AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 35 NUMBER 4 DECEMBER 2006

ISSN 1116—4077

Afr. J. Med. Med. Sci. (2006) 35, 461-467

Determination of trace elements status of Nigerians with sickle cell anaemia using INAA and PIXE

JO Ojo[1*], AF Oluwole[1*], RO Osoniyi[2], MA Durosinmi[3] and AO Aboderin[4]

Departments of Physics[1], Biochemistry[2], Haematology and Immunology[3] and Medical Microbiology[4], Obafemi Awolowo University, Ile-Ife, Nigeria

Summary

The clinical application of trace elements in the management of Sickle Cell Anaemia (SCA) has not become standard recommended practice despite decades of research. A major reason for this is the ambivalence in published results as to the relative importance of some of these elements in the disease. An attempt has been made in this work to correct some of the various factors that could contribute to such inconsistencies. Results from separate investigations carried out on Nigerian subjects by our group, using both INAA and PIXE methods have been holistically evaluated and compared. Trace and minor elements were determined in wholeblood, erythrocytes, plasma, headhair and nail obtained from SCA patients in steady state and compared with identical samples from normal controls. Twelve elements were determined in blood while 20 and 30 elements were analysed in nail and hair samples respectively from the total 225 subjects. The results indicate a general mild zinc deficiency, more serious for males, in Nigerian SCA patients. It is clear that the elements Na, K, Rb and Br play key roles in maintaining homeostasis in the steady-state SCA patients. Possible gender influence in the utilization of K, Br and Fe in SCA is also suggested.

Keywords: *INAA, PIXE, trace elements, sickle cell anaemia*

Résumé

L'application clinique des traces d'éléments pour les soins des drépanocytaires (SS) n'ai pas encore une pratique standard après des années de recherches du à l'importance relatif de ces éléments. Un essai dans cette étude était de qualifier certaine de ses facteurs pouvant contribuer à ces inconsistances. Les résultats séparés des investigations faite sur les sujets Nigérian utilisant les méthodes INAA et PIXE ont été évalués et comparés. Les traces et les éléments mineurs étaient déterminés du sang , des globules rouges du plasma ,des cheveux et des ongles des drépanocytaires à l'état normale et comparé avec les échantillons des individus sain . Douze éléments étaient déterminés du sang alors que 20 et 30 éléments étaient analysés des ongles et des cheveux respectivement sur un total de 225 sujets. Les résultats indiquaient un déficit général modéré de zinc , plus prononcé chez les males drépanocytaires Nigérian . Il est claire que les éléments tels que le Na, K, Rb et Br jouent des rôles important dans la maintenance de l'homéostasie dans l'état normale des drépanocytaires. Il est suggéré que le gendre pourrait influencer l'utilisation du K, Br et Fe chez ces patients.

Correspondence: Dr. Joshua O. Ojo, Department of Physics, Obafemi Awolowo University, Ile Ife, Nigeria. Email: jojo@oauife.edu.ng.

Introduction

The study of trace (and minor) elements in health and disease has grown into a major scientific field in recent years. The various applications include diagnostic (e.g. in early breast cancers [1], prognostic (e.g. in several malignant lymphomas [2], and therapeutic (e.g. Wilson and Menkes diseases [3,4]. Some elements are also known to cause other complications in some diseases. The involvement of trace elements in several diseases is actually, only indirect and the study of trace elements may be more important in elucidating aspects of the aetiology of those diseases rather than having direct clinical impacts. However, in Sickle Cell Anaemia (SCA), trace elements are directly involved in the key aspects and could therefore have direct clinical impacts. Sickle cell disease arises from the replacement of Glutamic acid with Valine on the 6th position of the beta chain in the normal adult haemoglobin (HbA), resulting in the sickle haemoglobin (HbS). Though the HbS is sufficiently efficient in its primary function of oxygen transport, it is further characterize by the tendency to form polymers with other HbS molecules. Many of the complications of sickle cell disease are secondary to the damage caused by this rapid polymerization of HbS in the red blood cells and the consequent impairment of the plasma membrane. Sound rationale for trace elements therapy has been established for prevention or repairs of these crucial primary damages.

