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Zinc and copper homeostasis is crucial to maintain the cellular health and their role in viral diseases including COVID-19

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Both, zinc and copper play important roles in human metabolic processes. In humans, zinc (Zn) is required directly for the chemical catalysis and/or maintaining the structure of nearly 10% of total body proteins. It plays a significant role not only in immune defence but also takes part in DNA and protein synthesis, growth and development throughout the life span as well as in tissue repair. On the other hand, copper (Cu) is crucial to strengthen the skin, epithelial tissue, connective tissue and blood vessels. Cu helps to increase the level of haemoglobin, melanin and myelin in our body. Both of these trace metals possess antioxidant like properties. However, it is necessary to balance the optimal concentration of Zn or Cu in blood serum to avoid the associated organ damage. Excess zinc intake increases the incidence of chronic kidney disease (CKD) which is harmful to normal renal function and thus elevated the risk of prostate cancer. Similarly, the one and only reason for heart, kidney and liver failure including Wilson disease is the excess amount of copper. Both of these trace metals are responsible to deal with brain diseases. Thus, there are many "faces" of Zn and Cu in the maintenance of cellular network including immunomodulatory regulation and infection prevention. Zinc appears to inhibit the enzymatic processes of viral protease and polymerase, as well as different physical processes for instance virus attachment, inflammation, and viral uncoating. Ideally, the clinicians should monitor zinc status of the individuals and advice for the supplements when necessary, otherwise deficiency of these micronutrients could lead to the onset of severe secondary diseases.

Keywords: Zinc, copper, immunomodulatory, signalling network, viral infection.

Introduction

It has been estimated that nearly two to four grams of zinc (Zn) is distributed throughout the human body[1]. Even though copper (Cu) is considered as the third most abundant trace metal [next to iron and zinc], its total amount in the human body ranges only between 75–100 mg[2]. Zinc and copper ions are involved with numerous aspects of cellular metabolism via electrochemical oxidation and reduction reactions. The proportion of copper to zinc is clinically more significant than the concentration of both of these trace metals[3]. Like oxidation and reduction reactions in electrochemical cell, copper and zinc metal ions play similar role in presence of human cell plasma, where the electron movement occurred in Zn and Cu through an electrically conducting pathway as an electromagnetic wave – leading to a better model for the cellular neurotransmission process.

Zn has versatile functions in physical improvement, enzymatic catalysis and signal transduction of biological system. Under certain circumstances the attachment of zinc to membrane is followed by binding with redox-active metals (like iron and copper), not only but also zinc acts as an essential component of both intracellular and extracellular Cu/Zn superoxide dismutase (Cu/Zn-SOD). The behaviour of zinc like an antioxidant is controlled by means of various regulators such as NF-κB, p53, AP-1, and some other enzymatic actions during cellular signal transduction at multiple cell levels. Several studies have shown that Zn supplement is helpful to improve the pathological conditions[4].

Zn is a crucial metal indispensable for proper assembly and progress for functioning of nearly about 2800 macromolecules and more than 300 enzymes. Around 83% of zinc proteins carry out enzymatic catalysis in prokaryotic organisms[5]. Eukaryotic organisms also use Zn for various biological purposes such as the regulation of zinc-related proteins in catalytic reactions (47%), DNA transcription (44%), protein transport (5%), and signalling pathways (3%). Zn is essential in stabilization of the membrane construction mechanism, and it supports to maintain membrane integrity against damages due to changes in osmotic potential, platelet aggregation, and other progressions[6]. Subsequently, the extensive shortage of Zn is associated with several health consequences for example weaknesses, growth and development retardation while it is required for proper immune, reproductive, and neurosensory systems[4,5].

