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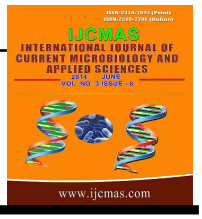

Review Article

Trace Elements Ratio in Patients of Haemoglobinopathie

Ramdas Malakar*, Manpreet Kour, Afroze Ahmed, S.N. Malviya and C.B.S. Dangi

Department of Biotechnology, RKDF University Bhopal (M.P.) India
**Corresponding author*

A B S T R A C T

Trace elements play an important role in many biological systems because they act as activators or inhibitors, hence competing with other elements and protein for binding site. Major effects due to micronutrients is observed which are required in trace amount but vital to the body. Haemoglobinopathies are inherited single gene disorders caused by genetic mutations that result in abnormal, disfunctional hemoglobin molecules or lower levels of normal haemoglobin molecules in red blood cells. The most common haemoglobinopathies are thalassaemias and sickle cell disease (SCD). The aim of this review is to scrutinize the relationship between serum trace elements. The role of trace elements like iron, copper, zinc, selenium, magnesium, chromium and iodine in Haemoglobinopathie patients reveals a significant change of these trace elements. The administration of selective antioxidants along with essential trace elements and minerals to reduce the extent of oxidative damage and related complications in Haemoglobinopathie still need further evaluation.

Keywords

Thalassaemias, sickle cell disease and trace element

Introduction

Haemoglobinopathies are inherited single gene disorders caused by genetic mutations that result in abnormal, disfunctional hemoglobin molecules or lower levels of normal haemoglobin molecules in red blood cells. The most common haemoglobinopathies are Thalassaemias and sickle cell disease (SCD) (Old, 2013). Thalassemia and other structural hemoglobinopathies are the major erythrocytic genetic disorders prevalent in certain parts of the world including India. While the general incidence of thalassemia trait and sickle cell hemoglobinopathy in India varies between 3-17% and 1-44%, respectively because of consanguinity and, caste and area endogamy, some communities show a very high incidence, making the disease as a major public health problem in our country (Balgir, R.S., 2005).

Thalassemia syndromes are a heterogeneous group of single gene disorders, inherited in an autosomal recessive manner, prevalent in certain parts of the world. Beta-thalassemia is the most common single gene disorder in our country. In fact beta-thalassemia has emerged as a huge public health problem worldwide. Increase in survival of patients with this disorder has led to

more prevalence of this disease. Reportedly, There are about 240 million carriers of Beta-thalassemia worldwide, and in India alone, the number is approximately 30 million with mean prevalence of 3.3%. The Sickle Cell Disease also known as Sickle cell anemia is very common among the tribal's in India and other parts of the world. Sickle Cell Anaemia is hereditary and occurs due to an inherited abnormal haemoglobin (Hb) gene passed through their ancestors from Africa, Saudi Arabia, and India. The Soliga tribe also has the Sickle cell anaemia disease which was first revealed by Dr. H. Sudarshan in B.R. Hills whereby treatment was started in the tribal hospital and the Sickle cell test was conducted to identify the number of individuals so as to enable a regular follow up of such affected patients (Madegovda c., *et al.,* 2013).

Trace element play rolls in haemoglobinopathie

Trace elements play an important role in many biological systems because they act as activators or inhibitors, hence competing with other elements and protein for binding site, influencing the permeability of membrane (Cavallo *et al.,* 1991). Trace elements play a pivotal role in the human body and participate in various bio-chemical reactions. Zinc is the abundant intracellular element with 85% of total zinc found in muscles and bone where as 0.1 % in the plasma. It forms structural part of more than 300 metalloenzymes like super oxide dismutase. Plays an essential role in human growth, development acts as an antioxidant synthesis, storage and secretion of insulin host defense mechanisms and in thyroid metabolism, its deficiency causes reduction in concentration of T3 in plasma (Kaur, M. *et al.,* 2013). Iron Copper and zinc are essential trace elements in human body and all are altered in different types of blood diseases including thalassaemia in which they may play a role in pathogenesis. The alteration of these elements combined with excess amounts of haemoglobin subunits enhance the generation of oxygen radicals after a chain of reactions leading to early death of the red cells and haemolysis.