Zinc is known to inhibit polymerization of HbS and has been demonstrated to unsickle, in vitro, already deformed cells [5]. Furthermore, the rapid formation of HbS polymers can also be drastically inhibited via trace elements- mediated prevention/ repair of red cell dehydration [6]. It is known from the kinetics of HbS polymerization that the delay time between the initial polymer formation and their explosive growth inside the erythrocyte is extremely concentration-dependent, being inversely proportional to the 15-35th power of HbS

concentration [7]. Dehydration serves to concentrate the polymerized HbS and if prevented or reversed, will help in achieving a prolonged delay time. If the delay time could be so prolonged to exceed the capillary transit time, polymerization and sickling would occur (if at all they do), only in the venulae, and therefore no vaso-occlusion would result. Furthermore, the crucial role of Zn in antagonizing Ca and thereby enhancing the integrity of the red cell membrane has also been abundantly demonstrated.[8,9]. This translates to less haemolysis and anaemia – a major clinical presentation in SCA. Trace elements are also important in determining the rheological properties of blood, not only as a result of the strong influence of the plasma proteins hosting these elements on blood viscosity, but also by the reduction of the abnormal adhesion of sickle erythrocytes to endothelial cells such as has been reported for Zn. [7]. Improved blood flow shortens the capillary transit time and together with the elongation of the delay time previously mentioned, would result in a tremendous synergistic improvement in the clinical course of SCA. In addition to the above, trace and minor elements play important roles in body's immunity to infections [10] which have been implicated as a major cause of morbidity in SCA among children [11]. Apart from these direct impacts of trace elements in SCA, there are other complications associated with SCA, which are linked to trace elements. For instance, Zn deficiency is associated with hypogonadism as well as delayed-healing of leg ulcers in SCA [12], while Pb is known to increase the susceptibility of children suffering from SCA to some neuropathy [13].

However, despite the fact that the study of trace elements in SCA has been going on for more than three decades, very few findings have resulted in routine clinical applications. The challenge has always been to deliver high enough concentrations of the required agent into the erythrocytes without causing any toxicity [14, 15]. This often depends, among other factors, on the status of other trace elements in the body. Unfortunately, many of the results published in this regard are not consistent. In recent times, probable reasons for this are becoming evident. They include limitations in the detection limits and accuracy of analytical procedures used in early works; inadequacies or inconsistencies in sample preparation (e.g. washing of hair samples, effect of diet, medication, etc); non-recognition of synergism in elemental interactions which would make the use of multi-elemental analytical techniques to be mandatory; differences in the sexes, ages, as well as other geo-social and genetic factors. All these make it difficult extrapolating results across different socio-cultural backgrounds. For example, it has been reported [16,17] that in Saudi SCA patients, there were no zinc deficiency or hyperzincuria; and in general, the clinical presentations are quite mild.

In this paper, we present the results of a series of studies carried out by our group using Nigerian subjects. Important parameters such as sampling and sample preparations protocols were consistent for the various investigations thus making it easy to compare the various results. Furthermore, two different highly accurate multi-elemental techniques, Proton-Induced X-ray Emission (PIXE) and Instrumental Neutron Activation Analysis (INAA) were used. Some aspects of these results have been previously published [18-21], but this is the first time the entire work will be holistically analyzed and discussed.

Materials and methods

The results being reported have been obtained as part of our on-going long-term study on trace elements in SCA. It involved the determination of trace elements in wholeblood, plasma, erythrocytes, head hair and nails of various sets of SCA patients (mostly with haemoglobin HbSS), drawn largely from the South West region of Nigeria. The HbSS subjects, all in steady state, were essentially drawn from volunteer patients attending the SCA clinics at the University College Hospital, Ibadan, and the Obafemi Awolowo University Teaching Hospital, Ile-Ife (OAUTH). Identical samples from normal controls were always analyzed together with samples from each set of patients. The normal controls included University students, lecturers, secondary school pupils, teachers, policemen, and other groups of people who showed interest in the work. Identification of blood genotypes was carried out at the OAUTH by haemoglobin electrophoresis at alkaline pH. None of the patients or controls had received blood transfusion in the previous six months

