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EFFECT OF MICRONUTRIENTS AND VITAMINS ON HEMOGLOBIN POLYMERISATION IN SICKLE CELL DISEASE

Priti V. Puppalwar[1], Prashant Adole[2], Archana Dhok[2], Priyanka Bhatkulkar[2]

[1]Dept. of Biochemistry, SVNGMC, Yavatmal, M.S.

[2]Dept. of Biochemistry, JNMC, Sawangi, M.S.

ABSTRACT

Protection of red cell membranes from free radical-mediated oxidative stress is crucial to the successful management of the sickle cell crisis.

Aims and objectives : To demonstrate in practical terms, the antisickling effectiveness of some micronutrients and vitamins in inhibiting sickle cell hemoglobin polymerization.

Material and methods : The antisickling effectiveness of crude extracts of copper, cobalt, magnesium, two fat soluble vitamins(A,E) and one water soluble vitamin (C) were investigated to ascertain the ability of ions to inhibit sickle cell hemoglobin polymerisation process. Mineral samples of concentration 1.0x10-1 mM, Vitamin A of concentration100 IU and 1 mg/ml for each of vitamins C and E were prepared and HbSS polymerization experiment was carried out by original methods of Noguchi and Schechter, 1978. Absorbance was took at 700 nm and rates of polymerization were calculated.

Observations and results : Copper (P value-0.253) elevated hemoglobin polymerisation by 33.13% while cobalt(P value-0.635) and magnesium(P value-0.099) inhibited polymerisation by 9.30% and 36.04% respectively. Vitamins A (P value-0.000), C(P value-0.001) and E (P value-0.015) inhibited hemoglobin polymerisation by 98.25581%, 62.2093%, 65.69767% respectively.

Conclusion : The results reveal nutritional and therapeutic relevance of antioxidant minerals and vitamins in the management of sickle cell anemia and trait.

Keywords : micronutrients, vitamins, hemoglobin polymerisation, sickle cell disease.

INTRODUCTION

Sickle cell disease (SCD) refers to a large group of hemoglobinopathies in which at least one sickle cell gene of the β-globin chain is inherited together with abnormal gene. A single base mutation results in an amino acid substitution at sixth position of beta chain, valine replacing glutamic acid, producing HbS [1]. The resultant loss of a negative charge allows deoxygenated HbS to polymerize. The rod like HbS polymers distort the red cell shape into the characteristic sickled appearance, impeding flow through the microvasculature, leading to ischemia, pain and death [2], features of sickle cell crisis (SCC).

Many metabolic changes which occur in SCD that lead to anemia include sickling, generation of reactive oxygen species and the general decrease in the rate of physical development. There is increased turnover of hemopoietic cells due to chronic hemolysis and cell death leading to tremendous red marrow expansion. These conditions lead to hyper-metabolic states and increase in nutrient and energy demand [3]. Nutrient deficiency, red cell dehydration, cell fragility which induce premature destruction of red blood cells and chronic haemolysis leads to anemia. Since late 1980s, under-nutrition has been identified as a critical feature of sickle cell disease [4, 5] but this focus has not been adequately addressed.

The first and most direct evidence of insufficient micronutrient intake demonstrated by clinical improvement following dietary intervention was reported by Heyman et al [6]

Micronutrients in the control of sickling and reactive oxygen generation in SCD

Protection of red cell membranes from free radical-mediated oxidative stress is crucial to the successful management of the sickle cell crisis. The sickle erythrocytes are fragile and dehydrated [3].

It has been shown that Magnesium (Mg) is effective in reducing not only the painful episode in SCD but also affects the hydration of RBC. Brugnarahas reported that K-Cl co transport is a major determinant in the dehydration of erythrocytes in SCD. Reduced erythrocyte-magnesium content with normal serum Mg is observed as a common feature of HbSS in sickle cell disease. When the internal Mg of the erythrocytes is increased, the activity of K-Cl co transport is markedly diminished. Therefore, blockage of this pathway by intracellular Mg could result in decreased dehydration and sickling in vivo[7]. Among the many important functions of Mg is its involvement, along with calcium, in the organization of membranes. Both cations are known to act as bridges between the neighbouring carboxylate groups in lipoproteins and such bridges stiffen the cell membranes [8].

