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RESEARCH ARTICLE

EFFECTS OF ASCORBIC ACID SUPPLEMENTATION ON THE HAEMATOLOGICAL STATUS OF TEENAGE PREGNANT WOMEN IN IBADAN, NIGERIA

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ABSTRACT

The effect of supplementing a regular diet with ascorbic acid on haematological indices was studied in teenage pregnant women attending antenatal clinics of three government hospitals in Ibadan, Nigeria. Informed consent was obtained from 45 subjects and this formed the sample for the study. The subjects were divided into two groups: experimental group consisting of 30 subjects and control which consisted of 15 subjects. Baseline records of four blood parameters were obtained from all the subjects before each member of the experimental group was given a daily dose of 100mg ascorbic acid supplement for four weeks. The initial records of the haemoglobin levels, haematocrit value, red blood cell count and Plasma Ascorbic concentration of the experimental subjects ranged between 6.4 to 19.6gl 100ml, 24% to 37%, 1.62 x 10^6 to 3.87 x 10^6/mm^3 and 0.00mg/100ml to 1.47mg/100ml respectively. In the control subjects, these range between 10.0 to 19.2g/100ml, 25 to 38%, 2.63 x 10^6 to 4.37 x 10^6/mm^3 and 0.05 to 1.46mg/100ml respectively also. The experimental group that was given a daily ascorbate load of 100mg above their normal dietary intake for four weeks period recorded a general increase in the blood parameters. The haemoglobin level, haematocrit, red blood cell count and plasma ascorbate levels rose from 7.1 to 20.0g/100ml, 30 to 39%, 2.14 to 4.22 x 10^6/mm^3 and 0.20 to 2.06 mg/100ml respectively. The low levels were still recorded in the control group after 4 weeks study period and this ranged between 10.3 to 18.2g/100ml, 25 to 37%, 2.94 x 10^6 to 3.64x$10^{6/}$$mm^3$ and 0.00mg/100 to 1.45mg/100ml in that order as well. The 4.8 x $10^{6/}$$mm^3$ which is the normal standard value of red blood cell and 40 - 47%, which is that of the haematocrit, were not met by all the subjects at the initial record. After 4 weeks of the supplementation (in the experimental subjects), these two values were still not met though there were some appreciable improvements. Generally, the data obtained from the study showed that ascorbate supplementation produced a positive haematological response in teenage pregnant women.

INTRODUCTION

Nutrition and health are inextricably interwoven; there is a great role played by nutrition in major national health policy. No clear-cut separation could be made in the therapeutic and preventive aspect of nutrition. Osamo (1983) opined that there are lots of benefits to be derived from the preventive aspect of nutrition, as the area has not been fully exploited for human health needs. Micronutrient malnutrition is a global problem manifesting in sub-clinical forms, which affects more than two billion individual worldwide (Tina, 1992). Effect of this form of malnutrition is specifically more pronounced in the vulnerable groups among which are pregnant teenagers. Pregnant teenagers are commonly seen in most maternity centers, traditional birth attendants homes, mission (Churches) maternity room etc. Childbearing at any age is a momentous event for a woman. For the teenagers, various problems often accompany it among which is iron deficiency anemia, prolonged labour, toxaemia (Sharon, 1988). Poor diets, inadequate antenatal care, and immaturity have been identified as the contributing factors. The consequences lead to increase maternal, infant morbidity and mortality rates.

According to Ajayi *et al.* (1989), nutritional anaemia is the most common public health problem in developing as well as in industrialized countries. Folate, Vitamin B12 and protein deficiencies have been identified as the causes of nutritional anaemia in tropical countries. Generally, anaemia of various kinds and degree results from inadequate food intake, especially from minerals and vitamin deficiencies. Anaemia occurrence has also been traced to infection with some disease condition (Enwonwu, 1983). The study further asserted that malaria parasite is one of the major causes of iron deficiency anemia most especially in children and pregnant mothers.

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Anaemia tends to be severe in those with inadequate intake of Vitamins C (ascorbate). It has been discovered that vitamin C plays a prominent role in the prevention of diseases such as scurvy apart from its role in the enhancement of haemopoeisis. In the work done by Nestle (1998) it was discovered that Vitamin C plays a role in the formation of collagen, the fundamental ingredient of the connective tissue, which holds the cell together. It also plays a part in the fixing of calcium. Likewise, ascorbate intake prevents haemorrhage and reduces the symptoms of common cold (Victor and Elizabeth 1994).