Importance of zinc in human biochemical reactions:

Zinc does not contribute to redox active reaction under physiological conditions in contrast to other transition metals. It is designated as the second most abundant trace metal, found in eukaryotes second only to iron. It operates as a Lewis acid to receive a pair of electrons. Zinc remains as a stable ion in an organic medium and it is reported to be a perfect metal cofactor for reactions like proteolysis and the hydration of carbon dioxide with the necessity of redox-stable ion. The crystallization of insulin with zinc was the first proof of Zn-proteins/peptides combination[7], while the zinc finger motif was first identified within the transcription factor TFIIIA of *Xenopus.* Human genome encodes almost 10% of the zinc proteins, which remarkably points out the physiological importance of zinc in cellular biology. After the initial recognition of zinc from erythrocyte carbonic anhydrase, it has been reported to appear within all six classes of enzymes defined in the enzyme commission (EC). The functioning of several enzymes, transcriptional factors, and other proteins shows an essentiality towards zinc; there exists a possible interaction between these proteins and Zn through definite regions like zinc-finger, LIM, RING finger domains etc. In the interior part of protein structure, zinc is coordinated by nitrogen, oxygen, and sulfur atoms with distinct coordination numbers[8]. It is suggested from zinc proteome analysis that nearby 9% of proteins in eukaryotes are zinc proteins with the number significantly enhanced in higher organisms. The number of zinc-binding motifs in zinc proteomes is governed by the intramolecular zinc binding sites although it is a difficult task to recognize them[9].

Physiological role of zinc in immune homeostasis:

The essentiality of zinc was first predicted in the year of 1869 while studying the growth of *Aspergillus niger*. At length of time, consistent growth of plants, rats and birds was found to be regulated by an indispensable role of Zn. Subsequently, different hazardous problems like immune deficiency, diarrhoea, alopecia, brain dysfunctions, uncoordinated healing process of wound, loss of appetite, liver disease, chronic inflammation, certain neuropsychological abnormalities such as emotional instability, irritability, and depression etc. are triggered by zinc deficiency, although up to 1961, zinc was not considered as a vital micronutrient for human body. Maximum zinc deficit cases are identified from the elderly, vegans/vegetarians people or the individual with chronic diseases like lymphopenia, liver cirrhosis or inflammatory bowel disease, defective lymphocyte responses from the developing countries like Africa and Asia while its global approximation varies from 17% to 20%. Mainly, zinc tends to be accumulated in the skeletal muscles and bones[10]. So, for the maintenance of proper Zn-balance in our body, zinc intake is necessary in our diet on a daily basis keeping it in mind that excess may be harmful. Different health related aspects like eye lesion and wound in skin, disorder in taste, alopecia, impaired immune function, deceleration in growth may arise from zinc deficiency while the excessive Zn-intake shows toxicity such as nausea, vomiting, fever, headaches and chronic kidney disease (CKD). Zinc supplementation is potential enough to reduce the occurrence of pneumonia in children from developing countries, diarrhoea, infections and to improve immunity[11]. It is also useful in boosting the growth of children and in reducing impaired vision as well as the muscle atrophy[12]. Zn also successfully reduced the time scale of symptoms caused by rhinovirus. Herpes simplex virus (HSV) infection can be effectively treated with zinc sulfate[13]. Infection caused by rhinovirus can be successfully inhibited by binding of zinc to virion. Reproductive cycle of HIV-1 virus[14], vaccinia virus[15], and polioviruses can also be hindered with *in vitro* zinc treatment. It was evidenced that zinc at its lowest concentration (10 μM), showed 800-fold reduction in RSV virus when studied *in vitro*. Zn transporters function as intracellular signal transducers and Zn also acts like a neuromodulator in synaptic transmission. Zn homeostasis is

maintained by a number of Zn transporters which signifies proper cellular functioning.

Zinc homeostasis in human body:

There are two different types of intracellular zinc pools (i) protein-bound zinc and (ii) loosely bound Zn^{2+} i.e. "free" zinc. Very small amount of zinc exists as free-zinc ions due to toxicity of zinc within cells and majority of zinc belongs to the first type. Mature human body possesses nearly 2–3 g zinc. Skeletal muscle cells can store upto 60% zinc, nearly 30% zinc is accumulated in case of bone cells, 5% in the cells of liver and skin while the rest 2–3% in other tissues. Less than 1% of the total body zinc is derived from blood serum. The albumin protein combines loosely with nearly 80% of serum zinc and the other 20% is tightly combined with α2-macroglobulin protein[16,17]. Human body can adjust with a ten-fold increased Zn, consumed on a daily basis for the maintenance of homeostasis. Almost, nearly 0.1% of the whole Zn is added in regular diet (breast milk for children). The strict regulation of Zn absorption from food mainly occurs in the duodenum and jejunum; the absorption rises up to 90% when there is inadequate accessibility of dietary Zn. In case of excess Zn intake, it is released from the gastrointestinal tract and is also discarded through shedding off mucosal epithelial cells[18] and renal excretion[19]. Generally, in mammals, over 30 proteins, together with ZnT and ZIP transporters, function properly to maintain systemic zinc homeostasis; however, humoral mediators have not been recognized in case of zinc mediated metabolism (Fig. 1).

