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Serum Folate and Vitamin B12 Levels among Pregnant Women Attending Antenatal Clinic in Sokoto North Western Nigeria

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Abstract: *Pregnancy is the fertilization and development of one or more offspring, known as an embryo or foetus in a woman's uterus. Pregnancy is a critical stage of development during which maternal nutrition can strongly influence obstetric and neonatal outcomes. American Congress of Obstetricians and Gynaecologist recommend that pregnant women take a variety of food including vitamins. Folate and vitamin B12 deficiencies have adverse effects on pregnancy outcomes. It is also associated with increased maternal morbidity and mortality. The aim of this case-control study was to determine the effect of pregnancy on the serum folate and vitamin B12 levels among pregnant subjects. The study included 74 pregnant women and 22 non-pregnant controls. Serum folate, vitamin B12 levels were determined using ELISA kits (Melsin Medical China). The mean age and income of the subjects were 28.00±8.295 years and 2760.42±7975.178 naira respectively. Folate and vitamin B12 levels were significantly higher among the pregnant subjects (25.2 ± 0.81 and 680.0 ± 81.0) compared to controls (17.0 ± 2.71 and 489.7 ± 50.2) p= 0.000 and 0.049 respectively. Folate and vitamin B12 level was compared based on trimester. The Folate and B12 levels were significantly higher among pregnant women in the 1st trimesters (28.1 ± 0.80 and 1170.3 ± 188.64) respectively compared to women to the second and 3rd trimester (p=0.000). This study has shown that the Folate and Vit B12 is higher among pregnant women compared to non-pregnant controls and that the levels are higher in pregnant women in the first trimester. This finding re-emphasizes the advocacy for supplementation of vitamins during pregnancy particularly among pregnant women of African descent. There is need for the implementation of a policy on the mandatory vitamin and micronutrient fortification of food in Nigeria.*

Keywords: *Serum Folate, Vitamin B12 Levels, Pregnancy, Antenatal Clinic, Sokoto, Nigeria*

1. Introduction

Pregnancy is a critical stage of development during which maternal nutrition can strongly influence obstetric and neonatal outcomes [1, 2]. Optimal nutrition is necessary to maintain the health of the mother, to help ensure a normal, healthy delivery, reduce the risk of birth defects, sub-optimal foetal development and chronic health problems in childhood [3]. Poor nutritional status and sub-optimal pre- and antenatal care are common in developing countries, often resulting in pregnancy complications and poor obstetric outcomes [4]. Pregnant women in Sub-Saharan Africa (SSA) are at particular nutritional risk as a result of poverty, food insecurity, political and economic instabilities, frequent infections, and frequent pregnancies [5]. The main nutritional issues impacting these women include maternal under- and deficiencies of key pregnancy micronutrients. Consequently, poor obstetric outcomes, such as anaemia, neural tube defects (NTDs), rickets, low birth weight (LBW) and maternal and neonatal mortality, are common in SSA. SSA is a region of intensive migration prompted by adverse economic, political and ecological conditions. Consequently, SSA immigrants represent a sizeable and growing immigrant population in many Western countries. For example, of the 4.6 million recorded Africans living in the European Union, at least one-third are from SSA and approximately 250,000 of these immigrants are living in the UK [6]. Several studies have reported that pregnant women of African origin are one of the immigrant groups at highest risk of pregnancy complications, such as hypertension and diabetes [7], and adverse birth outcomes, including preterm delivery, low birth weight infants, caesarean delivery and perinatal mortality [8-9]. Although the exact causes of such outcomes have not yet been clearly identified, it is possible that poor premigration health and nutritional status, high parity, closely-spaced pregnancies, pre-existing diseases and lower socioeconomic status in the host countries are contributing factors [8]. However, it should be noted that the majority of African migrants to Western countries are from the higher socioeconomic groups and thus, one would expect them to be healthy and well-nourished compared to the general populations of their native countries. In such cases, adoption of typical Western diets and lifestyles may worsen the nutritional status of immigrant SSA women [10] by predisposing them to obesity, which carries its own risk for pregnancy complications [11].

