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## Evaluation of Some Micronutrients among Sickle Cell Disease Patients in Benin City, Edo State, Nigeria.

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### Abstract

Sickle cell disease (SCD) is an inherited disorder of haemoglobin caused by a single nucleotide substitution of thymidine for adenine (GAG-GTG) of the  $\beta$ -chain that results in the amino acid valine instead of glutamic acid. This congenital haemoglobin mutation alters this balance and creates a pro-oxidant and micronutrient reactive milieu. This study investigated some micronutrients status of sickle cell patients. A total of one hundred (100) participants were recruited for this study which consists of fifty (50) sickle cell subjects and fifty (50) apparently healthy age -matched individuals were monitored as control. Blood samples were collected by venepuncture and some micronutrients (Iron, Zinc, Manganese, cobalt, Calcium and Magnesium) were analyzed using Atomic Absorption Spectrophotometer. All parameters were significantly decreased in sickle cell patients when compared with apparently controls ( $p < 0.05$ ). Considering the results obtained in this study, it could be inferred that sickle cell subjects are predisposed to nutritional deficiencies. We therefore advocate routine assessment of micronutrients in sickle cell patients. It may be necessary to routinely provide nutritional supplements for sickle cell disease patients in Nigeria.

**Keywords:** Micronutrients, Sickle Cell Disease, Benin City, Edo State, Nigeria.

### Introduction

Sickle cell disease (SCD) is an inherited disorder of haemoglobin caused by a single nucleotide substitution of thymidine for adenine (GAG-GTG) of the  $\beta$ -chain that results in the amino acid valine instead of glutamic acid (Steinberg *et al.*, 2001;

Ballas, 2002). This leads to alteration in the properties of the haemoglobin tetramer, with the tendencies to polymerize in the deoxygenated state. In homozygotes or when there is co-inheritance of a double heterozygous state (with haemoglobin C,  $\beta$ -thalassemia, D or O), the normal  $\beta$ -chains are replaced by the modified form of the  $\beta$ -chain. SCD is one of the commonest hereditary haemoglobinopathies.

Sickle cell anaemia is primarily a disorder of the red blood cells which are a significant source of free radicals in biological system (Platt *et al.*, 2004). Red blood cells have a rich oxygen supply and are densely packed with redox active haemoglobin residues (Wellems *et al.*, 2009). The bonding interaction between heme, iron and oxygen in oxygenated haemoglobin is association with an electron transfer. In response, there is an integrated network of the antioxidant system consisting of both enzymes and low molecular weight compounds which help to mitigate oxidative stress and injury to the red cell and tissues in general (Malowany and Butany, 2012). Congenital haemoglobin mutations may alter this balance and create a pro-oxidant and micronutrient reactive milieu (Kumar *et al.*, 2009). This may be contributory to the pathophysiology of the abnormalities that underlie the clinical course of sickle cell anaemia,

Micronutrients consist of vitamins and minerals required by the body in small quantities for the normal function of cellular metabolic processes (Benzie, 2013). Trace elements are usually defined as minerals that are required in amounts between 1 to 100 mg/day by adults (O' dell and Sunde, 1997). They usually function as essential cofactors in the

numerous enzyme catalysed reactions and their absence can result in impairments of metabolic functions which can lead to serious disease conditions (German, 1999). Micro minerals or trace elements include iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, molybdenum, calcium and other electrolytes such as sodium and potassium (Debelstein *et al.*, 2007).

Nutritionally essential trace metals are not antioxidants on their own, but are integral parts and are necessary for the proper functions of antioxidant enzymes like catalase, glutathione peroxidase and reductase, and superoxide dismutase (Chan *et al.*, 1999). Thus, it is necessary that the level of these nutritionally essential trace metals, which are important to these antioxidant enzymes, be assessed in sickle cell disease patients. There is paucity of information in literature concerning the micronutrients status of sickle cell disease patients in this locality. Therefore in this present study we evaluated some micronutrients status in sickle cell patients in Benin City, Nigeria.

### Materials and Methods

This study was carried out among patients visiting Sickle Cell Centre, Benin-City, Edo State. A total of one hundred (100) participants were recruited for this study which consisted of fifty (50) sickle cell subjects and fifty (50) apparently healthy age-matched individuals which served as control. Out of the total fifty subjects 30 were males and 20 were females. The control subjects comprised of 20 males and 30 females. Ethical clearance was obtained from the Ministry of Health ethical committee, Benin City and verbal informed consent was obtained from participants after explanation of the purpose and procedures of the study. Inclusion criteria include sickle cell disease and willingness to offer a verbal informed consent to participate in the study while exclusion criteria are absence of sickle cell disease and refusal to offer a verbal informed consent to participate in the study.