Iron

Iron is another essential trace element present in almost all cells of the body. Human body requires iron for the synthesis of oxygen carrying protein called haemoglobin found in red blood cells, and myoglobin which is also a protein found in muscles. It also takes part in the production of other important proteins in the body such as for DNA synthesis and cell division. Furthermore, iron is used in the connective tissues in our body, some of the neurotransmitters in our brain, and to maintain the immune system. Iron is transported through the blood by the serum protein, called transferrin. Transferrin is normally 30% saturated with iron. The total iron binding capacity (tibc) reflects the status of iron in the body and is defined as the amount of iron needed for 100% transferrin saturation (Shazia Q. *et al.,* 2012).

When iron is present in excess amounts in the body it will lead to hemochromatosis, which may be primary or secondary. Primary hemochromatosis is a genetic disorder characterized by increased iron absorption and consequent iron overload in the body. Secondary hemochromatosis occurs in diseases like thalassemia due to iron overload especially in thalassemia major where repeated blood transfusions

are required (Shazia Q et al., 2012). Beta thalassemia major patients require frequent blood transfusions which lead to iron overload in the absence of effective chelation therapy. This iron deposits in thalassemic patients can exceed from the storage and detoxification capacity of ferritin and also fully saturates transferrin and leads to the formation of free iron which accumulates in blood and tissues. This free iron will cause the formation of very harmful compounds, such as hydroxyl radical (OH). The hydroxyl radicals are highly reactive and attacks lipids to form lipid peroxides which contribute to oxidative stress (Raghuveer, R. *et al.,* 2009).

Copper

Copper is the other essential trace element present in our bodies. It mostly forms metalloprotiens which act as enzymes. Copper is the major component of hemoglobin which is a protein responsible for oxygen transport in blood cells. Along with vitamin C, it is responsible for the production of protein called elastin thus maintaining the elasticity of the skin, blood vessels, and lungs. It is antibacterial and bears important antioxidant properties (Shazia Q *et al.,* 2012). It is an essential element for humans and animals. In the human organism, copper exists in two forms -the first and second oxidation form, as most of the copper in the human organism is in the second form (Uauy *et al.,* 1998). Copper deficiency is quite rare in humans because it is a nutrient that is readily consumed and has a very low daily requirement (Williams 1983; Turnlund *et al.,* 1998). It can be found in almost every cell of the human organism. The highest concentrations of copper are discovered in the brain and the liver; the central nervous system and the heart have high concentrations of copper as well (Rosalind, 2005). Cu is an important component of proteins essential for neural function (Morton *et al.* 1976; Burkitt 2001; Cengiz *et al.,* 2004). Its unique aptitude to exist in distinct redox states, copper is able to function as a critical catalyst (Pardo *et al.,* 1995). It plays an integral role in many of our physiologic processes, including acting as a ligand to many proteins and enzymes (Turnlund, 1998) and a vital role in human health and is needed for a wide range of biological processes, including maintaining a healthy heart and liver bone strength and brain development. Too little copper in the body can actually lead to disease (Araya *et al.,* 2007). Copper is necessary in human nutrition for normal iron metabolism and the formation of red blood cells (Johnson *et al.* 1992).

Cu is a functional component of several essential enzymes, known as copper enzymes. That catalyzed many enzymatic reactions, which are essential for the proper functioning of the brain and the nervous system (Harris, 1997). Copper acts as Co-factor in various enzymes such as- cytochrome c oxidase, lysyl oxidase, feroxidase, 2-furoate-CoA dehydrogenase, amine oxidase, dopamine beta-monooxygenase, flavonol 2, 4-dioxygenase, superoxide dismutase, PHM (peptidylglycine monooxygenase hydroxylation) and others (Harris 1997; Uauy *et al.* 1998; Malte Rolff *et al.* 2008). The copper response to thalassemia was variable. The copper concentration in the brain, heart and spleen was significantly higher in thalassemic than nonthalassemic (Shuler *et al.,* 1990). They found lower serum zinc and magnesium levels and higher copper and potassium levels in thalassemic major patients as compared to controls. Zinc deficiency may be due to

hyperzincuria resulted from the release of zinc from hemolyzed red cells while hypercupremia occurs in acute and chronic infections and hemochromatosis which is the principal complication of thalassemia. A study done on status of thyroid function and iron overload in patients with beta thalassemia major on Deferoxamine in Jordan concluded that there is significantly high ($P < 0.05$) levels of serumferritin, FT3, zinc, and copper in patients with beta thalassemia major as compared to controls (Irshaid *et al.,* 2009).