Distribution of zinc within cell:

Nearly 50% zinc is distributed within cell cytoplasm, 30–40% in nucleus and 10% in membrane[20]. Total cellular zinc concentration varies between 10–100 μm range while the cytosolic concentration fluctuates between picomolar and low nanomolar range. In case of intracellular organelles, mitochondrial free zinc concentration have been quantified as 0.14 pM[21], 0.2 pM concentration have been reported from mitochondrial matrix[22], 0.9 pM from ER, and approximately 0.2 pM from the Golgi[23], although elevated level of zinc (~300 pM and 5 nM) concentrations have been reported from mitochondria and the ER[24]. So, the zinc balance is maintained through a complex sequence of uptake, distribution, storage, and efflux along with a central role of ZnT and ZIP transporters at cellular and subcellular level. Both of these transporters cause movement of zinc between vesicles and organelles of cell cytosol, leads to buffer disorder, a condition which is termed as "buffering" and "muffling"[25].

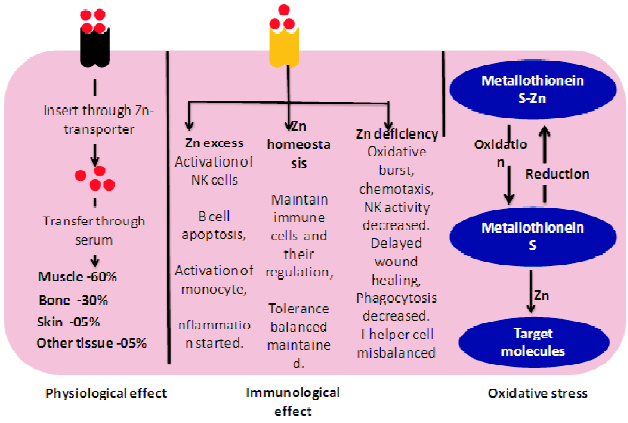

Fig. 1. Schematic representation of immuno-physiological effect of Zn homeostasis in cell.

Distribution of zinc within cellular vesicles/granules:

Excess amount of labile zinc is considered as chelating agent due to its accumulation within cells and tissues. Release of zinc from synaptic vesicles is predicted within the range of approximately 100 to 300 μM. Beside, soft tissues (200 nmol/g wt on average), zinc tends to be aggregated within the tissues of prostate gland at a 3- to 15-fold higher range than the range reported from other[26]. However, a massive reduction of higher zinc level found in prostate cancer and carcinoma is an indication of its significance in metabolic activity of prostate gland. Accumulation of higher concentration of zinc within the β-cells of pancreas is mandatory for crystallization of insulin molecule. Correspondingly, higher zinc content is also reported from GH containing dense-core secretory granules of anterior pituitary cell line, epithelial and myoepithelial cells of submandibular salivary gland, sperm cells, exocrine cells of pancreas, pigment epithelial cells of retina, paneth cells of intestine and mast cells. Various processes occur within subcellular compartments due to zinc accumulation. At the time of meiotic maturation from prophase I to metaphase, higher amount of zinc is introduced and concentrated within the cortical granules of oocytes, necessary for growth detention following meiosis I. During egg activation, accumulated zinc is discarded from the oocyte to reduce zinc bioavailability immediately after intracellular calcium oscillations, termed as "zinc spark"[27]. Zinc also tends to be aggregated within subcellular compartments during specific pathological situations.