### Sample Collection

Blood samples (5mls) were collected by venepuncture into an accurately labeled plain container for both subjects and control. The blood samples were centrifuged at 4000 rpm for 10minutes at room temperature within two hours of collection and the serum separated into clean plain containers, kept frozen until required for analysis. Analysis was

carried out for iron, manganese, zinc, cobalt and magnesium

### Biochemical Analysis

The concentrations of serum trace elements (iron, manganese, zinc, cobalt and magnesium) were determined using atomic absorption spectrophotometer (AAS) using direct method as previously described (Kaneko, 1999).

### Statistical Analysis

The Student's t - test at 0.05 level of significance was used to compare results in both the control and the test groups (all results were reported as mean  $\pm$  standard deviation); using a computer program named SPSS for windows release 21.0. Confident interval of values  $p \leq 0.05$  was considered significant.

### Results

Table I shows the mean  $\pm$  SD of micronutrients among sickle cell disease subjects and apparently health controls. The mean values of Iron, serum manganese, zinc, serum cobalt and serum calcium was significantly lower ( $p < 0.05$ ) among the in sickle cell subjects ( $86.66 \pm 10.84 \mu\text{g/ dl}$ ;  $7.10 \pm 0.72 \mu\text{g/L}$ ;  $75.20 \pm 10.84 \text{ I/g/L}$ ;  $0.24 \pm 0.03 \mu\text{g/L}$  and  $7.22 \pm 0.90 \text{ mg/dl}$  compared to that of the control ( $172.22 \pm 2.54.16 \mu\text{g/ dl}$ ;  $16.96 \pm 16.88 \mu\text{g/L}$ ;  $119.27 \pm 15.68 \mu\text{g/L}$ ;  $0.36 + 0.04 \mu\text{g/L}$ ,  $10.89 \pm 1.31 \text{ mg/dl}$  and  $4.69 \pm 0.64$ ) respectively. Serum magnesium was significantly higher among the sickle cell disease subjects ( $4.69 \pm 0.64$ ) compared to normal health controls ( $3.61 \pm 0.45$ ) ( $t = 5.68$ ;  $p < 0.05$ ). Table I shows the comparison of micronutrient status among Sickle Cell Disease Subjects and Controls participants. Table 2 shows the micronutrient parameters obtained for sickle cell male subjects and its comparison with the male control. There was significant difference ( $p < 0.05$ ) when the mean  $\pm$  SD of serum iron, serum manganese, serum zinc, serum cobalt, serum calcium and serum magnesium among sickle cell male subjects ( $87.65 \pm 9.28 \mu\text{g/ dl}$ ), ( $7.17 \pm 0.62 \mu\text{g/L}$ ), ( $76.20 \pm .28 \mu\text{g/dl}$ ), ( $0.24 \pm 0.03 \mu\text{g/ dl}$ ), ( $7.30 \pm 0.77 \text{ mg/ dl}$ ) and ( $3.65 \pm 0.39 \text{ mmol/L}$ ) were compared with that of the male control ( $131.10 \pm 13.52 \mu\text{g/ dl}$ ), ( $14.07 \pm 0.90 \mu\text{g/L}$ ), ( $119.65 \pm 13.52 \mu\text{g/L}$ ), ( $0.36 \pm 0.01 \mu\text{g/L}$ ), ( $10.92 \pm 1.13 \text{ mg/ dl}$ ) and ( $4.80 \pm 0.40 \text{ mmol/L}$ ). Table 3 shows the mean  $\pm$ SD obtained for sickle cell female subjects and its comparison with the female control. There was significant difference ( $P < 0.05$ ) when the mean  $\pm$  SD of serum iron, serum manganese, serum zinc, serum cobalt, serum calcium and serum magnesium among