Zinc

Zinc is an essential trace element for the growth, development and differentiation of all types of life, including microorganisms, plants and animals (Vallee, 1986; Ackland *et al*., 2006). After iron, zinc is the second most abundant trace metal in the human body; an average 70-kg adult human contains 2.3g of zinc (McCance and Widdowson, 1942). 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 (McCall *et al.,* 2000). Zinc occurs in a wide variety of foods, but is found in highest concentrations in animal sources, particularly beef, pork, poultry and fish, and in lesser amounts in eggs and dairy products. Zinc content is relatively high in nuts, legumes and whole grain cereals and is lower in fruits and vegetables (Lonnerdal, 2000). Zinc is present in all body tissues and fluids.

The total body zinc content has been estimated to be 30mmol (2g). Skeletal muscles content approximately 60% of the total body zinc. Plasma zinc represents only about 0.l percent of total body zinc content. High concentrations of zinc are found in the choroid of the eye 4.2 mmol/g (274 μg/ g) and in prostatic fluids 4.6-7.7 mmol/l (300- 500mg/l) (Hambidge, 1987). It is an important cofactor for more than 300 enzymes needed for cell function in the eyes, kidneys, muscles, skin, and bones (Sly *et al.* 1983; Prasad, 1995; Mahyar, 2005) and involved in nucleic acid metabolism such as DNA and RNA polymerases, reverse transcriptase and tRNA synthetases **(**Cousins, 1996**).** In the nucleus, Zn is a well-known cofactor for nucleic acid polymerases and is associated with the high mitotic activity that has a possible role in cell growth (Dewis *et al.* 1972; Fenton *et al.* 1985).

Zinc is a component of alcohol dehyrdogenase, which is important in the conversion of retinol to retinal (required for vision in humans) (Groff *et al.* 1995). Zinc plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity (Shankar *et al*. 1998), neutrophils, and natural killer cells (Prasad, 2008). It is an important immunoregulatory effect on lymphocytes, lymph tissue, neutrophiles, macrophages, mastocytes, and platelets (Bekaroglu *et al*. 1996). Zinc supports the work of numerous proteins in the body among them are the metalloenzymes, which are involved in a variety of metabolic processes (Hambidge, 1989). Zinc also frees the vitamin folate (so it can move across cell membranes), aids in the manufacture of heme, aids in essential fatty acid metabolism, and helps release vitamin A from its storage place in the liver (Groff *et al.* 1995). Zinc plays an important role in preventing osteoporosis as it help in normal collagen synthesis and mineralization of bones (Hyun *et al*. 2004). Zinc deficiency cause growth retardation, skeletal abnormalities, defective collagen synthesis and/or cross

linking, poor wound healing, night-blindness, impaired immune function, and impaired protein synthesis (Groff *et al.* 1995). Zinc is involved in the replication of the HIV virus (Cuajungco *et al.* 1997). Zinc supplementation has been successfully used as a treatment of many illnesses and disorders, including dwarfism, sexual immaturity, acrodermatitis enteropathica (inflammation of the skin and the small intestine), anorexia nervosa and bulimia nervosa (Bryce-Smith 1989).

In sickle cell subject the zinc level was analyzed in children (Karayalcin *et al.,* 1979) and zinc deficiency was found due to hyperzincuria [loss of zinc in urine] (Prasad *et al.,* 1975; Karayalcin *et al.,* 1979; Niell *et al.,* 1979).The plasma zinc levels in homozygous sickle cell anemic patients were drastically decreased (Muskiet *et al.,* 1984). Due to low level of zinc many complication like poor ulcer healing, growth retardation, delay in sexual development, immune deficiencies, and high irreversible sickle cell [ISC] are counted (Reed *et al.,* 1987). In sickle cell patient serum zinc dependent proteins alkaline phosphatase [AP] and retinol-binding Protein [RBP] were present in inferior amount. The serum zinc was low due to loss in urine; zinc malabsorption and chronic intravascular hemolysis (Phebus *et al.,* 1988). In homozygous sickle cell anemia zinc supplementation trim down irreverssible sickled cells (Muskiet *et al.,* 1991). Copper loss in excretion was less as compare to zinc where as the trace element concentration in plasma differing in case of sickle cell anaemic subjects (Kilinç *et al.,* 1991). The Zinc deficiency in sickle cell anaemic patients was compared with control and found marked retardation in growth and low level of red cell zinc and other vitamins even the dietary intake was similar in both cases (Gray *et al.,* 1992).

