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Effects of Vitamin C Supplementation on Sickle Cell Disease

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Abstract

Sickle cell disease is the general name for a group of disorders that affect haemoglobin, the red pigment in red blood cells that delivers oxygen to cells throughout the body. Fortunately, Vitamin C has been found to be very vital in improving and sustaining health. This study sought to determine the effect of vitamin C supplementation on the blood pressure and blood count in sickle cell patients. The study was a randomized double blind, placebo –controlled study. The treatment group received 300mg of vitamin C per day for 3 months. The data of 60 subjects were analysed. Vitamin C supplementation increased the mean haemoglobin concentration and haematocrit of the subjects with SC genotype by 2.4% and 2.5% respectively compared to baseline (even though not statistically significant). There was a statistically significant increase in the Mean Copuscular Haemoglobin Concentration of the subjects with SC genotype on vitamin C (from 29.61 to 31.32 g/dl) compared to those on placebo (from 30.44 to 28.5 g/dl) (P = 0.01). Vitamin C supplementation therefore has some beneficial effects on some full blood count parameters of sickle cell patients with SC genotype.

Keywords*: Sickle Cell Disease; Vitamin C.*

Introduction

Sickle cell disease is the general name for a group of disorders that affect haemoglobin, the red pigment in red blood cells that delivers oxygen to cells throughout the body (Harris and Graham, 2007). The most common types are SS (sickle cell anaemia), Sickle-Haemoglobin C Disease (SC), Sickle Beta-Plus Thalassaemia and Sickle Beta-Zero Thalassaemia. Usually, signs and symptoms of sickle cell disease begin in early childhood even though clinical manifestations including chronic anaemia, repeated infections and periodic episodes of pain depend on the genotype as well as several other conditions. According to WHO (2006), in West African countries, the frequency of the trait is 15% to 30% and sickle cell contributes the equivalent of 5% of under–five mortality in Africa. However, this unacceptable mortality rate can be reduced if more efforts are made to improve the immune system and general nutritional status of sickle cell patients. Clinicians therefore utilize current treatment guidelines to manage sickle cell disease and the use of nutritional supplements such as Vitamin C have been explored by many health care professionals today.

Vitamin C or L-ascorbic acid is a water-soluble vitamin needed by humans, non-human primates and other animals for vital metabolic functions. This essential nutrient is perhaps best known for its ability to strengthen the immune system. Vitamin C is not only a potent anti-oxidant that mops up free radicals from the body, but also plays an essential role in the production of collagen, bile, carnitine, hormones and norepinephrine. In essence, Vitamin C may be directly or indirectly beneficial in improving the health status of sickle cell patients by strengthening the immune system, producing new tissues and replacement of old tissues. This paper is based on my Mphil. in Nutrition thesis, at the University of Ghana, Legon (Adiiboka, 2012).

Main objective

To investigate effects of Vitamin C supplementation on sickle cell disease.

Specific objectives

To determine the effect of vitamin C supplementation on the following in sickle cell patients.

1. Red blood cell count.

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2. Haemoglobin concentration.
3. Haematocrit (percentage of RBC).
4. Mean corpuscular volume (MCV) (average size of RBC).
5. Mean corpuscular haemoglobin concentration (MCHC) (average concentration of haemoglobin inside a red cell).
6. Total white blood cell count.
7. Lymphocyte count.
8. Monocyte count.
9. Granulocyte count.
10. Platelet count.
11. Systolic and diastolic Blood pressure.
12. Body Mass Index.

These parameters were chosen because they are all known to have a role in the pathophysiology of sickle cell disease and its complications.

Methodology

Study area

This study was conducted at the Sickle Cell Clinic of the Centre for Clinical Genetics at the Korle-Bu Teaching Hospital, Accra. It is Ghana's first and biggest Sickle Cell clinic and attends to an average of 50 patients daily. The clinic also serves as a research center.

Inclusion criteria for subjects

1. Subjects must be diagnosed with sickle cell disease (SS or SC genotypes).
2. Subjects must give informed consent to participate in the study.
3. Subjects must not have any chronic medical condition apart from sickle cell disease (SS or SC genotypes).
4. Subjects must be 13 years or older.

Exclusion criterion for subjects

Subjects not fulfilling all of the inclusion criteria.

Study design

The study was a prospective study. A randomized double-blind, placebo-controlled trial was done with one experimental group and one control group.