Metallothionein and MTF-1 – storing house of zinc within cytosol:

61–68 amino acids together with 20–21 cysteines form MTs, which can combine up to 7 equivalents of zinc as well as another metal cations with a valence of two. 5–15% of zinc is tied up by MTs located in the cytosol. Approximately 12 MTs from humans and 4 MTs from mice are reported. In case of mammals, the MT molecule is divided into two zinc binding domains: α and β domains. MTs also serve as zinc acceptors and donors. In the promoter region of MTs excess zinc can tie up metal-response element-binding transcription factor-1 (MTF-1) and metal response element (MRE, 5'-TGCRCnCGGCCC-3')[28] leading to a significant increase in MT-I and MT-II expression in mice. In vertebrates MT genes are induced in response to zinc by metal-responsive transcription factor-1 (MTF-1) comprising 6 C_2H_2 zinc finger motifs which show a significant contribution towards zinc sensing and metal responsive transcriptional activation. Zinc finger motif also acts as DNA-binding domains with increased level of cellular zinc. MTF-1 maintains zinc homeostasis to increase the transcription of host genes – *MTs*, *ZnT1*, and *ZnT2*[28–30], which are related with the reduction of zinc induced toxicity as well as additional suppression of a set of genes such as zinc transporter *ZIP10*. But in later situation, Pol II movement is physically spoiled. MTF-1 is indispensable for liver development of embryo.

Antiviral effect of zinc:

Several *in vitro* studies have suggested the antiviral nature of zinc where concentrations are necessary for the measurement of antiviral activity. Antiviral zinc concentrations can extend upto mM concentrations whereas human plasma zinc concentration ranges between 10–18 μM. The antiviral potency of zinc depends on its availability although it is definitely virus-specific (Fig. 2). The details of antiviral effects of zinc are given below:

Herpesviridae: Restriction of the protein ubiquitination pathway can inhibit the herpes simplex virus reproduction and a reduction in NF-κB activity accompanied by zinc ionophore pyrithione. Remarkably a decreased repetition as well as extent of disease outbreak was revealed from the performance of several relevant zinc application studies in humans[31,32]. The effectiveness of recent implementation, along with *in vitro* studies indicates the coating of entire HSV virus particle by unbound zinc to prevent infection. Mechanistically, zinc ions retard Human alphaherpesvirus 3, usually referred to as the varicella-zoster virus by their *in vitro* inactivation. Both HSV and Varicella-Zoster virus, the members of *Alphaherpesvirinae* subfamily, are genetically related, and possess identical inhibition strategy.

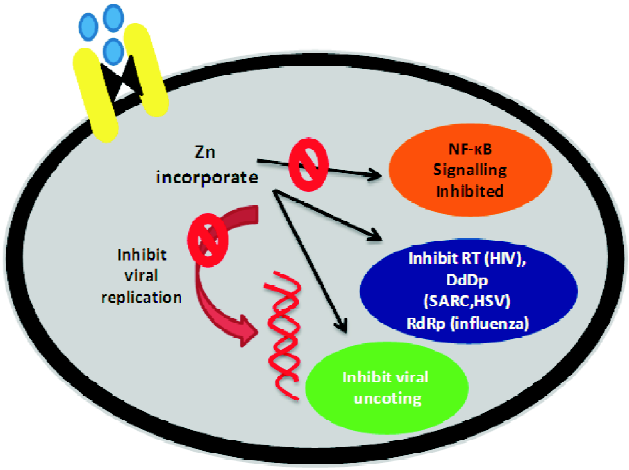

Fig. 2. Schematic representation of the mechanistic approach of Zn as antiviral agent.

Picornaviridae: Before 1980, it was revealed that zinc can inhibit picornavirus, encephalomyocarditis virus (EMCV), poliomyelitis causing virus – poliovirus, foot and mouth disease virus (FMDV). In case of coxsackievirus B3 which belongs to picornaviridae family, zinc hinders the autocatalysis to encode 3C protease from its precursor 3CD protease to inhibit viral polyprotein processing[33]. Zinc seems to bind and alter the viral polyprotein tertiary structure of EMCV[33].

Flaviviridae: Flaviviruses are mainly transmitted by insects. Mosquito-borne diseases caused by dengue virus and West Nile virus, as well as the hepatotropic virus such a hepatitis C virus are grouped under flaviviridae family. *In vitro* studies confirmed the diminished level of HCV replication (mainly inhibition of HCV RdRp) (approximately 50% by 100 μM $ZnSO_4$) by zinc salts; however, in case of *E. coli*, the IC_{50} value is near about 60 μM[34].