Some minerals and vitamins have been found beneficial in the control of anemia. These include iron (Fe), copper (Cu), and zinc (Zn) and folate [9] .Prasad et al [10], first reported zinc deficiency in adult patients with sickle cell disease. Also iron being very important in the synthesis of hemoglobin, copper and zinc play very important roles in iron metabolism [9]. Copper is a micronutrient that has been found at increased levels in the plasma of HbSS patients. The clinical significance is unclear, but it has been reported to occur in the event of decreased plasma Zinc levels [11]. A high Zinc intake sustained over weeks is reported to induce intestinal synthesis of metalothionein, a copper binding protein that traps copper within intestinal cells, blocking its absorption. Excess copper may contribute to free radical production and oxidative damage in HbSS [12]. Cobalt is known to stimulate the production of red blood cells [13]. Selenium as well as some antioxidants and vitamins, have been found to effectively relieve the oxidative stress that prevails in SCD. The vitamins include vitamins C, E, folate, vitamin B12 and B6 [14, 15, 16]. Other nutrients include tryptophan, lipoic acid and carotenoids [17].

Delicate balance of micronutrients and vitamins is required to maintain hydration and membrane integrity[3].Hence, this study was planned to observe the in vitro effects of micronutrients and vitamins on hemoglobin polymerisation in sickle cell disease.

AIMS AND OBJECTIVES

1. To demonstrate in practical terms, the antisickling effectiveness of micronutrients and vitamins in inhibiting sickle cell hemoglobin polymerization in sickle cell disease and sickle cell trait.

MATERIALS AND METHODS

Study type- Experimental study

Study design- Pilot study

Left over , routine blood samples (taken into the anticoagulant EDTA) collected from individuals homozygous for HbS (HbSS genotype, *n* = 15) and individuals heterozygous for HbS (HbAS genotype, n= 16) were used. Approval was obtained from the institutional Ethics committee. The study group comprised of 19 females and 12 males of age group 15 to 40 years.

MATERIALS

Sodium metabisulphite (2% solution),Vitamin A capsules of 25,000 IU(7.5 mg) each, Vitamin C tablets 1 gram each , Vitamin E capsules 200 mg each, mineral elements each as soluble salts.

As the patients were already on zinc therapy effect of zinc on hemoglobin polymerisation was not seen.

METHODS

One gram (1 g) of each of soluble chloride salts of copper, magnesium and cobalt, were prepared to give the gravimetric equivalent of 0.1mM (1.0x10-1 mM) final assay concentration of each metallic ion. [18]

Preparation of Vitamin Samples

Vitamin A capsule (25,000 IU) was dissolved in 25 ml ethanol to give a final assay concentration of 100 IU/ml. One gram (1 g) of vitamin C was dissolved in 100 ml of distilled water to give vitamin C solution of 10 mg/ml to give a final assay concentration of 1mg/ml. This solution was filtered using Whatman filter paper No.1. Vitamin E capsule (200 mg) each was dissolved in 20 ml of ethanol , to give a final assay concentration of 1mg/ml for vitamin E. These were prepared freshly and used immediately.

Preparation of Haemolysate

0.5 ml of EDTA blood sample was washed with 4 ml Normal Saline and centrifuged at 3000 rpm for 5min. The supernatant was removed and washing was repeated with normal saline twice. 500 ml of washed RBC were added to 1000 µl of distilled water. This was mixed and cooled for 10-15 minutes to lyse the RBC. 3 ml of chloroform was added to lysed RBC and centrifuged for 15 min at 3000 rpm. The supernatant lysate was removed

and used for the hemoglobin polymerization experiment.

Sickle Cell Hemoglobin Polymerization Experiment

The original methods of Noguchi and Schechter, 1978 were used for the HbSS polymerization experiment. HbSS polymerization were assessed by the turbidity of the polymerizing mixture at 700 nm using 2% solution of Sodium metabisulphite as a reductant or deoxygenating agent [19]; 4.4 ml of 2% Sodium metabisulphite, 0.5 ml normal saline (0.9 % NaCl) and 0.1 ml of hemoglobin were pipette into a cuvette, shaken and absorbance read in a (Spectrophotometer, Spectronic 20) at 700 nm for 30 minutes at 2 minutes Intervals. This served as blank for all assays. For the test assay, 0.5 ml normal saline was replaced with 0.5 ml antisickling agent or sample and readings taken as usual .The rates of polymerization were calculated from the formula of average change in absorbance against time in minutes [20].

Rp= ODf - ODi /t

Rp = ΔOD/t, where Rp = rate of polymerization

ODf = final absorbance, ODi= initial absorbance at time zero

ΔOD = change in optical density, t= time of reaction in minutes

OBSERVATIONS AND RESULTS

Rate of polymerisation, relative % polymerisation and relative percent inhibition by copper, cobalt and magnesium are shown in [Table1]. Rate of polymerisation, relative % polymerisation and relative percent inhibition by vitamins is shown in [Table2]. P value <0.05 was considered as statistically significant. Copper increased the polymerisation or sickling. Cobalt and magnesium inhibited polymerisation [Table1] but not significantly [Table3] whereas Vitamins A, C and E significantly inhibited HbSS polymerization[Table2, Table3].