Sampling methods

Subjects who met the inclusion criteria of the study were chosen for the study. Study subjects were randomly put into two pre-coded groups; treatment group (vitamin C) group and a control (Placebo) group. The treatment group was initially coded as R and the placebo group coded as Q until after the study ended and the data analyzed. Since the study was a blinded study, allocation of subjects to the two groups was done such that, neither the investigators nor the subjects knew who received the placebo and who received the vitamin C until after the study. Hence, the Production Manager at Ernest Chemist Ltd (who is not part of the study) was the only one who had detailed records of which subject codes received placebo (placebo group) and which subject codes received vitamin C (treatment group). However, the blinding process and the necessity for blinding was explained to the subjects before they decided to be part of the study or not. If during the study, it was discovered that one of the treatments (placebo or vitamin C) had significant adverse effects, the blinding would have been removed and the study stopped. There was however no such significant adverse reaction.

Data collection

A semi-structured pre-coded questionnaire was used to obtain information from the respondents. The first section of the questionnaire contained questions to solicit basic information such as age, sex, and history of medications being taken. The physical assessments such as the weight and height

measurements as well as the full blood count assessment were recorded in the second section of the questionnaire. The questionnaire was administered through personal interviews with the respondents.

The treatment group was supplemented with 300mg per day of vitamin C while the control group was supplemented with 300mg per day of placebo. Both groups were supplemented for three months (90 days). Data that were collected and the process of data collection at baseline, after the first month, after the second month and after the third month of supplementation are as follows

Full Blood Count (FBC)

Procedure for the FBC test

1. The patient was made to sit in a comfortable chair.
2. Allergies of patients were checked e.g. Antiseptics, adhesives, or latex by observing for armbands and by asking the patient.
3. The patient was well positioned in the chair with the arm hyper extended.
4. The patient's veins were palpated and traced with the index finger.
5. The tourniquet was applied 3-4 inches above the selected puncture site, without being placed too tightly or left on arm for more than 2 minutes. This was to avoid increasing risk for haemo concentration.
6. The patient was asked to make a fist without pumping the hand.
7. The venipuncture site was then selected.
8. The patient's arm was then prepared by cleansing with 70% alcohol in a circular fashion, beginning at the venipuncture site and working outwards. The alcohol was allowed to dry for a few seconds.
9. The patient's arm was firmly grasped to draw skin taut. The needle was then positioned to form a 15 to 30-degree angle with the surface of the arm and was swiftly inserted through the skin and into the lumen of the vein.
10. When the EDTA tube was filled to mark 2ml, the tourniquet was removed.
11. The needle from the patient's arm was then removed using a swift backward motion.
12. The patient was asked to press down the cotton on the arm once the needle was out of the arm, applying adequate pressure to avoid formation of a haematoma.
13. The used syringe was then disposed into a designated container.
14. The blood in the EDTA tube was then well mixed and labeled immediately.
15. The sample in the tube was transferred onto a mixer for full blood count analysis using an automated Haematology Analyzer (HORIBA ABX**)**. The automated analyzer is a machine that performs the full blood count automatically after is has been run with a reagent control. Blood pressure measurement:
16. A standardized digital Sphygmomanometer was used to measure the diastolic and systolic blood pressure of the subjects at rest. Blood pressure measurement was done for both the vitamin C group and the control group.

Sample size determination

A sample size of 50 subjects in the placebo (control) group and 50 subjects in the vitamin C (treatment), group made up of 30 SS genotypes and 20 Sc genotypes for both groups was recruited for the study. This sample size took into consideration attrition rate. Due to an attrition rate of 40%, the data of 30 subjects in the vitamin C group (18 SS and 12 SC genotypes) and 30 subjects in control group (18 SS and 12 SC genotypes) were analyzed and discussed.

The sample size was determined at a confidence level of 95% using the formula below by Jekel *et al.*, (2007).

$N = [(Z)^2 \times 2 \times (S)^2] \div d^2$

Z= value for alpha error (0.05)

S^2 = variance expected

d = Mean difference to be detected (smallest clinically important difference).

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Ethical clearance

Ethical clearance was obtained for the study from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research.

Statistical analysis

The Statistical Package for Social Scientists (SPSS) version 16.0 was used for data analysis and Microsoft Excel (2007 version) was used for the graphs and charts. Descriptive statistics such as means, medians and modes were used to describe continuous data such as blood pressure, age, haemoglobin concentration and full blood count parameters of the subjects. Student t-test was used to determine the effect of vitamin C supplementation on blood pressure, BMI and full blood count variables by comparing the control group with the treatment group. The variables assessed at the end of the study were also compared with their base line values using t-test. Statistical significance was set at a confidence level of 95% and p-value less than or equal to 0.05.

