

## Copper and Zinc Quotient in Haemoglobinopathies

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(Received: May 22, 2011; Accepted: June 27, 2011)

### ABSTRACT

The plasma copper (Cu) and zinc (Zn) levels act as measure in various haemoglobinopathies. The role of copper and zinc deficiency in various haemoglobinopathies remains poorly defined. Growth retardation and impaired immune function, suggest a relationship between haemoglobinopathies and level of copper and zinc. Fluctuations in the essential elements levels seem to be related to the different complications associated with the disease. In most of the Haemoglobinopathies there is severe hemolysis of red blood cells that results in release of zinc from red blood cells to blood and then excreted in urine leads to zinc deficiency. Hypercupremia occurs in acute and chronic infections and hemochromatosis, which is a principal complication of haemoglobinopathies. The zinc supplementation may be helpful in reducing the sufferings of complication in patients of haemoglobinopathies

**Key words:** Copper, Haemoglobinopathies, Zinc, Thalassemia.

### INTRODUCTION

Haemoglobin is a tetrameric protein with two  $\alpha$  and two  $\beta$  chain with four oxygen binding Haem groups. Haemoglobinopathies are the most common single gene inherited disorders in man, which are widespread in world due to immigration<sup>1</sup>. World wide 330,000 affected infants are born annually being 83% sickle cell disorders and 17% thalassemias<sup>1</sup>. The gender discrimination is not observed in Thalassemia as affected men and women are in equal ratio and occur in approximately 4.4 of every 10,000 live births<sup>2</sup> where in case of sickle cell anaemia male ratio is more as compare to female<sup>3</sup>. Haemoglobinopathies may occur due to the production of decreased amount of normal globin chain or structurally abnormal globin chain or persistence of fetal haemoglobin<sup>4,5</sup>. In Africa and Southeast Asia most of the persons are affected with alpha thalassemia where as in Mediterranean, African, and Southeast Asian descents, the natives are having beta-thalassemia<sup>2</sup>. Thalassemia is due to the defects in the synthesis of one or more of

the haemoglobin chains<sup>6</sup>. Alpha-thalassemia is prevalent with defect in  $\alpha$ -globin genes at short arm of chromosome 16<sup>7</sup>. Hb H disease is due to molecular defects of the alpha-globin gene that results in decreased expression of alpha-globin<sup>8</sup>. Hb Bart's, Hydrops fetalis associated with the absence of all the four  $\beta$ -globin gene. Beta-thalassemia is autosomal recessive disorders, characterized by absent ( $\beta^0$ ) or reduced ( $\beta^+$ ) production of  $\beta$ -globin chain of hemoglobin tetramer. Beta-thalassaemia major causes haemolytic anemia, poor growth, and skeletal abnormalities during infancy<sup>2</sup>.  $\beta$ -Thalassemia/Hb E is world's most common and important mutations [lysine for glutamic acid at position 26 of the  $\beta$ -globin chain]. The combination of Hb E and Hb S [Hb SE] results in a sickle cell disease syndrome similar to sickle beta [+]<sup>+</sup> Thalassemia. Hb Lepore is a structural  $\beta$  variant resulting from fusion of the  $\delta$  and  $\beta$  globin genes<sup>9,10</sup>. Haemoglobin S disorder is due to substitution of valine from glutamic acid at position 6 of the  $\alpha$ -globin chain with unending complication resulting in organ failure<sup>11</sup>.

### Common Complications in Haemoglobinopathies

The patients with various haemoglobinopathies suffer from various complications such as growth retardation<sup>12,13,14</sup>, impaired immune status, severe anemia, pubertal delay<sup>15</sup>, endocrine complications<sup>16, 15</sup>, rickets, scoliosis, spinal deformities, nerve compression, fractures, severe osteoporosis and painful vas-occlusive episode<sup>17</sup>. The most of the cases of haemoglobinopathies are reported to have severe endocrine dysfunction that results in many complications in organs. The increased pathophysiology in sickle cell disease and thalassemia is due to endothelial damage, inflammation and oxidative stress. The cumulative effects of various factors like trace elements, vitamins and growth hormones are involved in various haemoglobinopathies. Trace elements like copper and zinc play a vital role in preventing the oxidative stresses in human<sup>18</sup>.