Togaviridae: Togaviruses mainly involve the viruses which are transmitted by arthropod vectors like mosquitoes in the Semliki forest, Western equine encephalomyelitis causing agent, and Chikungunya virus. Receptor-mediated endocytosis, with the subsequent virus and endosomal membrane fusion, as well as the release of new virion particle into the cytoplasm are the steps involved in viral replication.

Zinc has been revealed as an efficient inhibitor of Semliki forest virus and sindbis virus's membrane fusion using liposomal vesicle[35], RBCs[36], and BHK Strain 21. To inhibit membrane fusion step of viral replication process, at low endosomal pH, zinc ions form a complex with one (i.e. envelope glycoprotein 1) of the two enveloped glycoproteins of virus through specific histidine residue[37].

Retroviridae: Viruses belonging to retroviridae family are identified on the basis of their capability for the transcription of RNA into DNA utilizing reverse transcriptase (RT) and therefore permitting incorporation of new DNA into the host cell genome. The integrated genome of virus called provirus within host cell DNA thus becomes the major barrier to virus healing pathway specifically for HIV-1, with the production of specific infection within host deprived of any symptom i.e. remains dormant. It is well established that there is a correlation between the cellular concentration of Zn ion and $CD4^+$ T cells count in HIV infection[37]. The inhibition of the HIV-1 protease[38], and viral transcription was reported to be inhibited by zinc. However, stimulation of zinc influx into monocytes by HIV remains inconsistent.

Papillomaviridae: HPVs, the oncogenic viruses, can stimulate proliferation in basal epithelial cells, give rise to swelling. Although cutaneous swelling is limited as well as non-dangerous, genetic variants of HPV (HPV-16 and -18) are the main source of cancer that occurs in the cells of the cervix[39]. E6 and E7 genes of HPV encode oncoproteins and both of them are important components for cell proliferation and apoptosis by reviving the degeneration of tumour suppressor's p53 and pRB, respectively[40]. Although nuclear zinc appears to boost up HPV replication, treatment with exogenic zinc (CIZAR, zinc chloride and citric acid anhydrous) can successfully prevents construction of E6 and E7 genes and regain the role of tumour suppressor's p53 and pRB causing cell death of cervical malignant cells. Although down regulating mechanism of E6 as well as E7 expression by zinc is unknown, but zinc induction may lead up to a barrier in different part of viral life cycle.

Zn-Cu homeostasis in health:

It has been reported that a competition between copper and zinc absorption occurs in small intestine[41]. After binding with protein metallothionein, both of these elements are absorbed by the cells facing the small intestine. Over expression of metallothionein production in the enterocytes is due to Excessive zinc ingestion. After tie up of zinc or copper with metallothionein, its movement through the enterocyte gets blocked. Zinc absorption is regulated by stimulating the synthesis of this protein. Copper possesses a higher affinity for metallothionein than zinc[42] and so it displaces zinc from metallothionein and thus undergoes a preferential binding to the metallothionein, with leftover in the enterocytes; when the intestinal cells are discarded, they lost in faces. Thus, due to mucosal blockage zinc gives rise to a negative copper balance. Failure to mobilize adsorbed Cu from the intestinal cells forms the basis of Menkes syndrome. High doses of zinc given for a longer period in patients, causes an abnormality with Wilson disease which is decreased by the incorporation of Cu into ceruloplasmin by the reduction in biliary excretion of Cu[43]. Myeloid hyperplasia has been shown in the bone marrow due to zinc-induced copper deficiency. Oral zinc ingestion causes suspiciously high toxicity comparative to copper – a process to influence copper deficiency. In case of humans, multiple antagonistic side effects involve a reduction in copper-dependent enzymes for instance superoxide dismutase, ceruloplasmin, and cytochrome c oxidase; fluctuations in immunological constraints, cholesterol, and its lipoprotein distribution.