Selenium

Selenium one of the essential trace elements in human plasma is selenium. Selenium was first discovered as a byproduct of sulfuric acid production. It is a well-known electrometalloid and is mostly famous due to its anti cancerous properties (Shazia Q. et al., 2012). It is an essential constituent of the enzyme glutathione peroxidase and also incorporates in various important proteins such as hemoglobin andmyoglobin. Selenium is also a component of the unusual amino acids selenocysteine which is essential for the production of various useful enzymes in the body. It helps in preventing free radical damage caused by ferrous chloride, and heme compounds. Its deficiency may affect the iron binding capacity of transferring which leads to increase iron stores and subsequent tissue damage. An age- and gender-matched case control study has been conducted on patients with beta thalassemia major on iron chelation therapy (Bartlay W.J. *et al.,* 2001).

Selenium is part of glutathione peroxidase which protects cell components from oxidative damage due to peroxides produced in cellular metabolism. Once absorbed, selenium interacts with the sulfur-containing amino acids (e.g., cysteine and methionine) to form the enzyme glutathione peroxidase, and for incorporation into various proteins, such as hemoglobin and myoglobin. Selenium supplements have been used in the treatment of anemia and growth problems which would not respond to other kinds of treatment (Al-Khazraji S.K., 2010). There is a study done to evaluate the *in vitro*

effects of vitamin C and selenium on natural killer cell activity of beta thalassemia major indicates a significant decreased in natural killer cell activity in all thalassemic patients as compared to control. The NK activity is increased by low-dose selenium treatment but no change is observed in control group. High-dose selenium decreased NK activity significantly in splenectomised patients. The result indicates the careful use of selenium dosage in thalassemic major patients (Atasever, B. *et al.,* 2006) the selenium concentration was markedly higher in liver and pancreas of thalassemic than of nonthalassemic (Shuler *et al.,* 1990).

Magnesium

Magnesium is another trace element which is essential for maintaining proper body functions. It is vital for body's immune system, cardiovascular, and musculoskeletal systems. Deficiency of this element will lead to hypertension, diabetes, and cardiovascular diseases. A study was carried out to evaluate the level of some essential elements in one hundred and five thalassemic blood transfusion-dependent patients and 54 healthy controls (Al-Samarrai A. H. *et al.,* 2008). They found lower serum zinc and magnesium levels and higher copper and potassium levels in thalassemic major patients as compared to controls. Zinc deficiency may be due to hyperzincuria resulted from the release of zinc from hemolyzed red cells while hypercupremia occurs in acuteand chronic infections and hemochromatosis which is the principal complication of thalassemia (Shazia Q. *et al.,* 2012). The same is true of manganese (Mg) which is known to play a fundamental role in the synthesis of glycoproteins, specially involved in bone formation. It is also known to be a component of pyruvate carboxylase and acts as a cofactor in the respiratory enzymes. These metabolic processes lead to energy production which is very much in demand in the sickle cell disease. Further discussions on these micronutrients will continue as we examine the other disease conditions that feature in this review (Okochi, V. I. *et al.,* 2005).

Iodine

Iodine is a trace element that is essential for the synthesis of thyroid hormones in vertebrates, although iodo-proteins are present in invertebrates (Frieden, 1984). Some regions of the world are naturally deficient in iodine due to the low availability of iodine from their soil or other climatic and environmental factors affecting iodine availability (Soetan K.O. *et al.,* 2010). Iodine The other vital trace element present in the body is iodine and is one of the powerful antioxidants present in the body. Bernard Courtois, a French chemist, was first discovered iodine in 1811. Iodine is present in almost every body tissue but found in greater quantities in thyroid, breast, stomach, liver, lungs, heart, adrenals, and ovaries. Iodine is important for mental and physical development and maintaining healthy immune system. It takes part in the production of thyroid hormones including thyroxin and triiodothyronine. These hormones are of primary importance in maintaining the body metabolism and brain development. Deficiency of this trace element may lead to cancer, diabetes, heart diseases, and multiple sclerosis. A study revealed increased sensitivity to the inhibitory effect of excess iodide on thyroid functions in 25 beta thalassemia major patients with normal thyroid functions (Alexandrides T. *et al.,* 2002).