sickle cell female subjects ( $84.94 \pm 13.44 \mu\text{g/dl}$ ), ( $6.99 \pm 0.90 \mu\text{g/L}$ ), ( $73.49 \pm 13.44 \mu\text{g/L}$ ), ( $0.23 \pm 0.04 \mu\text{g/L}$ ), ( $7.08 \pm 1.12 \text{mg/dl}$ ) and ( $3.53 \pm 0.56 \text{mmol/L}$ ) compared with that of the female controls ( $199.64 \pm 328.76 \mu\text{g/L}$ ), ( $18.83 \pm 21.81 \mu\text{g/L}$ ), ( $119.01 \pm 17.35 \mu\text{g/L}$ ), ( $0.36 \pm 0.05 \mu\text{g/L}$ ), ( $10.97 \pm 1.45 \text{mg/dl}$ ) and ( $4.61 \pm 0.73 \text{mmol/L}$ ) respectively. Table 4 shows the mean  $\pm$ SD values of micronutrients obtained for male and female sickle cell subjects. There was no significant difference ( $p > 0.05$ ) between the mean micronutrient levels

between male and female sickle cell disease subjects. The mean serum iron, manganese, serum zinc, serum cobalt, serum calcium and serum magnesium levels of sickle cell male subjects were ( $84.94 \pm 13.44 \mu\text{g/dl}$ ), ( $7.17 \pm 0.62 \mu\text{g/L}$ ), ( $76.20 \pm 9.28 \mu\text{g/L}$ ), ( $0.24 \pm 0.03 \mu\text{g/L}$ ), ( $7.30 \pm 0.77 \text{mg/dl}$ ) and ( $3.65 \pm 0.39 \text{mmol/L}$ ) compared with that of the female subjects ( $87.65 \pm 9.28 \mu\text{g/dl}$ ), ( $6.99 \pm 0.90 \mu\text{g/L}$ ), ( $73.49 \pm 13.44 \mu\text{g/L}$ ), ( $0.23 \pm 0.04 \mu\text{g/L}$ ), ( $7.07 \pm 1.12 \text{mg/dl}$ ) and ( $3.54 \pm 0.56 \text{mmol/L}$ ) respectively.

**Table I: Comparison of Micronutrient Status among Sickle Cell Subjects and Controls**

Parameters	Controls (N=50)	Test (N=50)	t-value	p-value
Iron ( $\mu\text{g/dl}$ )	172.22 $\pm$ 2.54	86.66 $\pm$ 10.84	43.24	0.000*
Mn( $\mu\text{g/dl}$ )	16.96 $\pm$ 16.88	7.10 $\pm$ 0.72	74.72	0.000*
Zn ( $\mu\text{g/dl}$ )	119.27 $\pm$ 15.68	75.20 $\pm$ 10.84	31.240	0.000*
Co( $\mu\text{g/dl}$ )	0.36 $\pm$ 0.04	0.24 $\pm$ 0.03	22.04	0.000*
Ca <sup>2+</sup> ( $\text{mg/dl}$ )	10.89 $\pm$ 1.31	7.22 $\pm$ 0.90	22.25	0.000*
Mg (mmol/l)	3.61 $\pm$ 0.45	4.69 $\pm$ 0.64	5.68	0.000*

\* Significant

**Table 2: Comparison of Micronutrient Status among Male Sickle Cell Subjects with Male Control**

Parameters	Male Control (n=20)	Male Test (n=30)	t-value	p-value
Iron( $\mu\text{g/dl}$ )	131.10 $\pm$ 13.52	87.65 $\pm$ 9.28	20.42	0.000 *
Manganese ( $\mu\text{g/dL}$ )	14.07 $\pm$ 0.90	7.17 $\pm$ 0.62	48.64	0.000 *
Zinc ( $\mu\text{g/dl}$ )	119.65 $\pm$ 13.53	76.20 $\pm$ 9.28	20.42	0.000 *
Cobalt ( $\mu\text{g/dl}$ )	0.36 $\pm$ 0.04	0.24 $\pm$ 0.03	19.95	0.000 *
Calcium (mg/dL)	10.92 $\pm$ 1.13	7.30 $\pm$ 0.77	20.39	0.000*
Magnesium (mmol/L)	4.80 $\pm$ 0.40	3.65 $\pm$ 0.39	12.95	0.000 *