Study limitations

The following are the limitations of this study

1. The study was limited to only SS and SC genotypes.
2. Due to time and resource constraints, the study lasted for only three months and so effects of vitamin C that may be observed after three months of supplementation were not seen.
3. Due to time and resource constraints, the urinary and blood ascorbate levels could not be determined at baseline and after the study. The study therefore relied on empirical evidence of the blood ascorbate and urinary levels of sickle cell patients (which were found to be low) in previous studies.

Results

At baseline, there was no statistically or clinically significant difference between the vitamin C group and the placebo group in terms of their characteristics such as the age distribution, sex distribution, blood pressure, Body Mass Index or full blood count variables. The average age for both groups was 28years.

The study showed that the vitamin C supplementation increased the mean haemoglobin concentration and haematocrit of the subjects with SC genotypes by 2.4% and 2.5% respectively compared to baseline (even though not statistically significant). By the end of the three months of supplementation, there was a statistically significant increase in the Mean Corpuscular Haemoglobin Concentration (MCHC) of the subjects with SC genotype on vitamin C (from 29.61 to 31.32 g/dl) compared to those on placebo (from 30.44 to 28.5 g/dl) ($P = 0.01$). There was also a statistically significant reduction of the lymphocyte count of the subjects with SS genotype on vitamin C supplementation (from 4.2 to 3.0×10^9/L) by the end of the 3 months of supplementation ($p=0.024$). There was however no significant effect of the vitamin C supplementation on the mean corpuscular volume, the platelet count, the total white blood cell count, monocyte count, granulocyte count, the systolic and diastolic blood pressures and the body mass index of the sickle cell patients with SS genotype or SC genotype.

Discussion

It is worth iterating for the purpose of this discussion that a prime symptom seen in sickle cell disease is chronic anaemia. Thus, since several reports have shown that chronic anaemia is one of the most problematic complications of sickle cell disease, this study sought to determine the effects of vitamin C supplementation on the haemoglobin concentration (and other parameters) of the subjects. The mechanism of action of vitamin C that makes it beneficial to haemoglobin concentration is that, vitamin C enhances absorption, utilization and metabolism of iron for the production of haemoglobin. At baseline the mean haemoglobin concentrations observed for subjects with the SS genotype as well as those with the SC genotype in both the vitamin C group and the placebo group were lower than the standard recommended levels of about 12.0 – 18 g/dl for males and 11.5 - 16 g/dl for females. This indicates that at baseline the subjects were mostly anaemic. However, evaluation at every other month

of the study showed that the mean haemoglobin concentrations still remained low. This is in spite of the observation that the vitamin C supplementation increased the mean haemoglobin concentration of the subjects with SC genotypes by 2.4%. This observation therefore raised several questions with several possible explanations.

In the first place, the consistently low haemoglobin levels in spite of the 3 months of supplementation could indicate that the supplementation dose of 300mg per day might be adequate for healthy adults, but inadequate for sickle cell patients with chronic anaemia. This however is most likely not the case since researchers have shown that supplementation doses of between 100mg to 300mg per day for at least 6 weeks improved some conditions in sickle cell disease. For instance, Jaja *et al*. (2002) investigated the effect of ascorbic acid supplementation on blood pressure, haematologic and erythrocyte fragility changes in children suffering from sickle cell anaemia. Their study involved 15 children within the ages of 4 to 11 years who were supplemented with 100mg ascorbic acid daily for 6 weeks. Ascorbic acid supplementation significantly reduced systolic blood pressure, diastolic blood pressure, mean arterial pressure and percent irreversibly sickled cells. It also significantly increased packed cell volume, haemoglobin concentration, percent foetal haemoglobin and the resistance of the cells to lysis. This implies that vitamin C supplementation dose of at least 100mg has some beneficial effects for sickle cell patients. It is however worth noting that the study by Jaja *et al*. (2002) involved children (4 to 11 years), who are actively growing. Actively growing children tend to have more fetal haemoglobin which is more resistant to lysis than normal haemoglobin of adults. Actively growing children are also more likely to be more responsive to treatments than adults. Thus, a dosage that is beneficial to children may not be beneficial to adults.