Sterile scalp vein needle with polyethylene catheter, polyethylene syringes and pre-washed heparinised (20 iu/ml of blood) polyethylene screw cap tubes were used for blood collection and storage. Ten ml of blood were collected from each subject after allowing the first 1-2ml to run into sequestrene tube for blood count in order to reduce contamination. The blood was separated to the different components within 2 hours of collection. About 2 - 3 ml of the blood were retained as whole blood and the rest was separated into the erythrocytes and plasma by centrifugation at 3000 rpm for 15 minutes. Each of the 3 products was frozen and then freeze-dried for 24 - 36 hours in their individual containers using an Edward vacuum freeze-dryer at the OAUTH. The wet and dry weights of each samples were noted. Hair samples were collected consistently from the left side of the nape, while nails were collected from the big toes. Hair and nail samples were first cleaned with a brush, and washed in an ultrasonic bath of deionised water.

The freeze-dried blood samples were crushed with polyethylene spatula into fine powder in their individual containers and pellets were made from the fine powder to give reproducible irradiation and counting geometries with sample masses ranging between 50 - 100mg dry weight. Analyses by PIXE were made by bombarding the samples with 2 MeV protons, and subsequent spectrometry of the X-ray fluorescence emitted, which is received by a Si(Li)

Table 1: Results of t-tests used for comparison of trace elements levels found in Male and female SCA subjects with normal controls using INAA and PIXE

	Female SS vs controls (INAA)		SS vs Controls (PIXE)		SS vs Controls (INAA)		Male SS vs Controls (PIXE)	
	Elevation	Depression	Elevation	Depression	Elevation	Depression	Elevation	Depression
Whole blood	Na (p<0.0005)	K(p<0.0005) Fe(p<0.0005) Rb(p<0.0005) Zn (p<0.001)	Cu(p<0.025) S (p<0.025)	K(p<0.0005) Fe (p<0.0005 Rb (p<0.0005) Zn(p,0.0005) P (P<0.0005) Mn(p<0.25) (a)		Fe (p<0.0005) Rb(p<0.0005) Zn(p<0.0005) Se (P<0.025 (K) (P<0.05)	Cu (p<0.001 Ca(p<0.01)	
	Br (p>0.01	Se(P.0)	Ca. Sec (p>01	Br. (p>01)	Na (P0.1	Br (p<0.1)	Br. S. Se (p>0.1)	Zn, Rb, P, K, Fe (p>0.1)
Erythrocyte	Br (p<0.01) Na (p<0.0005)	K (p<0.0005) Rb (p<0.0005) Fe (p<0.025) Zn (p<0.025) (b)	Cu (p<0.1)	Zn (p<0.0005) P (p<0.005) K (p<0.005) (a)	Na(p<0.0005)	Fe (p<01) Rb (p<0.001) Zn (p<0.1) (c)	Cu (p<0.0005) Br (p<0.0005)	
		Se (pp<0.1)	Ca, Se, Br (p>0.1)	Fe, Rb (p>0.1)	K (p<0.1)	Br (p>0.1) Br (p>0.1)	P (p<0.1) (Ca, Se)*	Zn, Rb (p>0.1) (K, Fe)*
Plasma	Rb (p<0.1)	Zn(p<0.025) Fe (p<0.1)* (a)	Fe (p<0.005)	Ca (p<0.1) (a)	Fe(p<0.0005) Rb (p<0.1)	Zn (p<0.025) (c)		
	K*	Br, Na, Se (p>0.1)	K, Rb (p<0.1)	P, Zn (p>0.1)	Se (p>0.1)	Br, Na (p>0.1) K*	(P, K, Rb, Fe, Se)*	(Ca, Zn)*

a - Age (p<0.005)
b - Age (p<0.005), Weight (p<0.05)
c - Age (p<0.0005), Weight (p<0.0005), Height (p<0.0005)
**incomplete data or unequal variance*

detector. For Instrumental Neutron Activation Analyses, the samples were irradiated in reactor neutron fluxes, and gamma spectrometry of the resulting activated samples followed. The details and reliability of these methods as used in our research group have been published in the references already cited. [18-23].