Pregnancy is a period of increased metabolic demands, with changes in the woman's physiology and the requirements of a growing foetus [12]. During this time, inadequate stores or intake of vitamins or minerals, referred to collectively as micronutrients, can have adverse effects on the mother, such as anaemia, hypertension, complications of labor and even death [13]. Furthermore, the foetus can be affected, resulting in stillbirth, pre-term delivery, intrauterine growth retardation, congenital malformations, reduced immunocompetence and abnormal organ development. The essential nature of micronutrients has been recognized through the identification of clinical conditions associated with severe deficiencies of particular vitamins or minerals, and through subsequent animal experiments.

American Congress of Obstetricians and Gynaecologist recommend that pregnant women take a variety of food including vitamins. Vitamins are organic substances required by the body in minute quantities to maintain life and health of the pregnant woman and her developing foetus [14]. They act as catalysts in the formation of hormones, enzymes, blood cells, neurotransmitters, and genetic material. They are essential to complete the metabolism of carbohydrates, proteins, and fats. The body's need for vitamins is met by the diet. The fat-soluble vitamins, A and E have antioxidant properties and as such either block the initiation of free radical formation or inactivate (scavenge) free radical [15].

Pregnant women are particularly vulnerable to deficiencies in micronutrients and vitamins because of the increased metabolic demands imposed by pregnancy [16]. Folate, and vitamin B12 deficiencies have adverse effects on pregnancy outcome. It is also associated with increased maternal morbidity and mortality [17]. Folate deficiency during pregnancy is associated with low birthweight and neural tube defects [18]. Strong associations of vitamin B12 status with intrauterine growth retardation have been documented [19-20]. A previous report indicated that 67.7%, 26.3%, and 74.1% of the women had poor iron, folate, and vitamin B12 stores, respectively [21]. Folate is a B vitamin that is needed for healthy growth and development. Folic acid is important for pregnancy, as it can help to prevent birth defects known as neural tube defects, including spina bifida. Folate deficiency is one of the micronutrient deficiencies of global public health concern, especially among women of child bearing age [22]. Folate deficiency in pregnant women increases the risk of neural tube defects (NTDs), premature births, intrauterine growth retardation, congenital heart defects and oro-facial cleft defects in newborns [23-24]. There is paucity of data on folate and vitamin B12 levels among pregnant women in Sokoto, North Western Nigeria. This study was undertaken to determine the serum folate and vitamin B12 levels among pregnant women attending antenatal care in Sokoto, North-West Nigeria.

2. Materials and Methods

2.1. Study Area

This study will be conducted at the Specialist Hospital Sokoto (SHS), North-West Nigeria. The hospital is a secondary health institution located in Sokoto metropolis committed to the provision of quality healthcare services to people in Sokoto State and its environ. The state is located between longitudes 11° 30ʺ to 13° 50ʺ East and latitude 4° to 6° North. It has a land area of about 28,232.37sq kilometer and stands at an altitude of 272 m above sea level near to the confluence of the Sokoto River and the Rima River. Sokoto state is at the extreme Northwest of Nigeria forming a border with Niger Republic. The state is in the dry Sahel surrounded by sandy terrain and isolated hills with an average annual temperature of 28.3°C (82.9°F). The weather is characterized by two seasons the wet and dry seasons. Rainfall (wet season) starts late around June and ends in September sometimes extending into October. The average annual rainfall is 550 mm with peak rainfall usually recorded in the month of August. The highest temperatures of 45°C during the hot season are experienced in the months of March and April. Harmattan, a dry cold and dusty condition is experienced between the months of November and February. Sokoto state had a population of 4.2million as at the 2006 census. The metropolis is estimated to have a population of 427,760 people 19 made up of Hausa and Fulani majority and a minority of

Zabarmawa and Tuareg and other non-indigenous settlers. The two major languages in the state is Hausa and Fulfulde are spoken among the Fulani. The main occupation of the people is grain production and animal husbandry. Majority of the indigenous people practice agriculture. Crops produced include commercial crops like millet, sorghum, beans, rice and maize. Other occupations commonly practiced are dying, blacksmithing, weaving, carving, trading, and cobbling. Sokoto ranks second in livestock production in Nigeria. Modern Sokoto city is a major commercial center in leather crafts and agricultural products. Occupation of city inhabitants also include trading, commerce, with a reasonable proportion of the population working in private and public sectors.