### Role of Copper and Zinc

#### Copper

It is trace element which exhibits the ubiquitous property. Copper deficiency is quite rare in humans because it is a nutrient that is readily absorbed and has a very low daily requirement<sup>19</sup>. Cu is an important component of proteins essential for neural function. Copper plays an important role in development of neural tube, it is possible because of its participation in oxidative stress<sup>20-22</sup>. Copper plays an integral role in many of our physiologic processes as it acts ligand in many proteins and

enzymes. Copper plays essential role in the absorption, storage and metabolism of iron. Copper acts as Co-factor in various enzymes such as:-

1. Cytochrome oxidase [EC 1.9.3.1], terminal oxidase of the electron –transport chain.
2. Ceruloplasmin or ferroxidase I [EC 1.16.3.1], oxidation of the ferrous iron of body to ferric form and thus iron is incorporated into apotransferrin which is transported to bone marrow.
3. Ferroxidase II [EC 1.16.3.1], copper containing enzyme of high relative molecular mass, it oxidises iron thus allowing it to be mobilized and transported from liver to bone marrow to be used in erythropoieses<sup>23</sup>.
4. Dopamine  $\beta$ -hydroxylase [Ec.1.14.17.1], Dopamine–hydroxylase helps in conversion of dopamine to norepinephrine which helps in mediating many neurological functions<sup>24</sup>.

### Copper Metabolism

Absorption is mainly through the mucosa of the stomach and proximal duodenum, most of it is absorbed in small intestine. A critical component of copper gastrointestinal balance involves enterohepatic circulation. At least one-half of the amount of copper absorbed from small intestine reappears in the bile as strongly bound compounds<sup>25</sup>. Copper half-life is 13-33 days. In biological systems biliary excretion is major route of elimination<sup>26</sup>. Urinary route of excretion plays trivial role in copper clearance, principal route of excretion includes saliva, sweat and through stool<sup>27</sup>. The steps of copper metabolism are depicted in Fig. 1.

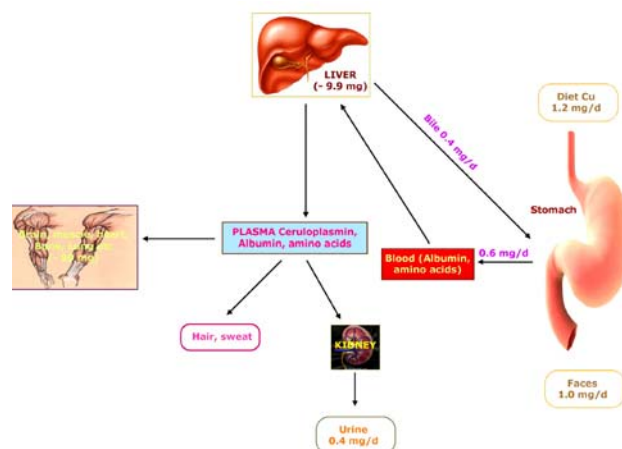


Fig. 1: Copper (Cu) Metabolisms

## Zinc

Zinc an essential trace element for the growth, development and normal enzymatic function in multiple metabolic pathways<sup>28</sup>. Zinc is second most prominent trace metal in human body after iron<sup>26</sup>. Zinc, belongs to first row of transition metals and contains partially filled d orbital [d10] therefore acts as Lewis acid [it will accept a pair of electron] in all catalytic sites<sup>29</sup>. Redox activity makes  $Zn^{2+}$  a stable ion in biological medium. Normal human body contains 2.3 gm of zinc<sup>29</sup>. High concentrations have been found in brain hippocampus, many medical researchers believe that zinc is a neurotransmitter<sup>30</sup>. It plays vital role in closure of the human neural tube<sup>31</sup>. It plays an essential role in syntheses of RNA, DNA and cell division<sup>32-35</sup>. Zinc is important for maintaining the DNA integrity<sup>35, 36</sup>. Zinc remains an vital component of all DNA and RNA polymerases<sup>32</sup>. Zinc plays an in key role in immunity by activating the gene of lymphocytes and plays role in function of cell mediated immunity<sup>34, 37</sup>. Zinc is crucial for normal salt-taste perception, wound healing, general growth and development<sup>32, 38</sup>. Zinc finger proteins are implicated in the genetic expression of steroid hormone receptors<sup>39, 40</sup>. Zinc also has anti-apoptotic<sup>41</sup> and antioxidant properties<sup>42</sup>. Zinc can counteract the oxidation by binding sulphhydryl

groups in proteins and by occupying binding sites for iron and copper in lipids, proteins and DNA<sup>43, 42</sup>. Zinc plays an important role in preventing osteoporosis as it help in normal collagen synthesis and mineralization of bones<sup>44</sup>. In human body, zinc has vital role in metabolic regulation of thyroid<sup>45</sup>. Reticulate Binding Capacity [RBC] of Zn acts as imperative indicator of mean thyroid hormone of patient who is under strict medication<sup>46</sup>. Less amount of zinc interferes with the deiodinase enzyme conversion of T4 to T3<sup>47</sup>. Zinc is a cofactor in 300 enzymes<sup>48, 29, 35</sup>, involved in a large number of enzymatic functions, fulfilling both structural [maintaining protein structure and stability] and catalytic roles [chemical catalysis]<sup>28, 29</sup>.