\* Significant

**Table 3: Comparison of Micronutrient Status among Female Sickle Cell Subjects with Female Control**

Parameters	Female Control (n=20)	Female Test (n=30)	t-value	p-value
Iron ( $\mu\text{g/dl}$ )	131.64 $\pm$ 328.76	84.94 $\pm$ 13.44	28.30	0.000 *
Manganese ( $\mu\text{g/dl}$ )	18.83 $\pm$ 21.81	6.99 $\pm$ 0.90	43.82	0.000 *
Zinc ( $\mu\text{g/dl}$ )	119.01 $\pm$ 17.53	73.49 $\pm$ 13.44	11.23	0.000*
Cobalt ( $\mu\text{g/dl}$ )	0.36 $\pm$ 0.05	0.23 $\pm$ 0.04	11.21	0.000 *
Calcium (mg/dl)	10.87 $\pm$ 1.45	7.08 $\pm$ 1.12	11.23	0.000 *
Magnesium (mmol/L)	4.61 $\pm$ 0.73	3.53 $\pm$ 0.56	6.23	0.000 *

\* Significant

**Table 4: Comparison of Micronutrient Status based on the gender of the Sickle Cell Subjects**

Parameters	Male (n=30)	Female (n=20)	t-value	p-value
Iron (µg/dl)	87.65+928	84.94+13.44	0.67	0.519**
Manganese (µg/dl)	7.17+0.62	6.99+0.90	0.67	0.516 **
Zinc (µg/dl)	76.20+9.28	73.49+13.44	0.67	0.519 **
Cobalt (µg/dl)	0.24+0.03	0.23+0.04	0.47	0.647**
Calcium (mg/dl)	7.30+0.77	7.08+1.12	0.66	0.526**
Magnesium (mmol/L)	3.65+0.39	3.53+0.56	0.66	0.526**

\*\* Non- Significant

### Discussion

Sickle cell disorder is a hemoglobinopathy caused by point mutation in the β- chain of the globin gene. It is an autosomal recessive inheritance, and clinical severity varies widely from the sickle cell trait (heterozygous) to sickle cell anaemia (homozygous) (Wang, 2004). Our results show a significantly low level of serum Iron in sickle cell patients when compared to the apparently healthy controls. This is in tandem with the study of Olaniyi and Arinola, (2010). The serum iron levels was higher among male sickle cell subjects compared to female sickle cell patients. The difference was however not statistically significant. A plausible explanation for this low level of iron may be due to constant use of iron in the formation of normoblast after occlusive crisis experience by the patients. Iron is important in the synthesis of Haemoglobin while copper and zinc play very important roles in iron metabolism (Chan *et al.*, 1999, Okochi and Okpuzor, 2005). Therefore continuous use of iron in haemoglobin synthesis in SCD patients may explain low plasma iron in these patients. Also women shed iron containing red red cell during their monthly menstruation. This may be responsible for the lower serum iron status of female sickle cell disease subjects compared to male subjects.

Manganese is an antioxidant as it is incorporated into the antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase (Halliwell and Gutteridge, 2007). Our report observed a significantly decreased level of manganese in sickle cell patients when compared with their apparently healthy counterparts. Also male and female sickle cell patients were compared Serum manganese level

was higher among sickle cell disease male subjects compared to females. The difference however was not statistically significant. These reduced levels of manganese could be due to consumption as antioxidants to ameliorate oxidative stress, which follows free radical load in sickle cell disease patients.

Our results show, a significantly decreased levels of serum zinc concentration in sickle cell patients when compared with apparently healthy controls. Our finding is in agreement with previous reports (Prasad *et al.*,1967; Bot *et al.*, 2013) who reported that zinc deficiency was a common occurrence among sickle cell disease patients. This low zinc levels among sickle cell disease patients may be responsible for manifestations such as growth retardation, hypogonadism in males, hyperammonaemia, abnormal dark adaptation and cell mediated immune disorder. Similarly, the biochemical evidence for zinc deficiency in patients with SCD includes low zinc concentrations in plasma, erythrocytes, lymphocytes and granulocytes (Zemel *et al.*, 2002, Singhi *et al.*, 2003). In a report by Prasad *et al.*, (1967), low activities of zinc dependent enzymes such as carbonic anhydrase, alkaline phosphate and thymidine kinase have also been observed. Due to the low level of Zinc in sickle cell patients, a higher than normal activity of plasma ribonuclease has been observed in patients with sickle cell disease, because zinc is a known inhibitor of this enzyme activity (Parad *et al.*, 1967). Zinc deficiency can also be the result of the adverse effect of hydrourea which increase zinc excretion as previously reported by Silliman *et al.* (1993). In a study on sickle cell disease by Parad *et al.*, (1975) on the effect of zinc



supplementation on the growth and body composition of children with sickle cell disease, they observed that zinc deficiency, resulted in growth retardation, which is a major clinical problem in sickle cell patients. Prasad *et al.*, (1975) therefore affirm that zinc supplementation could help sickle cell patients resume normal growth. In addition, these patients showed a reduction in crippling attacks of severe abnormal pain and vomiting, which had hitherto caused them to be hospitalized more often than not.