2.2. Study Design

The study population for this case-control study consist of 74 pregnant women patients and 22 non-pregnant controls. The study population will be recruited from among pregnant women attending the antenatal clinic at Specialist Hospital Sokoto (SHS).

2.3. Sampling Techniques

2.3.1. Inclusion Criteria

All consenting legal adult (>18years) pregnant woman who are attending antenatal clinic without any history of hypertension and diabetes mellitus were consecutively recruited into the study.

2.3.2. Exclusion Criteria

Non-consenting and non-legal adults (<18years) pregnant woman and those with history of hypertension and diabetes mellitus attending antenatal clinic will be excluded from participating in the study.

2.3.3. Sample Size Estimation

Using the formula $n=Z^2pq/d^2$

Where n= minimum required sample size in a population >10,000

Z= standard normal deviation

P= proportion of success or prevalence

q = proportion of failure (1-p)

d= precision, tolerable margin of error, expected difference

Documented prevalence of 15% from the previous study was used. Consecutive recruitment of all consenting pregnant women was done to prevent bias. A structured questionnaire will be used to collect socio-demographics and obstetric-related data from subjects.

$n = Z^2pq/d^2$

Z = 95% (1.96)

P = 15% (0.15)

Q = 1 – 0.15 = 0.85

D = 5% (0.05)

Therefore n = (1.96)2 x 0.15 x 0.85 / (0.05)2

= 195.9

Sample size = 196

2.4. Study Instrument

2.4.1. Study Participation Consent Form

Written informed consent will be obtained from all participants in the study according to the Declaration of Helsinki. Example of the informed consent form for use in this study is included below.

2.4.2. Determination of Serum Folate and B12

Vitamin B_{12} levels and serum folate levels were determined using Enzyme Linked Immunosorbent Assay (ELISA) (Melsin Medical, China). The principle of the assay is based on delayed competitive enzyme immunoassay.

2.4.3. Statistical Analysis

The data obtained will be analysed using SPSS version 20 (SPSS Inc., Chicago, IL., USA, 2011). The result will be expressed as percentage and Mean + SD. Comparison will be made using analysis of variance (ANOVA), paired comparison will be carried out using the student t-test and a p-value of equal to or less than 0.05 ($p<0.05$) will be considered as significant.

3. RESULTS

This case-control study investigated the serum folate and vitamin B12 levels among 74 consecutively -recruited pregnant women aged 18 to 44 years and mean age and mean income of 28.00 ± 8.295 years and 2760.42± 7975.178 naira respectively. Twenty-two non-pregnant age-matched women were monitored as controls. Table 1 shows the mean values of some socio-demographic variables among the subjects. Table 2 shows the distribution of the subjects based on obstetric (trimester, parity) and socio-demographic variables (ethnicity, educational status and age). Table 3 shows the mean values of vitamins among pregnant subjects and non-pregnant controls. The serum folate and Vit. B12 levels was significantly higher among the pregnant subjects compared to the non-pregnant controls (25.2 ± 0.81 and 680.0 ± 81.0 versus 17.0 ± 2.71 and 489.7 ± 50.2), p=0.000 and 0.049 respectively. The mean folate and vitamin B12 levels among pregnant subjects were compared based on trimester. There was a statistically significant variation in the folate and Vit.B12 levels of the pregnant subjects based on trimester (p =0.000).

Table1. *Mean Values of Socio-demographic Data of subjects*

Parameter	Mean
Age (Years)	28.00±8.295
Income (Naira)	2760.42± 7975.178

Table2. *Distribution of Subjects based on socio-demographic and obstetrics indices of subjects*

Parameter	frequency	%
Trimester		
First	25	33.78
Second	25	33.78
Third	24	32.44
parity		
Prima	39	40.6
Para	14	14.6
Multi Para	43	44.8
Ethnicity		
Hausa	59	61.5
Fulani	11	11.5
Igbo	3	3.1
Yoruba	9	9.4
Others	14	14.6
Educational Status		
Non - Formal	36	37.5
Primary	12	12.5
Secondary	38	39.6
Tertiary	10	10.4
Age (Years)		
<24	31	41.89
25-34	34	45.95
35-44	9	12