## Zinc Metabolism

Zinc is released from food as free ions during digestion. These liberated ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum<sup>49</sup>. Gastrointestinal loss of zinc is half of the total, which is from unabsorbed dietary zinc, a small contribution from intestinal cell shedding, and 1-2 mg from the pancreatic and biliary secretions<sup>49, 50</sup>. The steps of zinc metabolism are depicted in Fig. 2.

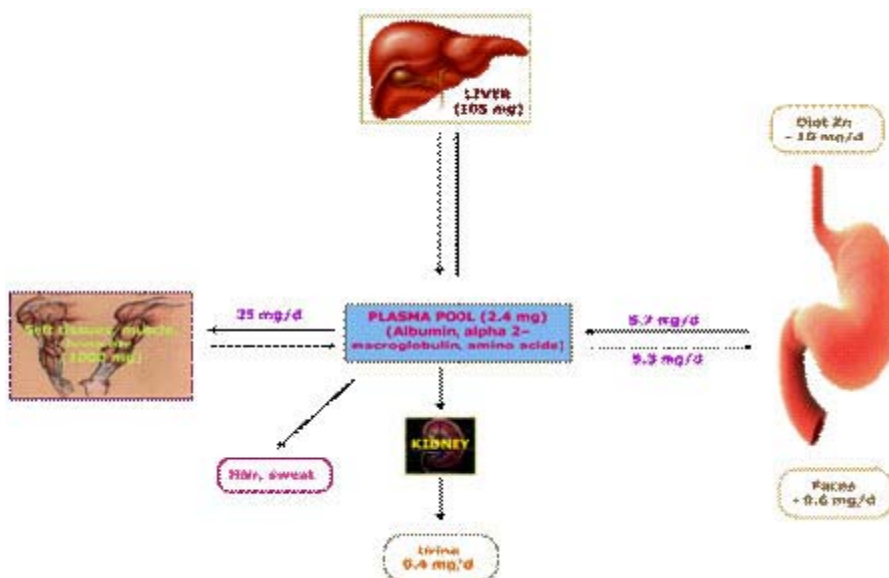


Fig. 2: Zinc (Zn) Metabolism

### The determination of Copper and zinc ratio

The copper and zinc are determined by the help of atomic absorption spectrophotometer (AAS). The plasma sample is used for determination of respective elements<sup>58,68</sup>.

### Role of Copper and Zinc [Cu:Zn]ratio in deficiency diseases

Trace elements play an important role in numerous biological systems through their action as activators or inhibitors, thus competing with other elements and protein for binding site, influencing the permeability of membrane<sup>51</sup>. Past reports indicate that serum levels of Cu: Zn is abnormal in patients with diseases like Cancer<sup>52</sup> and various haemoglobinopathies. So Cu: Zn has received substantial consideration in thalassemia, sickle cell anemia and various other hemoglobinopathies. Persons having disease related to hemoglobin have poor immune status<sup>53</sup>. Major effect is observed due to micronutrients which are essential to the body but are required in trace amount. The plasma Cu:Zn ratio is more valuable indicator of state of disease in affected persons. The serum zinc level of thalassemia patients was less as compared to serum copper level<sup>54</sup>. The marked zinc deficiency in thalassemia subjects was due to hyperzincuria [loss of zinc in urine]<sup>32</sup>. Deficient linear growth in beta-thalassemia is more prominent in children having low plasma zinc level<sup>55-57</sup>. The improvement in growth was observed in patients treated with zinc supplementation<sup>55</sup>.

Thalassemia patients when treated with intravenous desferrioxamine [an iron chelator]<sup>58</sup> results in zinc deficiency [due to chelation of zinc also] results in retinal abnormalities.

Level of calcium, copper and zinc was assessed in patients of beta-thalassemia/hemoglobin E, It was found to be less in both red cells and plasmas compare to control<sup>59</sup>. Zinc deficiency was studied in thalassemic natives of Tehran<sup>60</sup>. Zinc deficiency increases of inability of pancreas to secrete insulin in response to glucose stimulus in Beta Thalassemia subjects<sup>61</sup>. Study conducted in Thalassemia patients of Mosul, Iraq showed low level of serum zinc compared to normal subject on contrary serum copper level was high<sup>62</sup>. Zinc deficiency is also considered a causative

factor of osteoporosis and endocrinopathies in Thalassemic subjects<sup>63</sup>. Osteoporosis is common even in well-treated thalassemia patients<sup>64</sup> thus making subjects more prone to fractures. The bone mineral density shows strong correlation with serum zinc<sup>60,65</sup>. The results were supported by study carried on thalassemia adolescents and outcome showed low zinc level as compare to control ones, affecting bone mineral density<sup>66</sup>. Serum Zinc has positive effect on height/ age of thalassemic major patients<sup>67</sup>.