Our result shows significantly decreased level of serum cobalt in sickle cell patients when compared with apparently healthy subjects. Cobalt is a part of the vitamin B<sub>12</sub> molecule as cobalamin and has no other known function in humans (Farell *et al.*, 2010). Free cobalt cannot be incorporated in body's' vitamin B<sub>12</sub> pool, hence diet has to supply body's B<sub>12</sub> needs. Therefore the significant decrease observed for cobalt in sickle cell subjects could suggest low dietary intake of vitamin B<sub>12</sub>.

We observed a significantly decreased level of serum magnesium in sickle cell disease subject when compared with the control. Our finding is in agreement with previous reports (Defrancheschi *et al.*, 1994; Nnodim *et al.*, 2014). This probably contributes to red blood cell dehydration and a concomitant increase in the symptoms of sickle cell disease. Sickle red blood cells are fragile and dehydrated. It has been shown that magnesium is not only useful in reducing the painful episode in sickle cell subjects, but also affects the hydration of red blood cells.

Also, our study observed a significantly decreased serum calcium levels in sickle cell subjects when compared with the control. This is in accordance with the reports by Nduka and colleagues (1995) who reported a similar trend of calcium in sickle cell subjects. These observations could suggest that calcium homeostasis is affected in sickle cell disease (Nduka and Ekeke, 1987; Al-Dabbagh *et al.*, 1989; Muhammed *et al.*, 1993). A tendency towards hypocalcaemia in these sickle cell subjects has been found in others studies (Nduka and Ekeke, 1987; Al-Dabbagh *et al.*, 1989; Muhammed *et al.*, 1993). The fact that patients with sickle cell disease are not universally hypocalcaemic may be explained by the complex nature of calcium homeostasis and the possible role of vitamins and hormones, as suggested by Al-Dabbagh and his colleagues (1989). The similarity of our results to those of earlier studies (Nduka and Ekeke, 1989; Al-Dabbagh *et al.*, 1989; Muhammed *et al.*, 1989) and the absence of

variation in the calcium levels between crisis and steady state periods of sickle cell disease strongly suggest that the commonest presenting feature of the disease is the bone manifestations which is an inherent aspect of the disease (Nduka and Ekeke, 1987; Muhammed *et al.*, 1993).

Genetic diseases especially hereditary blood disorder such as Sickle Cell Disease (SCD) is a significant problem in many countries. Their chronic nature with no prospect for cure, make them important causes of morbidity and mortality. Considering the results obtained in this study, it could be inferred that sickle cell subjects are predisposed to nutritional deficiencies. This is supported by the significant decrease levels of iron, manganese, zinc, cobalt, calcium, magnesium.

### Recommendations

We advocate that management of sickle cell disease should include consideration for early and prompt diagnosis and improvement on general health conditions such as improved sanitation, healthy nutrition, immunization, prophylaxis against infection and public education programmes aimed at preventing early mortality. Research programmes are also needed to develop comprehensive treatment plan tailored to Africa with particular reference to Nigeria. It is advisable to include trace elements supplementation as adjuvant therapies used for the management of sickle cell disease patients.

### References

1. Al-Dabbagh, A.A., Acquaye, J.K., Al-Baghdadai, T.M. and El-Zarnzamy M.M. (1989): Radiological bone changes of sickle cell anaemia in Western Saudi Arabia. *Saudi Medical Journal*; **10**: 367-371.
2. Ballas, V.V., Kalinyak KA. and Bean, J.A. (2002): Hyperhomocysteine. nia is associated with low plasma pyridoxine levels in children 'with sickle cell disease. *J. PediatrHemntolOncol.* 24(5):374-379.
3. Bot, Y.S, Benjamin, A., Nyango, D.Y., Ventmack, D.T., Eunice. C.B. Etukudo, N.S. and Obeta, M.D. (2013): Analyses of Cu and Zn in serum of sickle cell disease patients in Jos. *African Journal of Medical Biochemistry*; **1** (1): 1-4.
4. Chan, A.C., Chow, C.K. and Chiu, D. (1999): Interactions of oxidants and their implications. in genetic anaemia. *Proceedings of the Society for Experimental Biology and Medicine*; **222**: 274-282.
5. De Franceschi, L., Sandane, N., TrudeL M., Alpers, S.L., Brugnara, C and Beuzard, Y. (1994): Treatment with oral clotrimazole blocks Ca<sup>2+</sup> activated K<sup>+</sup> transport and reverses erythrocyte dehydration in transgenic SAD mice. A model for therapy of sickle

