the fact that supplementation is compulsory for all pregnant women attending antenatal clinics, more so as there were no differences between the anaemic and non-anaemic pregnant women.

In studies done in Northern Province (now known as Limpopo province) it was found that 48% of pregnant women in the third trimester had folate deficiency[25] and Ubbink and his group[34] found that in the same area folate deficiency was also present in 21% of non-pregnant women. In the present study a prevalence of 10% was found using serum folate. But on a subset of samples where RCF was determined, the prevalence of folate deficiency is much lower at 4.6%. This low prevalence of folate deficiencies contrasts with previous findings in Limpopo province where high prevalences of folate deficiency were found in both pregnant women and women of childbearing age. Possible explanations include increased dietary and supplemental folate (5 mg/day as given by the antenatal clinics) intake among the participants in our study. Additionally it should be kept in mind that the study was undertaken in a rural area where the population relies mainly on subsistence farming and as such may have regular access to leafy vegetables known to be a rich dietary source of folate. This theory is supported by the findings of Koebnick and colleagues[35] who found that long-term consumption of vegetables leads to improvement in folate status and a decreased folate deficiency during pregnancy.

The supposition regarding a regular satisfactory intake of dietary folate is unlikely since Steyn *et al.*[36] found that on average women's dietary intake of folate was below the recommended daily allowance in this area. It was also observed that consulting the clinic early in pregnancy and regular visits for antenatal care were associated with higher levels of both serum folate and RCF. This finding suggests that the folate supplements given to pregnant women are effective as a means of reducing levels of folate deficiency in pregnancy.

The findings with regard to folate supplementation do not, however, apply to the iron status of the pregnant women since high percentages of iron deficiency were seen in both the anaemic and non-anaemic women. This might be explained by what has been shown in several studies, namely that ferrous sulfate, the iron supplement given to pregnant women, causes nausea after ingestion.[2,17,37,38] Consequently it is possible that women only took the folate supplement, and left the iron supplement, fearing the outcome of taking them. Since we did not monitor the supplement usage in these women it is difficult to say this with certainty. Zavaleta and colleagues[39] attributed low supplement compliance in pregnancy to: *(i)* inadequate patient motivation; *(ii)* low motivation of health personnel; *(iii)* poor access to health services; *(iv)* adverse effects; and *(v)* inadequate supply of supplement tablets. Other possible contributing factors may be: *(i)* that the women were already deficient before conception and as such the supplements are inadequate to return iron levels to normal; *(ii)* most women went to the clinics in the second trimester and the mean number of clinic visits during pregnancy was 4.1 (SD 2.18), so the time might not have been sufficient for iron stores recovery to occur; and *(iii)* the women might be taking the pills within a diet high in phytates and tannins, known to reduce iron absorption.

Vitamin B_{12} deficiency is rare in pregnancy except in populations that are mainly vegetarian.[40] In the present study the prevalence of vitamin B_{12} deficiency was found to be 16.4%, with no significant differences between the anaemic and non-anaemic groups (Table I). These findings suggest that the study population did not have a diet with rich sources of vitamin B_{12}. This supposition is supported by a study done by Steyn *et al.*[36] where none of the top 10 commonly eaten foods were really rich in vitamin B_{12}.

Several studies have shown that some socio-demographic characteristics have an effect on the prevalence of anaemia.[41, 42] Young age has been shown to be associated with poor nutrient intakes, often including poor micronutrient intake.[43,44] Inadequate nutrient intake is also known to be compounded by parity, and poor education has been shown to influence the type of diet consumed by pregnant women.[42] In the present study, however, we found that women who were married, with increased parity and with electricity in their households were more likely to be anaemic. On the other hand age and educational status did not seem to influence the women's anaemic status. This might have resulted from the fact that the study population comprised mainly young adults who had a secondary level of education (Table III).

In conclusion, comparing the present study with earlier studies shows that anaemia is now less prevalent among pregnant women in the study area. The main cause might be iron deficiency due to an inadequate dietary intake, despite dispensing of iron supplements by the antenatal clinics. The situation with regard to anaemia and iron deficiency has to be interpreted cautiously, bearing in mind the lack of information on: *(i)* the burden of infectious diseases in the studied area; *(ii)* the actual time at which supplementation was started as well as duration of intake and compliance on the part of the women; and *(iii)* the knowledge of health workers on the importance of supplementation, and the quality of their training in this regard.

Also of note is the high prevalence of vitamin B_{12} deficiency, which might be due to the mainly vegetarian diet consumed. It is therefore recommended that nutritional education for pregnant women be a priority at health care centres in this area in order to inform women about foods rich in iron (especially haeme iron, which is the most readily absorbed form of iron) and vitamin B_{12}.

On the other hand, if we compare our findings with those of Baynes and colleagues[25] and Ubbink *et al.*,[34] the prevalence of folate deficiency seems to be declining over time. This shows that with respect to folate supplementation health care services are effective, and as such it is recommended that greater effort be made to ensure that this steady progress is also effective with regard to iron supplementation and vitamin B_{12} intake. To further clarify the situation, studies should look at availability/dispensing of supplements at health care centres and focus on the compliance of women with regard to these supplements.

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