DISCUSSION

The nutritional approach to the management of sickle cell disease has been the most modern and most effective protocol adopted in the management of the syndrome. Many studies have provided humanity with reliable data on the deficiencies of various nutrients some of which are exacerbated by the sickling episode. Some of these deficient nutrients include Zinc, Magnesium, Copper, vitamin A, vitamin C and vitamin E, resulting in many pathophysiological complications of the syndrome [21,22].

Table 1 : The effect of micronutrients on the rate of Polymerization, the relative % polymerization and the relative %Inhibition of sickle cell hemoglobin

Sample	Final assay (conc mM)	Rate of polymeri-sation	Relative % polymeri-sation	Relative % inhibition
Control	----	0.0172±0.0	100	00
Copper	0.01	0.0229±0.0	133.1395	-33.1395
Cobalt	0.01	0.0156±0.0	90.69767	9.302326
Magne-sium	0.01	0.011±0.0	63.95349	36.04651

Table 2 : The rates of polymerization, relative percent polymerization and the relative percent inhibition by the Vitamins

Sample	Final assay (conc mM)	Rate of polymeri-sation	Relative % polymeri -sation	Relative % inhibitio n
Control	----	0.0172±0.0	100	00
Vitamin A	100I U/ ml	0.0003±0.0	1.744186	98.25581
Vitamin C	1 mg /ml	0.0065±0.0	37.7907	62.2093
Vitamin E	1 mg /ml	0.0059±0.0	34.30233	65.69767

Table 3 : Statistical significance of effect of micronutrients and vitamins on haemoglobin polymerization in sickle cell anemia.

Pair	P value
Control-Copper	0.253
Control-Cobalt	0.635
Control-Magnesium	0.099
Control-Vitamin A	<0.001
Control-Vitamin C	0.001
Control-Vitamin E	0.015

Copper increases HbSS polymerisation by 33.13%(Table1).This finding is in accordance with Nathan et al., 1992, who found that the excess copper may contribute to free radical production and oxidative damage in HbSS. Magnesium is the second most abundant intracellular cation[23] and its deficiency has been associated with several disorders including sickle cell disease. The use of magnesium salts to stabilize the red cell membrane and to prevent dehydration of the membrane has been advocated [24].

Cobalt inhibits polymerisation by 9.3% comparatively less to that of inhibition by magnesium, which was 36.04%(Table1) and found to be in agreement with Nwaoguikpe RN and Braide W [18]. In present study both cobalt and magnesium did not inhibit the polymerisation significantly ($p>0.05$).

Relative percent inhibition of polymerisation by Vitamin A, C and E were 98.255, 62.20 and 65.69 respectively. Beta carotene is known to be an antioxidant but role of vitamin A supplement as an antioxidant is not known. The mechanism by which Vitamin A very significantly inhibited polymerisation ($p<0.001$) in sickled cells could not be revealed. Antioxidant vitamins, vitamin C and E were also found to be potent inhibitors of sickle cell haemoglobin polymerisation (Table 3). Similar findings were reported by Nwaoguikpe RN and Braide W[18]. Ohnishi and Ohnishi (2001) [25] proposed that a cocktail of antioxidants would be effective in alleviating the incidence and severity of crisis in sickle cell patients.

The relationship between SCD and nutrition has been systematically reviewed and documented. Root crops, legumes, fruits and vegetables have been prescribed for sicklers (Ekeke,1997) [26].The beneficial micronutrients and vitamins from natural resources would be effective in reducing polymerisation in sickled cells. Thus daily consumption of good amount of nutritious food will not only reduce pathophysiological complications of syndrome but also promote good health for sickle cell disease patients.

Studies using direct measure of nutritional status [27,28,29], indirect assessment of nutritional status[30,31,32], and application of nutritional supplementation[6, 33,34] have established the association between SCA and the presence of nutritional deficiency among patients with the disease. These studies showed that although intake might be sufficient when measured against the recommended daily dietary allowance (RDA) for age and sex, it is still insufficient for the individual with SCA due to the increased nutritional demand imposed by the disease

CONCLUSION

Present study shows that the nutritional approach to the management of sickle cell disease is novel and remains the current and the most promising approach in the management of sickle cell disease. Further in vivo controlled randomized blinded studies of individual nutrients and combination of nutrients are needed so as to develop special RDAs for HbSS patients. A well balanced nutritious daily diet would help sickle cell anemia patients to lead a better life.

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