- cell disease. *Journal of Clinical Investigation*; **93**:1970-1676.
6. Farell, J.J. (2010): Digestion and Absorption of nutrients and vitamins. In: Feldman M, Friedman LS, Brandt LJ (eds). *Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 9th ed.* New York:423-427.
  7. Halliwell, B. and Gutteridge, J.M.C. (2007): *Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death*, 3<sup>rd</sup> ed. New York Oxford University Press: 187-267.
  8. Kaneko, J.J. (1999): *Clinical biochemistry of animals*, 4th edition (ed Kaneko. J.J). Academic Press Inc, New York: 923.
  9. Kumar, V., Abbas, A. K., Fausto, N. and Aster, J. (2009): *Robbins and Cotran Pathologic Basis of Disease, Professional Edition: Expert Consult - Online (Robbins Pathology) (Kindle Locations 33498- 33499).* Elsevier Health. Kindle Edition.
  10. Malowany, J. I. and Butany, J. (2012): "Pathology of sickle cell disease". *Seminars in Diagnostic Pathology*; **29** (1): 49-55.
  11. Muhammed, S., Addae, S., Suleiman, S., Adzaku, F., Annobil. S. and Kaddoumi, O. (1993): Serum calcium, parathyroid hormone and vitamin D status in children and young adults with sickle cell disease. *Annals Clinical Biochemistry*; **30**:45-51.
  12. National Institute of Health (1995): Management and Therapy of Sickle Cell Disease; **95**: 2116- 2117.
  13. Nduka, N. and Ekeke, G. (1987): Serum calcium and protein in haemoglobin- SS patients. *Folia Haematology*; **114**:508-511.
  14. Nduka, N., Kazem, Y. and Saleh, B. (1995): Variation in serum elect. and enzyme concentrations in patients with sickle cell disease. *Clinical Pathology*; **48**:648-651.
  15. Nnodim, J.K., Meludu, S.C., Dioka, C.E., Onah, C., Chilaka, UJ. and Obi, P.C. (2014): Altered Membrane Potential and Electrolyte in Sickle Cell Anemia. *Journal of Krishna Institute of Medical Sciences*; **3** (1): 70-73.
  16. O'Dell, B.L. and Sunde, R.A. (1997): *Handbook of Nutritionally Essential Mineral Elements*. New York. Marcel Decker Inc: 2-6.
  17. Okochi, V.I. and Okpuzor. J. (2005): Micronutrients as therapeutic tools in the management of sickle cell diseases, malaria and diabetes. *African Journal of Biotechnology*; **4**(13): 1568-1579.
  18. Olaniyi, J. A. and Arinola, O. G. (2010): Nitric oxide and trace metals in relation to haemoglobin F concentration in Nigerian sickle cell disease patients. *Turkish Journal of Medical Science*; **40** (1): 109-113.
  19. Platt, O.S.; Brambilla, D.J. and Rose, W.F. (2004): Mortality in sickle cell disease, life expectancy and risk factors for early death. *New England Journal of Medicine*; **330**(23): 1639-1644.
  20. Platt, O.S. (2008): "Hydroxyurea for the treatment of sickle cell anemia". *New England Journal of Medicine*; **358** (13): 1362-1369.
  21. Prasad, A .S., Oberleas, D. and Wolf, P.L. (1967): Studies on zinc deficiency. *Journal of Clinical Investigation*; **46**:549-557.
  22. Steinberg, M.H., Barton, F., Castro, O., et al. (2003): "Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment". *Journal of American Medical Association*; **289** (13): 1645-5116.
  23. Wellems, T.E., Hayton, K. and Fairhurst, R.M. (2009): "The impact of malaria parasitism: from corpuscles to communities". *Journal of Clinical Investigation*; **119** (9): 2496-2505.
  24. Wang, W., Elliot-Mills, D. and Powars, D. (2004): Renal failure in sickle cell Anemia: Hematology and Oncology Clinics of North America; **10**(6): 1321-1331.