The subjects (ages 10 – 60 years) included 10 SS patients and 11 controls whose hair and nail were analyzed for trace elements by INAA at the Washington State University (WSU), Pullman, USA [18]; 30 SS patients and 58 controls whose blood and hair were analyzed by INAA at the Imperial College Reactor Centre, Ascot, England [19]; 19 SCA patients and 42 controls whose blood were analyzed by PIXE at the University of Surrey, Guildford, England [20]; 25 SCA patients and 30 controls whose blood were analyzed by INAA at WSU [22]. Further details are supplied in the cited references.

In blood, the elements Ca, Cu, Mn, S, and P were analyzed by PIXE only, while Na was analyzed only by INAA. All the other elements (Br, Fe, K, Se, Rb, and Zn) were detected by both PIXE and INAA.

Results

A summary of our results on trace elements in blood is presented in table 1. Student t-test has been used to compare trace elements levels in patients and control subjects. Unlike in our previous reports, statistical comparisons are now made between sex-matched patients and controls. Cases where age, body weight, or height are

Table 2: Mean and percent standard deviations of Na and K in erythrocytes and plasma of subjects with HbAA and HbAs, and HbSS.

	RBC (concentrations in μg/mL)			Plasma (concentrations in μg/mL)		
	AA	AS	SS	AA	AS	SS
Na	848.8 ± 25.1%	985.1 ± 36.6%	1602.6 ± 58.4%	2762.2 ± 16.9%	2817.2 ± 12.8%	2724.1 ± 18.8%
K	3399 ± 40.2%	3653.4 ± 36.9%	2627 ± 42.4%	193.4 ± 41.1%	287.5 ± 33.4%	329.7 ± 86.9%

Table 3: Correlation coefficients and levels of significance p, between some elements in the Red Blood cells and plasma for both SCA patients and control subjects

		Control subjects (with HbAA)			SCA Patients (with HbSS)		
RBC	Plasma	n	r	p	n	r	p
Br	Br	26	0.5940	<0.001	20	0.1650	>0.1
Na	Na	25	0.3904	<0.05	21	-0.0609	>0.1
Se	Se	26	0.1777	>0.1	19	0.3968	>0.1
Zn	Zn	26	-0.0861	>0.1	20	-0.1886	>0.1
Rb	Rb	26	0.0680	>0.1	19	-0.1239	>0.1
K	Rb	13	0.6999	<0.01	12	0.3638	>0.1

not matched between subjects and controls are noted on the table. The results obtained with the two techniques (INAA and PIXE) have been treated and tabulated separately for wholeblood, erythrocytes and plasma. In general, only elements that are significantly different for the two groups of subjects are shown in the table. However, elements that are not significantly different in a particular situation (e.g. for a particular technique or sex) are still included if the result conflicts with another situation.

In the RBCs, Na is consistently significantly elevated in the SS patients. INAA results, but not PIXE, also showed that Rb is also significantly elevated in the patients. Both the results from INAA and PIXE show that K and Zn are significantly depressed in the female SS patients, but not in male patients. Likewise Cu is significantly elevated in male subjects but not in females. INAA data indicated that Br is elevated in female patients, but not males, whereas the PIXE data indicated that the element is elevated in male patients, but not females, relative to the controls. This is an example of the ambivalence referred to earlier in this paper.

In the wholeblood, Ca and Cu are significantly elevated in all SS groups relative to the controls. Na is very significantly elevated in females ($P < 0.0005$), but not at all in males ($P > 0.1$). Further in the wholeblood, K, Fe, Rb, and Zn are all significantly depressed in almost all cases in the SS patients relative to the controls. Se is significantly depressed in male SS patients (INAA only), but is non-significantly depressed in all cases by PIXE data. P is also significantly depressed in female SS patients only. The level of significance of Fe depression in wholeblood is very high ($P < 0.0005$) compared to the depression in the erythrocytes ($P < 0.03$ and $P < 0.1$ for females and males respectively).

In the plasma, PIXE analyses were poorer than INAA, being characterized by high uncertainties. Coupled with the small number of samples from male SS patients analysed by PIXE, most results under this column in Table 1 are not statistically reliable. The only consistent picture in the plasma is a marginal depression of Zn levels in the SS patients relative to the controls.