Secondly, it possible that the general dietary iron intake levels of the subjects might be so low that the effect of vitamin C in enhancing absorption and utilization of iron to produce haemoglobin will not be realized. Furthermore, an iron deficiency can be reflected in microcytic anaemia, a condition in which the red blood cells are smaller than normal. However, the results of this study showed that the mean size of the red blood cells of the subjects were within normal or adequate range throughout the study period.

Mean Corpuscular Haemoglobin Concentration (MCHC) measures the amount and concentration of haemoglobin in the average cell. The study results showed that vitamin C supplementation increased the MCHC of the subjects with SC genotype by 5.8% and at the end of the third month of supplementation, the MCHC of the vitamin C group was statistically higher that the MCHC of the placebo group ($p=0.01$). This observed increase was in spite of the fact that their corresponding haemoglobin concentration still remained low. Thus, even though the amount of haemoglobin per cell increased, the amount of haemoglobin per unit amount of blood remained low. The most likely explanations for this observation therefore are that, either the total numbers of red blood cells were low or the size of each red blood cell (MCV) was low.

According to Cox (2009), reduced MCV (less than 78 fl) can indicate anaemia of chronic disease or iron deficiency anaemia whiles elevated MCV can indicate vitamin B12 deficiency, folate deficiency, thyroid problems, liver problems or marrow dysplasia/aplastic anaemia. None of the study subjects had thyroid problems, liver problems or aplastic anaemia. A high MCV can indicate megaloblastic anaemia, where red blood cells are large and pale. This can be caused by a shortage of folic acid. However, almost all the subjects were on folic acid supplements as part of standard care by their Health Care Providers. The study findings showed that the mean MCV of all the subjects were within the normal range of 78 fl – 104 fl throughout the 3 months of supplementation. Thus, the average size of the red blood cells was normal. This therefore ruled out MCV as the cause or contributing factor for the low haemoglobin concentration.

Essentially, the consistently low haemoglobin concentration, in spite of the increased haemoglobin per cell (MCHC) is most likely as a result of the very low RBC counts. The low RBC count in turn was also most probably caused by red blood cell breakdown. This is because, several relevant literatures have shown that increased red blood cell break down is a common problem in sickle cell disease.

The study results showed that the mean WBC levels remained within the normal range (4 – 10 $\times 10^9$/L) for the patients with SC genotypes, throughout the study, whereas the subjects with SS

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genotype recorded WBC levels higher than the normal range throughout the study. Vitamin C has been shown to stimulate both the production and function of white blood cells (Higdon and Drake, 2011). The apparent lack of effect of the vitamin C supplementation on the white blood cell count of the SC patients is probably because the levels were within the normal range. Thus, the body tries to maintain a normal level of white blood cells unless there is infection, a disease condition or the effects of some drugs.

Finally, after 3 months of supplementation, there was no statistically significant effect of vitamin C on.

1. Red blood cell count (low throughout the study).
2. White blood cell count.
3. Monocyte count (within normal range throughout the study).
4. Granulocyte count (within normal range throughout the study).
5. Platelet count (within normal range throughout study).
6. Systolic blood pressure (within normal range throughout study).
7. Diastolic blood pressure (Low throughout study).
8. Body Mass Index (Within normal range throughout study).

It is important to note that, stringent measures were put in place to ensure compliance of the subjects in taking their full dosage though out the study period. For instance, supplements and placebo were given to the patients in collaboration with their Medical Doctors. This placed emphasis and urgency on the need for them to comply strictly with taking the tablets. Secondly, all the study participants were reached via mobile phone calls twice each time they were given a dose before the next set of doses to remind them and listen to any concerns and reservations they had. Subjects who were discovered to have difficulty in complying with taking their vitamin C or placebo voluntarily dropped out of the study. Investigations in this study showed that none of the patients had bone marrow failure and as one of the management protocols at the Sickle Cell Clinic, the patients are prescribed with nutrient supplements especially folic acid supplements. Further studies should be carried out with vitamin C supplementation longer than 3 months to see the long-term effects of vitamin C supplementation on sickle cell disease.

Conclusions

Sickle disease has devastating effects on the wellbeing and quality of life of those who suffer from it. Since, this study showed some beneficial effect of vitamin C, Health Care Providers should therefore include Vitamin C in the prescribed nutritional supplements for sickle cell patients. Further research on ways to improve the health and wellbeing of sickle cell patients and should also be fostered.

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