Zinc and copper are the most important element which act as ion cofactor in receptors, proteins, different enzymatic reactions and hormones[44]. This Zn-Cu duo can reduce the oxidative stress by stimulation of metallothionein synthesis to form structural ions of SOD[45–47]. This duo has the essential role in redox potential mechanism and their imbalanced ratio may be the main cause for an improved susceptibility to oxidative damage[48–50], although acute Zn depletion causes a decrease in innate and adaptive immunity, chronic insufficiency with increased inflammation[51]. Contrarily, excess Cu may be related with an inflammatory response, though it is not clear whether copper has pro-oxidant or antioxidant like effects. Deactivation of unbound radical as well as prevention of associated damage promoted by the antioxidant properties of copper. In opposition to that, unbound radical induced cellular damage may be triggered by the pro-oxidant like behaviour of copper. This is because ceruloplasmin, as the key copper-containing protein, has been exposed to act both as an antioxidant and pro-oxidant in different situations[52].

Maintenance of the dietary ratio of copper-zinc is very important as higher intake of Zn can affect Cu absorption

and the raised levels have been reported to introduce a negative impact on health status. Zinc acts as a signalling molecule to promote different types of cellular proliferation and growth. If the Zn level decreases with the increased level of Cu, intracellular and extracellular anti-oxidant defence mechanism will be highly affected. According to a research, metabolic and endocrine alterations with enhanced aging might be an indication of "survival" reactions to genotoxic stress that could stimulate tumorigenesis[53].

Physiochemical effect of Zn on COVID-19:

Since December 2019, there was a rapid outbreak of a virus named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease-19 (COVID-19) to almost every country of the world. But until now there is no inhibition strategy to keep control of SARS-CoV-2 infection due to lack of approved vaccines or pharmaceutical therapies. Due to the role of zinc as immune modulator in infection as well as its nature like antiviral agents, it is regarded as one of the optional treatments for COVID-19. Inhibition of proteolytic processing of replicase polyproteins by Zn was reported previously. In fact, it was shown that zinc can inhibit the RNA dependent RNA polymerase (RdRp) activity of Hepatitis E virus. Moreover, it was also shown that zinc ionophores blocked coronavirus RdRp activity as well as coronavirus replication[54]. Chloroquine (CQ) and hydroxychloroquine (HCQ), which are generally prescribed for the treatment of malaria and associated inflammatory conditions, might be an alternative tactic because both of these drugs behave like weak bases with an elevated pH level and tend to accumulate within endosomes, lysosomes, or golgi vesicles[55]. In case of SARS-CoV-2, during the replication procedure within host cell, specifically the increased pH of lysosomes, could restrict the pH-dependent phases like membrane fusion and viral uncoating. This elevated pH within intracellular compartments seems to inhibit SARS-CoV-2 replication, because it requires acidification of endosomes for appropriate functioning. Therefore, it is assumed that an inhibiting effect of CQ and HCQ might be important for the treatment of SARS-CoV-2 infected patients. Earlier findings revealed that chloroquine as a zinc ionophore, increases Zn^{2+} flux into the cell[56]. Treatment with zinc supplementation without chloroquine shows some positive effects in treatment[57]. Theoretically, such effectiveness of Zn with substantial lower toxicity may also be detected by means of additional zinc ionophore activity of quercetin and epigallocatechin-gallate[58]. Targeting Zn ions in viral protein structure is another approach for modulation of COVID-19. Zn helps in protein destabilization of MERS-CoV and SARS-CoV[59]. Zn-ejecting agents (e.g. antialcoholism drug disulfiram) may be used as potential antiviral agents for SARS-CoV-2 treatment by ejecting Zn^{2+} from the predicted target site to inhibit viral replication. SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) for entry into host cells. So, modification of ACE2 receptor was also thought as the potential therapeutic strategy in COVID-19. It is also reported the susceptibility of 100 μM zinc to reduce the activity of recombinant human ACE-2 in rat lungs. The consequence of zinc on SARS-CoV-2 and ACE2 interaction appeared to be only imaginary even though this concentration is close to the physiological values of total zinc[60]. Impaired mucociliary clearance caused by HCoV 229E, induced ciliary dyskinesia although neither HCoV 229E nor HCoV-OC43 infection triggered a substantial reduction in ciliary beat frequency. Improvement of length of cilia from bronchial epithelium of Zn-deficient rats[61], as well as increased ciliary beat frequency was boosted by Zn supplementation. We therefore postulate that zinc supplement will enhance nCoV-2019 induced dysfunction via mucociliary clearance.