In hair and nail for the paediatric subjects (3-12 years), out of the 13 elements investigated, viz Se, Hg, Cr, Fe, Zn, Co, Au, Br, As, Sb, Cu, Na and Sc; only Cu was found to be significantly different (elevation, $p < 0.05$) in SCA patients relative to controls. For the adult group (hair only), no element was significantly elevated in hair of SS patients with respect to normal controls. However, the following elements were depressed: Br ($P < 0.01$), Na, Zn, La, Cs, Sb, Tb (all at $P < 0.025$), and a third group, Au, Eu, Fe, Ba (at $P < 0.05$).

Table 2 shows the mean and percent standard deviations of Na and K in erythrocytes and plasma of the subjects investigated; while Table 3 shows the correlation coefficients between some elements in the Red Blood Cells and Plasma for the subjects.

Discussion

Our results show that there are changes in the trace elements contents in the erythrocytes from steady-state SCA patients (with HbSS) compared with those from normal controls (with HbAA or HbAS). Comparing levels in the

RBCs and in the plasma, the changes can be seen to involve an influx of Na, Ca, Cu and Br into the cells and an efflux of K, Rb, and Zn out of the cells of the subjects in SCA to maintain ionic and chemical equilibrium. Na and Ca have higher extra-cellular concentration and their influx probably follows some disturbances at the cell membrane as demonstrated by Ney *et al* [9]. One of the major events associated with the influx of calcium is the displacement of phosphorus from the cell, which is also accompanied by a drop in the level of Adenosine Triphosphate (ATP) for which P is a precursor. This in turn affects all energy-requiring processes including the activities of the ion pumps which leads to movements of the ions down their concentration gradients [23] thus accelerating the vicious cycle. The influx of Br could be explained by its affinity with Na, just as the efflux of Rb is expected due to its chemical similarity with K. The involvement of Na and K might suggest that the sites involved are the Na-K-ATPases. The breakdown in regulation of Na within the RBC of SS patients, and K within the Plasma is vividly demonstrated by the wide ranges of concentrations observed for these elements in the two compartments for SCA patients. This is indicated in Table 2 by the percent standard deviations from the mean of the distributions: for intracellular Na, 58% (SS) versus 25% for AA subjects and for plasma K, 87% (SS) versus 41% for the AA subjects. Values for subjects with heterozygous HbA+S (sickle cell trait, AS) are also shown in the table for comparison. Furthermore, whereas the levels of Na and Br within the RBC correlated with the corresponding levels in the plasma for the control subjects, no such correlation was found in the SCA patients. This indicate that the movement of these elements between the extracellular and intra-cellular compartments was ordered for the control subjects, but random in the SCA patients where they could be playing some regulatory functions. The same picture is true for RBC-K and Plasma-Rb in the two groups. The strength of these correlations between elements in the RBC and those in plasma for one set of our subjects is shown by the p values in Table 3. We have previously reported details of the methods used for these computations [21].

It is known that the permeability of a normal red cell membrane increases significantly during deformation and de-oxygenation [9]; and that this is marked by Na influx and corresponding K efflux [24]. The influx of Ca and Cu across the weakened membrane is probably to be associated with Zn efflux since both Ca and Cu are known to compete for the same biochemical binding sites as Zn in the RBC [25]. The existence of negative correlations (antagonism) between RBC-Zinc and both Ca and Cu in normal RBCs has been previously shown by us, [23,26], and also by Taylor *et al* [27].

The finding that RBC-K is significantly depressed (relative to controls) only in SS females and not males is surprising but crucial. The significantly increased level of K in the plasma of both male and female patients however confirms K efflux from RBC. It might be that this efflux of K (accompanied by Rb and other ions) is insignificant compared with the high levels of K in the RBC of males with HbSS. K is one of the few elements that are significantly higher in the RBC of Nigerian males relative to the females [22]. This observation on K has been masked in our previous reports in which we did not separate the sexes [19,20]. The result is however crucial because efflux of K has been shown to be the important in the two main pathways for dehydration of the erythrocytes in SCA, a major clinical presentation in the disease. These two pathways are the Gardos K channels and K-Cl co-transport [7,15]. The clinical response to trace elements therapy might therefore be different for the two genders.