The comparative study on Cu: Zn in Hemoglobin H, Beta –thalassemia/ Hemoglobin E disease indicates the lower level of zinc to copper in affected individuals. The zinc insufficiency was observed due to hemoysis causing hyperzincuria [loss of zinc in urine] resulting in undergrowth in these patients<sup>68</sup>. Cu: Zn status of Hemoglobin H disease, Beta–thalassemia/HbE and homozygous beta-thalassemia was evaluated, Zinc level in plasma and hair was lower as compare to erythrocytes where as copper level was higher in plasma and erythrocytes<sup>69</sup>. As serum zinc level is decreased, serum Zinc binding capacity [ZnBC] is increased in nutritional zinc deficiency but in thalassemia patient's serum zinc level is decreased and ZnBC does not increase in chorus<sup>70</sup>, this result is confirmed by improvement of anemia with high serum zinc level<sup>71</sup>.

In sickle cell subject the zinc level was analyzed in children<sup>72</sup> and marked zinc deficiency was found due the loss of zinc in urine [hyperzincuria]<sup>73, 72, 74</sup>. Homozygous sickle cell anemic subjects plasma zinc levels were significantly decreased<sup>75</sup>. The many complication in sickle cell disease like poor ulcer healing, growth retardation, delay in sexual development, immune deficiencies, and high irreversible sickle cell [ISC] counts are related to low level of the zinc<sup>76</sup>. Zinc-dependent proteins alkaline phosphatase [AP] and retinol-binding protein [RBP] were present in lower amount in serum of sickle patients. The serum zinc was low due loss in urine, zinc malabsorption and chronic intravascular hemolysis<sup>77</sup>. Zinc supplementation in case of homozygous sickle cell anemia reduces irreversible sickled cells<sup>78</sup>. The zinc loss in excretion was more as compare to copper where as plasma concentration of there trace elements were contrary in case sickle cell anaemic

subjects<sup>79</sup>. The Zinc deficiency in sickle cell anaemic patients was compared with control and found that even the dietary intake was similar in both cases but marked retardation in growth and low level of red cell zinc and other vitamins were present in former<sup>80</sup>. In the comparative study of homozygous and heterozygous Sickle cell anemia in Eutrophic children, zinc level in plasma of homozygous Sickle cell anemia was less in comparison to heterozygous Sickle cell anemia and control subjects, reverse condition was observed in the case of plasma level of copper in same subjects<sup>81</sup>. Decrease level of zinc in plasma of sickle cell anemic patients results in decrease in linear growth, skeleton growth, muscle mass and skeleton maturation<sup>82</sup>. The Sickle cell patients when treated with zinc the improvement was observed both in growth and level of zinc in plasma<sup>83, 84</sup>. In the case of haemoglobinopathies subjects are more prone to oxidative damage due to chronic redox imbalance in Red blood cell. Uninhibited production of ROS [Reactive oxygen species] often leads to damage of DNA, protein and lipids<sup>85</sup>. In sickle cell patients copper level is inversely proportional to zinc in plasma that result in fabrication of reactive oxygen thus enhancing the pathophysiological condition in the patients<sup>17</sup>. Zinc supplementation facilitates in improving the immune status thus preventing the nosocomial as well other infections<sup>86</sup>.

Some contradicting reports state that copper and zinc was normal in Sickle cell anemic patients of eastern Province of Saudi Arabia but

opposite condition was observed in sickle cell anemic subjects of North American Black<sup>87</sup>. In case of the comparative study carried on Serum copper and zinc levels in sickle cell anaemia and beta-thalassaemia in North Jordan, copper levels were significantly increased in beta-thalassaemia and sickle cell anaemia where as zinc levels were significantly increased in beta-thalassemia but significantly decreased in Sickle cell anaemia [SCA]<sup>88</sup>. Only 3 of the 68 thalassaemic patients had zinc deficiency in population study<sup>89</sup>. Zinc status was found normal in Beta-thalassemia subjects who are getting regular blood transfusion and chelators<sup>90</sup>.

## CONCLUSION

Wide discrepancy seen by various workers and mentioned in different reports, the cause in variation endorsed due to person to person variation, natural factors and protocol of treatment, blood transfusion and chelators. Anemia parameters showed significant positive correlation indicating that anemia improves in patients having high serum Zn level. Patients of Haemoglobinopathies with complications showed higher plasma Cu:Zn ratio than the patients with normal development. These results indicate the usefulness of using this ratio more efficiently than using each one alone. This conclusion based on decreased level of serum Zn and increase Cu:Zn ratio hence Zn supplementation is recommended for patients having different hemoglobinopathies with complications and delayed milestones of development.

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