Table3. *Mean Values of vitamins among pregnant subjects and non-pregnant controls*

Parameter	Mean Values of Subjects	Mean Values of Controls	t-value	p-value
Folate (µmol/L)	25.2 ± 0.81*	17.0 ± 2.71	-3.928	0.000*
Vitamin B12	680.0 ± 81.0*	489.7 ± 50.2	-1.997	0.049*

Table 4 shows the Mean Values of vitamins among pregnant subjects based on Trimester. The folate level was compared among the pregnant subjects based on parity, marital status age and type of marriage. The folate level was significantly higher among multiparous women (p=0.010), single (p=0.015), younger women (24-35 years old) (p=0.042) and those in monogamous marriage (p=0.013).

Table 5 shows the effects of socio-demographic and obstetric variables on the serum folate.

The vitamin B12 level was compared among the pregnant subjects based on marital status, type of marriage and age. The B12 level was significantly higher among married women (p=0.38), those in monogamous relationship (p=0.045) and younger women (< 24 years).

Table 6 shows the effects of socio-demographic and obstetric variables on the serum vitamin B12 level of subjects.

Table4. *Mean Values of vitamins among pregnant subjects based on Trimester*

Parameter	1st Trimester	2nd Trimester	3rd Trimester	F-value	p-value
Folate (µmol/L)	28.1 ± 0.80*	23.3 ± 1.57*	23.9 ± 1.61*	6.888	0.000*
Vitamin B12	1170.3 ± 188.64*	436.0 ± 57.80	391.0 ± 38.04	11.697	0.000*

Table5. *Effect of socio-demographic and Obstetric variables on the level of serum folate*

Factor	Mean ± SD	F	p-value
Trimester			
1st	28.13 ± 4.14	0.072	0.001
2nd	23.44 ± 7.54		
3rd	25.05 ± 6.79		
Parity			
Prima	21.87 ± 8.85	27.333	0.010
Para	24.97 ± 6.45		
Multi Para	26.66 ± 5.55		
Marital status			
Single	29.55 ± 2.58	7. 411	0.015*
Married	25.38 ± 6.61		
Type of Marriage			
Monogamous	25.95 ± 6.32	26.845	0.013*
Polygamous	23.83 ± 7.40		
Ethnicity			
Hausa	26.74 ± 5.34	14.619	0.099
Fulani	22.35 ± 8.44		
Igbo	22.43 ± 14.81		
Yoruba	25.45 ± 4.03		
Others	25.12 ± 7.21		
Level of Education			
None	26.64 ± 7.24	32.612	0.052
Primary	26.45 ± 4.31		
Secondary	24.96 ± 6.41		
Tertiary	24.06 ± 7.93		
Age (Years)			
<24	25.19 ± 6.37	29.195	0.042*
25 – 34	26.02 ± 6.25		
35 – 44	24.98 ± 8.64		

Table6. *Effect of socio-demographic and obstetric variables on the serum Vitamin B12 levels of subjects*

Factor	Mean ± SD	F	p-value
Trimester			
1st	1138.30 ± 968.10	0.072	<0.001**
2nd	497.72 ± 412.16		
3rd	391.98 ± 186.37		
Parity			
Prima	478.16 ± 262.21	32.736	0.203
Para	676.70 ± 646.43		
Multi Para	734.12 ± 781.92		
Marital status			
Single	358.87 ± 113.92	10.051	0.038*
Married	693.40 ± 708.06		
Type of Marriage			
Monogamous	736.81 ± 753.92	44.395	0.045*
Polygamous	435.69 ± 259.82		
Ethnicity			
Hausa	785.39 ± 804.34	16.173	0.103
Fulani	398.47 ± 206.36		
Igbo	251.65 ± 207.66		
Yoruba	616.23 ± 588.52		
Others	709.48 ± 676.10		
Level of Education			
None	709.48 ± 777.10	0.466	0.76
Primary	581.50 ± 582.80		
Secondary	695.88 ± 674.72		