The patients were given 20mg of iodine three times daily for three weeks. They found significant decrease in concentration of thyroid hormones and significant increase in TSH concentrations with 56% of the patients reached to hypothyroid levels. They concluded that beta thalassemia major patients should not be given excess iodide due to increased sensitivity to inhibitory effects on thyroid functions as it may lead to permanent hypothyroidism. A study was carried out on long-term intensive combined chelation therapy on thyroid function in 51 beta thalassemia major patients after they achieved negative iron balance (Farmaki k., 2008; and Shazia Q. *et al.*, 2012).

Chromium

Chromium is required for normal glucose metabolism (Das, 1990, Anderson, 1998). According to Das (1990), chromium functions as a glucose tolerance factor but occurs in foods in varying different forms of which it is not yet clear in which form it is most effective. However, a particular form containing an organic moiety not fully characterized has been considered as the "glucose tolerance factor" (GTF) (Okochi V.I. *et al*., 2005). Chromium is another mineral that has been found beneficial in the management of sickle cell disease. The involvement of chromium in SCD is not clearly defined but it is known that it potentiates insulin action. It has been proposed that chromium acts as a cofactor in the initial reaction of insulin with the receptor sites of insulin sensitive cell membrane (Das, 1990). If the effect of chromium is associated with insulin action, it is probable that its beneficial effect might be as a result of its involvement in carbohydrate metabolism which is a source of energy (Okochi, V. I. *et al.,* 2005).

Chromium is an essential element for animals and humans (Frieden, 1984). It has been found in nucleoproteins isolated from beef liver and also in RNA preparations (Uppala et al., 2005). It could play a role in maintaining the configuration of the RNA molecule, because Cr has been shown to be particularly effective as a cross-linking agent for collagen (Eastmond *et al.,* 2008). Cr has also been identified as the active ingredient of the glucose tolerant factor (Brown, 2003), a dietary factor required to maintain normal glucose tolerance in the rat. Trivalent chromium is a constituent of ''glucose tolerance factor'' (GTF), which binds to and activates/potentiates insulin action (Wennberg, 1994; Murray *et al.*, 2000) (Soetan K.O. *et al.,* 2010). Thalassemia caused small increases in the concentration of chromium in the kidney, liver, and spleen. (Shuler *et al.,* 1990).

Studies on trace elements like iron, copper, zinc, magnesium, selenium, chromium and iodine, reveal significant change in plasma concentration of these trace elements in beta thalassemia major patients. Zinc levels in beta thalassemia major patients were significantly decreased in most of the studies as compared to the controls. The reason proposed being hyperzincuria due to the release of zinc from hemolysed red cells. The patient ssuffering from beta thalasemia major do not survive for more than 5 years without blood transfusion (Shekhar H. U. *et al.,* 2007). A contrary study showed significantly reduced levels of serum zinc in beta thalassemia major patients (Nasr M. R. *et al.,* 2002). Copper, another essential trace element, was found to be significantly decreased [Nasr M. R. *et al.,* 2002, Shamshirsaz A. A. *et al.*, 2003) on thalassemia major patients but high levels of copper as compared to

controls. This increased level of copper may be due to acute or chronic infections and hemochromatosis that occurs as complications in thalassemia major (Al-Samarrai, A. H. *et al.,* 2008). There is one prospective study indicating no change in serum copper levels in thalassemia major patients. Iron being the most important of all minerals was found to be significantly increased in beta thalassemia major patients (Mahyar A., *et al.,* 2010). Probably due to repeated blood transfusions and increased iron absorption from gastrointestinal tract. Studies also showed significantly decreased plasma concentrations of selenium in thalassemia major patients.

The involvement of chromium in SCD is not clearly defined but it is known that it potentiates insulin action. It has been proposed that chromium acts as a cofactor in the initial reaction of insulin with the receptor sites of insulin sensitive cell membrane (Das, 1990). If the effect of chromium is associated with insulin action, it is probable that its beneficial effect might be as a result of its involvement in carbohydrate metabolism which is a source of energy. Another important trace element is magnesium that plays an essential role in maintaining body's immune system as well as cardiovascular and musculoskeletal system found to be significantly higher in patients with beta thalassemia major as compared to controls (Al-Samarrai, A. H. *et al.*, 2008). Studies have shown that excess of iodine which is vital for the production of thyroxin and tri-iodothyronine may cause permanent hypothyroidism in beta thalassemia patients (Tantawy A. A. *et al.*, 2008). In addition, hypocalcaemia was found in beta thalassemia major patients than in controls (Soliman A. *et al.*, 2008).

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