The same explanation (relatively large plasma pool) probably holds for the observation that the depression in Plasma-Na, despite its efflux out of the plasma into the RBC, is also not significant. In fact, in males there is no clear pattern at all. The observation that Rb is significantly elevated in the plasma of SS males and not in the females may also suggest that Rb is playing some gender-sensitive role in SCA. Rb is known to substitute for K in several situations [28,29].

Results with PIXE showed no significant differences in the RBC-Fe levels. This is consistent with findings by several workers including Davis [30] who determined iron in individual red blood cells. INAA suggests a marginal, statistically insignificant difference ($P<0.1$) in the Iron contents of patients with HbSS and normal controls. Both techniques however show a highly significant difference in the Fe in the wholeblood of patients with HbSS compared with normal subjects ($P < 0.0005$). Compared with the situation in the RBC, it is then clear that the decrease in Fe observed in wholeblood is due to the expected decrease in the haematocrit in SS patients as a result of the anaemia. It therefore does not represent a decrease in 'blood iron', but rather a decrease in the red blood cells within the blood of our steady state SCA patients.

The elements Ca, Br, and Se, which were elevated at various levels in the RBCs were depressed at corresponding levels in the plasma, suggesting simple inter-compartmental transfer. However, Zn is depressed both in RBC and plasma (as well as in wholeblood and hair), indicating a general Zinc deficiency.

Our results on hair show that Na, Br, Fe, and Zn (among others) are depressed in adult SS patients, but not in children, where only Cu was significantly different (elevated, $P < 0.05$). Levels of trace elements measured in hair reflect long-term effects and might be age-dependent. Our results for the children is in agreement with a previous work by Olatunbosun *et al* [31].

Clinical Implications and Conclusions

In this work, several imbalances in the trace element milieu in the compartments investigated, attributable to SCA, have been identified. It has also been demonstrated that gender-sensitive factors are probably very important in the influence of trace elements in SCA and must not be overlooked. Unfortunately, most previous studies (including ours) did not give this consideration the right attention it deserves. The depressed level of Zn in wholeblood, erythrocytes, plasma, and hair of Nigerian SCA subjects in this study strongly suggests that members of this group are generally zinc-deficient. The usefulness of Zn supplementation for such category of subjects has been well established. In fact, at the Seventh Ann Arbor Conference on The Red Cell (1989), Ananda Prasad and George Brewer [11] suggested that any clinician who fails to administer Zn supplement to their SCA patients who are confirmed to be zinc-deficient ought to be charged for malpractice! Although the ingestion of inorganic Zn over prolonged periods of time could upset the balance of Zn with other essential elements [3, 31], it is agreed that the benefits may outweigh the ill-effects [5,32-34]. The problem posed by the significant elevation of Ca in the erythrocytes can also be solved by zinc supplementation based on the reported antagonism between Ca and Zn. The adverse impact of Ca on the plasma membrane of the erythrocytes has been mentioned earlier in this paper. Many methods for preventing K efflux and thereby inhibiting the accompanying dehydration have been suggested. The use of dietary Mg has been reported to be promising and a number of drugs based on this principle are currently on trial [7, 24].

Se is an essential element, which as part of the enzyme Glutathione Peroxidase, could help to protect the cell membrane against oxidation damage. Stress and oxidation damage are two factors which in synergy put the erythrocytes into great jeopardy in SCA [9,25]. It might then have been supposed that Se supplementation would be beneficial in SCA. However our results have indicated that the intracellular Se level is normal in SCA. There is therefore no basis for such 'intuitive' Se supplementation, especially in view of the toxicity of Se beyond a narrow normal range [35].