Tertiary	672.06 ± 811.30		
Age (Years)			
<24	803.38 ± 854.633	42.590	0.020*
25 – 34	650.35 ± 600.66		
35 – 44	365.75 ± 170.70		

4. Discussion

This study investigated the level of serum folate and Vit B12 levels among pregnant women of African descent in Sokoto, North Western Nigeria. The folate and vitamin B12 levels were significantly higher among the pregnant subjects compared to controls (p=0.001). This finding may be because antenatal women in Nigeria are offered folic acid supplementation. Our findings are consistent for a population of pregnant women seeking ANC following implementation of mandatory peri-conceptional folic acid fortification. In South Africa, the prevalence of folate deficiency among non-pregnant rural women of child bearing age was found to have reduced from 27.9% to 0% nine months after mandatory fortification of maize and wheat flour was introduced [25]. In Canada, a national survey conducted following the start of mandatory fortification reported a prevalence of 1% among the Canadian women of reproductive age [26]. However, our findings differ from studies in three African countries conducted before mandatory fortification including a community based study in Ethiopia among women of child bearing age which found 46% of women deficient [27], a study in Benin among HIV negative pregnant women at the time of first ANC visit which found 31% of women folate deficient [28] and a study conducted in Eastern Sudan among pregnant women that found 57.7% of women folate deficient [29]. A Hungarian randomized controlled trial conducted in 5,453 well-nourished women found that peri-conceptional consumption of a multivitamin/mineral supplement that contained 800 µg of folic acid reduced the incidence of first occurrence NTDs by about 90% compared to a supplement containing only copper, manganese, zinc, and vitamin C [30]. Multivitamin/mineral supplements marketed in the US commonly contain 400 µg of folic acid, and many prenatal supplements marketed in the US contain 800 µg of folic acid. Folic acid may also be present in the food supply. Several countries now have programs of mandatory folic acid fortification of all enriched grain products. This evidenced – based best practice of peri-conceptional intake of folic acid is known to reduce a woman's risk of having an infant affected by a neural tube birth defect (NTD) [31]. Moreover, folic acid supplementation in the form of a daily multivitamin may be more effective in reducing NTDs than when used alone [30]. Doses of greater than 1 mg/day of folic acid are used pharmacologically to treat hyperhomocysteinemia and to prevent reoccurrence of NTDs [30]. Women who have had a previous NTD-affected pregnancy may be advised to consume up to 4 mg/day (4,000 µg/day) of folic acid if they are planning a pregnancy, but the level of supplementation should be prescribed by their medical provider. Inadequate folate status may also be linked to other birth defects, such as cleft lip, cleft palate, and limb malformations, but the support for these findings is not as clear or consistent as the support for NTDs [32]. However, results of some case-control studies [33] and controlled trials [34] have suggested that periconceptional supplementation with a multivitamin containing folic acid may protect against congenital cardiovascular malformations, especially conotruncal (outflow tract) and ventricular septal defects. Folic acid supplementation of women has significantly enhanced the physiologic reduction in plasma Hcy that occurs during pregnancy, when the supplements were provided during the 2nd and 3rd trimesters [35]. Plasma Hcy appears to respond to supplementation with folic acid up to about 500 to 600g of folic acid per day. In addition to poor B vitamin status, other risk factors for elevated plasma Hcy include a high intake of coffee, smoking and no use of vitamin supplements during pregnancy. More attention needs to be paid to vitamin B-12 status of women during pregnancy and lactation. It has become apparent that there is a high global prevalence of low plasma vitamin B-12 concentrations in infants, children, and adults. A recent review of available data from Latin America revealed that at least 40% of individuals in all age groups studied had low plasma vitamin B-12 [36]. Other reports of a high prevalence of vitamin B-12 deficiency include those among pregnant women in Nepal [37]. The cause of these low plasma vitamin B-12 concentrations is most likely low dietary intake of the vitamin. While it is commonly believed that only strict vegetarians (vegans) are at substantial risk of developing vitamin B-12 deficiency, several studies have revealed that even lacto-ovo vegetarians [38] or individuals who consume low amounts of meat [39] have lower plasma vitamin B-12 and are at greater risk of vitamin B-12 deficiency compared with omnivores. Vitamin B12 status during pregnancy is critical since maternal vitamin B12 deficiency can affect the pregnancy outcome for both mother and the offspring. For women who want to get pregnant, a vitamin B12 deficiency means an increased risk of developing preeclampsia, intra-uterine growth retardation, preterm labor [40]. Children of deficient mothers are at risk for low birth weight [41-42]. Recent studies have also found an association between low vitamin B12 status in mothers and neural tube defect. This suggests an increased risk for birth defects when starting pregnancy with a deficient or inadequate vitamin B12 status, although this association clearly does not prove any causal relationship [40]. The need for folate and vitamin B12 supplementation particularly among African women cannot be over emphasized. Infants who are exclusively breastfed by mothers who have a low dietary intake of vitamin B12 such as vegans and vegetarians are therefore at risk for developing vitamin B12 deficiency possibly influencing cognitive and psychomotor development [43]. A severe vitamin B12 deficiency in the infant can cause neurological symptoms, including irritability, failure to thrive, apathy, anorexia and haematological symptoms such as megaloblastic anaemia [44]. At a later age developmental regression, like impaired growth, gross motor function, poor school performance and other adaptive skills has been suggested to be a consequence of a poor maternal vitamin B12 status during pregnancy [45].