Conclusion

There is a well-organized system within human body for proper management and regulation of vital trace metals. But once this system fails to operate accurately, anomalous levels of trace metals can be a significant threat to human health. Zinc as a vital trace element, performs many fundamental activities of cellular metabolism, significant to all forms of life. Generally, over 300 enzymes possess the essentiality of zinc for their proper functioning. This trace metal is also assisted with improved immune mechanism, faster wound curing, synthesis of DNA or protein and cell division. The antioxidant like properties of zinc may defend against faster aging. On the other hand, copper permits many critical enzymes to function properly and thus achieves an important place in metabolism. It basically maintains the firmness of skin, blood vessels, epithelial and connective tissue all over the body. It also takes part in the production of hemoglobin, myelin, melanin and collagen. Copper behaves both as an antioxidant and a pro-oxidant. Zinc acts like an intracellular signalling molecule with an important role in cell-mediated

immune response and oxidative stress. At the same time, zinc deficiency is the cause of many long-term illnesses which is needed to be altered to evade complications. Different types of diseases can be prevented with supplements, and at the same time certain types of medications cause disturbed Cu and Zn concentrations which may results in onset of other diseases. Therefore, it is very much necessary to keep a well-maintained balance between Zn and Cu than their individual concentration in blood serum.

References

1. M. Dardenne, J. M. Pleau, B. Nabama, P. Lefancier, N. Denien, J. Choay and J. F. Bach, *Proc. Natl. Acad. Sci. USA,* 1982, **370**, 5373.
2. M. S. Willis, S. A. Monaghan, M. L. Miller, R. W. McKenna and W. D. Perkins, *et al., Am. J. Clin. Pathol*., 2005, **123**, 125.
3. J. Osredkar and N. Sustar, *J. Clinic. Toxicol.,* 2019, **S3**, 001.
4. A. Hamnett and R. J. Mortimer, *J. Electroanal. Chem. Interfacial. Electrochem.,* 1987, **234**, 185.
5. J. Osredkar and N. Sustar, *J. Clinic. Toxicol.,* 2011, **S3**, 001.
6. S. R. Lee, *Oxid. Med. Cell. Longev*., 2018, **2018**, 9156285.
7. D. A. Scott and A. M. Fisher, *J. Clin. Invest*., 1938, **17**, 725.
8. T. Kochanczyk, A. Drozd and A. Krezel, *Metallomics.,* 2015, **7**, 244.
9. C. Andreini, I. Bertini and A. Rosato, *Acc. Chem. Res.,* 2009, **42**, 1471.
10. M. J. Jackson, "Physiology of Zinc: General Aspects", Springer Publishers, London, 1989, pp. 1-14.
11. Z. A. Bhutta, R. E. Black, K. H. Brown, J. M. Gardner, S. Gore,
12. A. Hidayat, F. Khatun, R. Martorell, N. X. Ninh, M. E. Penny, J. L. Rosado, S. K. Roy, M. Ruel, S. Sazawal and A. Shankar, *J. Pediatr.,* 1999, **135**, 689.
13. Age-Related Eye Disease Study Research Group, *Arch Ophthalmol.,* 2001, **119**, 1417.
14. A. Wahba, *Acta Dermatol. Venerol.,* 1980, **60**, 175.
15. Y. H. Haraguchi, S. Sakurai, B. M. Hussain and H. Hoshino, *Antivir. Res.,* 1999, **43**, 123.
16. E. Katz and E. Margalith, *Antimicrob. Agents. Chemother.,* 1981, **19**, 213.