It has been observed that larger and increasing number of pregnant mothers and children suffer from nutrient (iron) deficiency anaemia. Complicating the iron deficiency anaemia is malaria, which further destroys already deficient number of red blood cells (WHO 1992). WHO (1993) further asserted that malaria initially causes haemolytic anaemia and later iron deficiency during the period of recovery from malaria. In the light of the above, it behooves nutritionist to find means of reducing all forms of anaemia that are nutritionally related. This is especially necessary in teenage pregnancy. Ajayi *et al.* (1989), discovered that there is a positive haematological response to supplementations of riboflavin and ascorbic acid in Nigerian young adults. In another study Latunde and Agboola (1995), carried out an extensive study on the effects of vitamin C, Ferrous sulphate and calcium supplementation on the haematological status of some University students in Nigeria. It was discovered that vitamin E and ferrous sulphate have positive haemopoetic activity while calcium has haemolytic effect.

Furthermore, several studies have shown correlation between supplementation of folic acid and ferrous sulphate and prevention of anaemia in pregnancy. It is probably the results of the studies that warrant routine administration of these two nutrients (in drug form) from second trimester of pregnancy till birth. This is now widely practice in many parts of the world. However, ever increasing numbers of anaemic cases in pregnant teenagers, as observed by Lanre and Blaw (1998), poses a challenge to health care providers. To complement the effect of folate and iron supplementation, ascorbate is been looked into. Since little work is known to have been done on the test of the haemopoectic function of ascorbate in this regard, this study therefore focused on the following:

i. To what extent does the supplementation of ascorbic acid affect blood formation?
ii. To what level does this supplementation affect the plasma ascorbate, haemaglobin concentration, haematocrit value and the red cell count of individual subject?
iii. What are the differences in plasma ascorbate level, haemoglobin concentration, haematocrit value and red cell count in those subjects with ascorbate and in those with no ascorbate supplementation?

MATERIALS AND METHODS

A total number of 45 subjects were selected, as sample size, from three (4) government health facilities in Ibadan. (ie.15 subjects per health facility). This consisted of experimental group "E" of 10 subjects and 5 subjects as control group "C" thus making a total number of 30 for "E" and 15 for "C" groups respectively. The 3 maternity centres chosen was based on their accessibility as each of them contain large number of teenage pregnant women all the year round. All subjects were clinically healthy and they continued with their usual diet and daily activities. Each subject in group E received a table containing 100mg ascorbic acid (after the midday meal) daily for 4 weeks. Members of the C group received no table throughout the study period. Both E and C groups were giving daily dose of folate and iron and a weekly dose of 25mg Daraprim (antimalaria). Blood sample from each subject was taken (from members of the two groups) first before and then after 4 weeks of Vitamin C supplementation. The weight and height of subjects were taken on the day the first round of blood was taken. That same day, each subject answered questionnaires. This was to determine their respective baseline nutritional status. This was done in the department of Human Nutrition Laboratory, University of Ibadan. The blood of each subject was analysis first before vitamin C supplementation and then after 4 week post supplementation.

(A) Hemoglobin Concentration

The hemoglobin (Hb) was determined by the cyanmethaemoglobin method. The reagents used were Drabkin's solution which was made up of 0.2g of potassium cyanides (KCN), 0.2g of potassium ferricyanide (K3Fe(CN)6 and 1g of sodium bicarbonate (NaHCoz) dissolved in distilled water.

B) Haematocrit (PVC) estimation

The packed cell volume (PVC) was measured by the microhaematocrit centrifuge method.

C) Plasma Ascorbate

In a test tube containing one millitre of plasma was added 1ml of 5% trichloroacetic acid (TCA). The content of the tube was thoroughly mixed. The mixture was centrifuged at 3000 revolution per minute (RPM) for 15 minutes.

D) Red Cell Count

The Red Cell Pipette was employed to dilute a small known quantity of blood. The pipette was graduated at 0.5, 1 and 101. The bulb of this pipette has a volume of 100units and sucked up to the 0.5 mark.

RESULTS

The Analysis of the data was done in two batches.

A Questionnaire

From the 45 questionnaires served it was discovered that 41(91.1%) of the respondents were within the ages of 16-19 years with a mean 17± 0.68. 28 (62.2%) of the subjects had primary school education, while 82.2% (37) are Yorubas from predominantly Muslim family background- i.e. 38(80.2%). 49.1% (22) of the respondents were jobless while the

remaining 50.9% (23) engaged in petty trading and other occupations such as barbing, hairdressing, sewing etc. Also 31 subjects (68.9%) were primigravida while 5 (11.1%) were primipara.