The folate level was compared among the pregnant subjects based on parity, marital status age and type of marriage. The folate level was significantly higher among multiparous women (p=0.010), single (p=0.015), younger women (24-35 years old) (p=0.042) and those in monogamous marriage (p=0.013). Similarly, the vitamin B12 level was compared among the pregnant subjects based on marital status, type of marriage and age. The B12 level was significantly higher among married women (p=0.38), those in monogamous relationship (p=0.045) and younger women (< 24 years). Previous report [46] indicated that serum levels of vitamins decrease with parity while others reported no difference [47-48]. Multiparous women are likely to have depleted vitamin storage from frequent pregnancies at short intervals [46].

In this study we observed that the folate (p=0.001) and B12 levels were significantly higher among women in the first trimester compared to the second and third trimesters. Our finding is consistent with previous reports which indicated that from the first through the third trimester of pregnancy there is a gradual decline in the serum concentration of vitamin B12 [49-50]. Another study performed in a Japanese population reported that homocysteine levels were only significantly higher in the third trimester compared to in the second trimester [51]. The lowest concentration of vitamin B12 is seen at 32 weeks of pregnancy and before delivery it increases again to reach a normal level after birth. The concentration of the active part of vitamin B12, holotranscobalamin, remains unchanged during pregnancy [52]. The reasons for this decline are thought to include; haemodilution, alterations in renal function, hormonal changes, and alterations in the concentration of vitamin B12 binding proteins, and placental transport of vitamin B12 to the foetus. Increased placental and foetal demands are also likely to contribute to this drop, and there is evidence of increased maternal catabolism of folate as pregnancy progresses, coinciding with periods of rapid cell proliferation and equivalent to a turnover of nearly 400 μg per day by the third trimester [53-54]. This doubled folate requirement could result in a pronounced negative folate balance in women who are not on additional folate intake during pregnancy, and may contribute substantially to the development of clinical folate deficiency.

Our finding re-emphasizes the need for supplementation of folate and B12 among pregnant women particularly of African descent. It has been suggested that women should start pregnancy with serum vitamin B12 concentrations of 221pmol/L and that a concentration above 295pmol/L is desirable to minimalize the risk of developing complications [40].

5. Conclusions

This study has shown that the Folate and Vit B12 is higher among pregnant women compared to non-pregnant controls and that the levels are higher in pregnant women in the first trimester. This finding re-emphasizes the advocacy for supplementation of Vitamins during pregnancy particularly among pregnant women of African descent. There is need for the implementation of policy on the mandatory vitamin and micronutrient fortification of food in Nigeria.

Recommendations

This finding re-emphasizes the need for the implementation of universal supplementation of vitamins during pregnancy particularly among pregnant women of African descent. There is need for the implementation of policy on the mandatory vitamin and micronutrient fortification of food in Nigeria. There is need for public enlightenment program to educate pregnant women on the need to maintain a balanced diet containing sufficient amounts of micronutrients and vitamins containing food.

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