Apart from therapeutic applications of trace elements in the management of SCA as suggested above, trace elements status could also have prognostic values. This work has shown that the balance among trace elements is disrupted in Sickle Cell Anaemia. The nature of these imbalances, including ratios of elements in various body compartments, if carefully studied using rigorous statistical techniques such as Cluster analysis may yield the prognostic factors currently being searched for that could sort out patients at greatest risk for the most serious complications in SCA from potentially benign ones. This would also be significant in the management of the disease. For example there are several patients whose lives might depend on stroke-preventive bone-marrow transplantation being carried out as early as possible. However the risks (and costs) involved in this procedure at the present time makes it mandatory that it be restricted only to the cases with the worst outlooks [36].

References

1. Dabek JT, Hyvonen-Dabek M., Kupila-Rantala T, *et al.*, Ultrafiltrable, Dietary, total and ceruloplasmin copper fractions separate early breast cancer patients from controls. Presented at the Vth COMTOX symposium on Toxicology and Clinical Chemistry of Metals. Vancover BC, Canada. 10 – 13 July, 1995. (No 120 in Book of Abstracts).
2. Hrgovic, M, Tessmer, CF, Brown, BW, *et al.*, Serum Copper studies in the lymphomas and acute leukemia. In: Ariel IM, ed. Progress in Clinical Cancer. New York: Grune and Stratton, 1973; 5:121 – 153.
3. Hill GM, Brewer GJ, Prasad AS, *et a.*, Treatment of Wilson's disease with Zinc I. Oral Zinc therapy regimens. Hepatology, 1987; 7:522-528.
4. Sarkar B. Specificity of metal-binding in normal physiology and biochemical alterations in disease conditions: Wilson's and Menkes' Diseases. in Vohora SB, Khan SY (eds). Proceedings of the First International Conference on elements in health and disease, New Delhi, 6-1- Feb, 1983. Institute of History of Medicine and Medical Research, New Delhi 1983; 27-41.
5. Brewer GJ, Brewer LF and Prasad AS. Suppression of irreversibly sickled erythrocytes by zinc therapy in sickle cell anaemia. Journal of Laboratory Clin. Med., 1977; 90: 5479-5482.
6. Mueller BU, Brugnara C., Prevention of red cell dehydration: a possible new treatment for sickle cell disease. Pediatr. Pathol. Mol. Med. 2001 Jan-Feb; 20: 15-25.
7. Brugnara C. Erythrocyte dehydration in pathophysiology and treatment of sickle cell disease. Curr. Opin. Hematol. 1995; 2: 132-138.
8. Schatzmann HJ and Vincenzi FF. Calcium movements across the membrane of human red cells. Journal of Physiology 1969; 201: 369-395.
9. Ney PA, Christopher M.M, and Hebbel R. Synergistic effects of oxidation and deformation on erythrocyte monovalent cation leak. Blood 1990; 75: 1192-1198.
10. Chandra RK and Dayton DH. Trace element regulation of immunity and infection. Nutrition Research, 1982; 2: 721-733.
11. Prasad AS, Kaplan J, Brewer G, and Dardenne M. Immunological effects of zinc deficiency in sickle cell anaemia. The Red Cell: Seventh Ann Arbor Conference. Alan R. Liss Inc. 1989; 629-649.

Prasad Ananda. Studies in Sickle Cell Disease. Final Report on Contract RFP-NHLI, 72-5. Reproduced by National Technical Information Service (US), 1977.

Erenberg G *et al*. Lead Neuropathy and Sickle Cell Disease. Pediatrics 1974; 54: 438-441.

Steinberg MH, Brugnara C., Developing treatment for sickle cell disease. Expert Opin. Investig. Drugs. 2002; 11: 645-659.

Brugnara C, De Franceschi L, Armsby CC, *et al*, A new therapeutic approach for sickle cell disease. Blockade of the red cell Ca(2+)-activated K+ channel by clotrimazole. Ann. N Y Acad. Sci. 1995; 763: 262-271.

Alayash AI. Zinc and some Zinc Dependent Enzymes in Sickle Cell Anemia. Internat. J. Vit. Nutr. Res. 1989; 59: 388-389.

Perrine RP, Weatherall DJ and May A. Benign Sickle-Cell Anaemia. The Lancet, Dec 2, 1972; 1163-1167.