The Nutritional Status of Respondents

64.4% (29) of the respondents eat more than 3 times per day and 11.1% (5) of them skipped one of the daily meals occasionally for maintenance of desirable posture and sometimes due to work pressure. The dietary evaluation using 24 hours re-call method exposed not only their food consumption but also their food habit. The Table 1 showed the food items consumed by the respondents as established by the 24 hour dietary re-call method. Table 2 revealed the percentage recommended dietary allowance (RDA) met by the experimental group in comparison with the recommendation proposed by the Nutrition Committee of the British Medical Association. The dietary intake of respondents showed that the ascorbic acid daily consumption ranged form 42mg to 47mg. This accounts for 105% to 118% of the 40mg recommendation as recommended by Nutritional Committee of the British Medical Association 1950. (Passmore and Eastwood' 1998). Other dietary data of respondents showed that the protein consumption ranged from 55 to 120g. This accounts for 59% to 118% of the recommended 93g to 102g respectively. Also the total calories consumption ranged from 1900kcal to 3100kcal. This accounts for 76% to 113% of the recommended 250kcal to 270kcal respectively. The iron consumption record ranged from 13mg to 19mg. This represented 108% to 126% of the recommendation, which ranged from 12mg to 15mg.

Table 1. Distribution of the subject based on the 24 hour Dietary Recall Method

Food Items	Respondent	Percentage
Yam Flour (Amala	44	98%
Ewedu	32	71%
Fish	38	84%
Meat	44	98%
Bread	29	64%
Stew	45	100%
Moinmoin	41	91%
Rice	33	73%
Beans	39	87%
Yam	19	42%
Akara (Bean Cake) 28		62%
Okro (ila)	14	31%
Vegetable	29	64%
Cassava Flour (lafun) 19		42%
Maize Flour (Tuwo) 22		49%
Fruits- Orange 40		89%
Apple (Pineapple)	21	47%
Banana	30	67%
Grape fruits	19	42%
Beverage - Bournvita 2		4%
Vitalo	38	84%
Coffee	3	7%
Milo	10	22%
Drinks – Soft Drinks 39		87%
Alcoholic Drinks	14	31%

B. Results of the Blood Parameters

For the 45 subjects, the haemoglobin (Hb) concentration, haemoatocrit (PCV) value, red blood cell count and the plasma ascorbic acid concentration at the beginning of the study were compared with the levels of the same blood parameters at the end of 4 weeks ascorbate supplementation.

Table 2. Range of Daily Dietary Allowance Met by the Experimental Group

2A	ENERGY KCAL	PROTEIN (g)	VIT. A & CAROTENE	IRON
RDA STANDARD	2500-2750	93-102	6000	12-15
RDA MET	1900-3100	55-120	7100-7800	13-19
%RDA MET	76-113%	59-118%	118-130%	108-126%

2B	CALCIUM (mg)	RIBOFLAVIN (mg)	ASCORBIC ACID (mg)
RDA STANDARD	0.8-1.5	1.5-1.6	40
RDA MET	0.7-1.9	1.2-1.5	42-47
%RDA MET	88-126%	80-94%	105-118%

(i) Haemaglobin (Hb) Concentration

The initial record of the Hb concentration in the experimental subjects ranged between 6.4g/100ml to 19.6g/100ml. After 4 weeks ascorbate supplementation there was a slight increase in the Hb level. The Hb increase value ranged between 7.1g/100ml to 20.0g/100ml in the experimental subjects. This accounts for an increase percentage of 10.9% to 2.1% respectively. Similarly in the control group which also started off with the initial level of 10.0g/100ml to 19.2g/100ml, recorded a ranged of 10.3g/100ml to 18.2g/100ml after 4 weeks study period. This accounts for an increase percentage of 3% in the lowest records and a decrease of 5.5% in the highest records. The 3% increases may be because the only subject (with this increase) had a better feeding pattern during this period. The normal standard value ranges from 11.4g. - 15g/100ml. Out of the 45 subjects, 22 (48.9%) had Hb level lower than 11.4g/100ml and does not meet the standard value. i.e 16(53%) of the subjects in the experimental group and 6 (40%) of the control subjects. After 4 weeks. 7(23%) of the experimental subjects and 3(20%) of the control subject had Hb value lower than 11.4g/100ml and this does not meet the standard value also.

(ii) Haematocrit (Pack Cell Volume - PCV) Value

In the experimental subjects the initial record of PCV ranged from 24% to 37%. After 4 weeks ascorbate supplementation, there was a significant increase. The PCV value ranged from 30% to 39%. This represented an increase percentage of 5.4% to 25% respectively. In the control group, which started off with the initial PCV range of 25% to 38%, recorded even a slight fall range of 25% to 37% after 4 week study period. The lowest records of both periods were the same while there was a slight decrease of 2.6% in the two highest subjects. The normal standard value ranges from 40% to 47%. Therefore, the PCV value ranges obtained in all the subjects, both at the initial record and after 4 weeks, did not meet the standard.

(iii) Red Blood Cell (RBC) Count

The initial record of RBC counts in the experimental subject ranged between $1.62x10^6/mm^3$ to $3.87x10^6/mm^3$. After 4 weeks ascorbate supplementation, there was a slight increase in the RBC count. The RBC count value ranged between 2.14x 10^6mm to 4.22x106mm. This accounts for an increase percentage of 9% to 32% respectively. Also the control group which began with the initial RBC count of 2.63x 10^6/mm to $4.37x10^6/mm^3$ recorded a ranged of $2.94x10^6/mm^3$ to 3.85 $x10^6/mm^3$ at the end of the study period. This represented an increase percentage of 11.8% in the lowest value of both period and decrease of 20% in the highest values of the two periods. The increase of 11.8% in the lower values may be because the subjects (4 of them) had a better feeding pattern during this period. The normal standard value is $4.8x10^6/mm^3$. All the subjects, both at the initial stage and after 4 weeks had RBC count value lower than $4.8x10^6/mm^3$. This did not meet the standard. This must be a reflection of their diet at this period.

iv) Plasma Ascorbate Concentration

In the experimental subjects, the initial record of plasma ascorbic acid concentration ranged between 0.00mg/100ml to 1.47mg/100ml. The plasma ascorbate concentration increased significantly after 4 weeks ascorbate supplementation. The plasma ascorbate concentration increased value ranged between 0.20mg/100ml to 2.06mg/100ml. This accounts for an increase percentage of 40% to 100%. In the control group which had initial record of 0.05mg/100ml to 1.46mg/100ml, plasma ascorbate concentration range of 0.00mg/100ml to 1.45mg/100ml were recorded after l4 weeks study period. This accounts for a decrease of 0.70% to 100%. The normal standard value ranged between 0.4mg/100ml to 1.5mg/100ml. Out of the 45 subjects, 14 (47%) of the experimental subject and 7 (47%) of the control subjects too had plasma ascorbate level lower than 0.4mg/100ml and this did not meet the standard. After 4 weeks, 5 (16.7%) of the experimental subjects and 6 (40%) of the control subjects had plasma ascorbate level lower than 0.4mg/100ml and this did not meet the standard. But 25(83.3%) of the experimental subjects and 9 (60%) of the control subjects had plasma ascorbate value higher than 0.4mg/100ml and this met the standard.

DISCUSSION

The supplementation of ascorbate acid among other nutrients, exert a beneficial effect on haemopoesis. Latunde and Agboola (1995) discovered in their study that vitamin C and ferrous sulphate have haemoposis activity. The data obtained from the present study have shown that ascorbate supplementation produced positive hematological responses. Significant increase were discovered in haematocrit values and plasma ascorbic acid level while slight increase were discovered in haemoglobin (Hb) concentration and red blood cell counts after 4 weeks ascorbate supplementation. The reason behind the slight increase noticed in Hb concentration and red blood cell counts might be due to the short duration (4weeks of given the supplementation).

From the result, it could be deduced that the hematological response will be higher if the supplementation period went on longer. Available report (Wilson, 1990) have shown that the presence of some haemopoetic factors such as vitamin C, vitamin B, folic acid, iron and copper facilitate production of normal blood cells. Since vitamin C supplementation increases significantly the heamatocrit value and the plasma ascorbate concentration in the experimental subjects, it is evident that the contribution of the nutrients in the maintenance of adequate blood cell level is of utmost importance. It is therefore recommended that ascorbic acid supplementation should be given alongside with the routine folate and iron from second trimester of pregnancy till birth of the baby.

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