Oluwole AF, Asubiojo OI, Adekile AD *et al*. Trace Element Distribution in the Hair of some Sickle Cell Anemia Patients and Controls. Biological Trace Element Research. (Nuclear Analytical Methods in the Life Sciences, eds Rolf Zisler and V.P. Guinn). The Humana Press Inc. (1990). Clifton, New Jersey. 479-484.

Durosinmi MA, Ojo JO, Oluwole AF, *et al*: Trace Elements in Sickle Cell Disease. Journal of Radioanal. Chem. 1993; 168: 233-242.

Ojo JO, Oluwole AF, Durosinmi MA, *et al*: Studies in the compartmentalization of trace elements in the blood of patients with Sickle Cell Anaemia disease using PIXE technique. Nucl. Instr. and Meth. in Phys Res. 1993; B79: 408-412.

Ojo JO, Oluwole AF, Durosinmi MA, and Asubiojo OI. Correlations of Trace Elements levels in Nails and Hair of SCA Patients and Controls in Dim LA *et al* (eds), Proceedings of First National Conference on Nuclear Methods. ABU Press Zaria, 1994; 135-143.

Ojo JO. Application of Nuclear Analytical Techniques to Studies of Elemental Models in Health and some Diseases in Man. Ph.D. thesis of the Physics Department, Obafemi Awolowo University, Ile-Ife, Nigeria. 1995.

Ojo JO, Durosinmi MA, Oluwole AF, *et al*. Baseline levels of elemental concentrations in wholeblood, plasma, and erythrocytes of Nigerian subjects. Biological Trace Element Research 1994; 43/44: 461-469.

24. Brugnara C., Membrane transport of Na and K and cell dehydration in sickle erythrocytes, Experientia. 1993 Feb 15; 49(2): 100-109.

25. Adeyefa I, Atinmo T and Jeje O.M. Trace Element Status of Patients with Sickle Cell Anaemia. Nig. J. of Nutr. Sc. 1986; 7: 39-46.

26. Ojo JO, Osoniyi RO and Aboderin AO: Study of uptake of zinc into blood from a Nigerian diet using INAA. Czechoslovak Journal of Physics, Vol 53, (Suppl A), A201-A208, 2003.

27. Taylor CJ, Moore G and Davidson DC. The Effect of Treatment on Zinc, Copper and Calcium Status in Children with Phenylketonuria. J. Inher. Metab. Dis. 1984; 7: 160-164.

28. Leggett RW, and Williams LR. A biokinetic model for Rb in Humans. Health Physics 1988; 55 (4): 685-702.

29. Selin E and Teeyasoontranont V. Rubidium: a companion of Potassium or an essential trace element of its own? Beitr Infusionther. Basel, Karger, 1991; 27:86-103.

30. Davis David: Analysis of Iron content in individual human red blood cells by electron microprobe and scanning electron microscope, Micron 1978; 9: 175-190.

31. Olatunbosun D, Morris S, Lichte F, *et al*: Trace Elements content of hair from Nigerians with Sickle Cell Anaemia. Trace Substances in Environmental Health 1977; 10: 383-388.

32. Leonard A and Gerber GB, Zinc toxicity: Does it exist? J. Am. Coll. Toxicol. 1989, 8: 1285-1290.

33. Brewer GJ, Oelshlegel FJ and Prasad AS. Zinc in Sickle Cell Anemia. In Brewer GJ. (ed), Erythrocyte Structure and Function. Proceedings of the 3rd International Conference on Red Cell Metabolism and Function held at the University of Michigan, Ann Arbor, October 16-19, 1974. Alan R. Liss, Inc., New York. 417-435.

34. Gupta L and Chaubey BS, Efficacy of Oral Zinc therapy in the management of Sickle Cell Crises. Indian J. Med. Res. 86, Dec 1987, 803-807.

35. Yang, G., Zhou S., Yin D., *et al*. Studies of safe maximal daily dietary selenium intakes in a seleniferous area in China. J. Trace Elem. Eletrolytes Health Dis. 1989; 3: 77-87.

36. Piomelli S. Bone Marrow Transplantation in Sickle Cell Disease: A plea for a rational approach. Bone Marrow Transplant. Suppl. 1. 1992; 10:58-61.

Received: 04/05/05
Accepted: 10/01/07