17. M. J. Jackson, Springer, 1989, pp. 1-14.
18. J. P. Barnett, C. A. Blindauer, O. Kassaar, S. Khazaipoul, E. M. Martin, P. J. Sadler and A. J. Stewart, *Biochim. Biophys. Acta,* 2013, **1830**, 5456.
19. C. M Taylor, J. R. Bacon, P. J. Aggett and I. Bremner, *Am. J. Clin. Nutr.,* 1991, **53**, 755.
20. M. Hambidge and N. F. Krebs, *Annu. Rev. Nutr.,* 2001, **21**, 429.
21. H. Rink, *Biofactors,* 2014, **40**, 27.
22. J. G. Park, Y. Qin, D. F. Galati and A. E. Palmer, *ACS Chem. Biol.,* 2012, **7**, 1636.
23. B. J. McCranor, R. A. Bozym, M. I. Vitolo, C. A. Fierke, L. Bambrick, B. M. Polster, G. Fiskum and R. B. Thompson, *J. Bioenerg. Biomembr.,* 2012, **44**, 253.
24. Y. Qin, P. J. Dittmer, J. G. Park, K. B. Jansen and A. E. Palmer, *Proc. Natl. Acad. Sci. USA.,* 2011, **108**, 7351.
25. P. Chabosseau, E. Tuncay, G. Meur, E. A. Bellomo, A. Hessels, S. Hughes, P. R. Johnson, M. Bugliani, P. Marchetti, B. Turan, A. R. Lyon, M. Merkx and G. A. Rutter, *ACS Chem. Biol.,* 2014, **9**, 2111.
26. R. A. Colvin, W. R. Holmes, C. P. Fontaine and W. Maret, *Metallomics*., 2010, **2**, 306.
27. M. C. Franz, P. Anderle, M. Burzle, Y. Suzuki, M. R. Freeman, M. A. Hediger and G. Kovacs, *Mol. Aspects. Med.,* 2013, **34**, 735.
28. A. M. Kim, M. L. Bernhardt, B. Y. Kong, R. W. Ahn, S. Vogt, T. K. Woodruff and T. V. O'Halloran, *ACS Chem. Biol.,* 2011, **6**, 716.
29. G. W. Stuart, P. F. Searle and R. D. Palmiter, *Nature*, 1985, **317**, 828.
30. L. A. Lichten, M. S. Ryu, L. Guo, J. Embury and R. J. Cousins, *PLoS One.,* 2011, **6**, e21526.
31. H. R. Godfrey, N. J. Godfrey, J. C. Godfrey and D. Riley, *Altern. Ther. Health Med.,* 2001, **7**, 49.
32. B. B. Mahajan, M. Dhawan and R. Singh, *Indian J. Sex. Transm. Dis. AIDS,* 2013, **34**, 32.
33. S. Shishkov, T. Varadinova, P. Bontchev, C. Nachev and E. Michailova, *Met. Based Drugs*, 1996, **3**, 11.
34. S. A. Read, G. Parnell, D. Booth, M. W. Douglas, J. George and G. Ahlenstiel, *J. Viral Hepat.,* 2018, **25**, 491.
35. J. Corver, R. Bron, H. Snippe, C. Kraaijeveld and J. Wilschut, *Virology*, 1997, **238**, 14.
36. E. Zaitseva, A. Mittal, D. E. Griffin and L. V. Chernomordik, *J. Cell. Biol.,* 2005, **169**, 167.
37. C. Y. Liu and M. Kielian, *J. Virol.,* 2012, **86**, 3588.
38. E. Mocchegiani and M. Muzzioli, *J. Nutrition*, 2000, **130**, 1424S.
39. Y. Haraguchi, H. Sakurai, S. Hussain, B. M. Anner and H. Hoshino, *Antiviral Res*., 1999, **43**, 123.
40. K. Hoppe-Seyler, F. Bossler, J. A. Braun, A. L. Herrmann and F. Hoppe-Seyler, *Trends. Microbiol*., 2018, **26**, 158.
41. S. N. Bae, K. H. Lee, J. H. Kim, S. J. Lee and L. O. Park, *Biochem. Biophys. Res. Commun.,* 2017, **484**, 218.
42. E. R. Broun, A. Greist, G. Tricot and R. Hoffman, *JAMA,* 1990, **264**, 1441.
43. V. Yuzbasiyan-Gurkan, A. Grider, T. Nostrant, R. J. Cousins and G. J. Brewer, *J. Lab. Clin. Med*., 1992, **120**, 380.
44. N. Kumar, "Fifty Neurological Cases from Mayo Clinic", Oxford University Press, Oxford, England, 2004, 131.
45. J. C. Fleet, "Biochemical and Physiological Aspects of Hu-

man Nutrition", Philadelphia, PA, Saunders, 2000.

46. M. Fukuoka, E. Tokuda, K. Nakagome, Z. Wu, I. Nagano and Y. Furukawa, *J. Inorg. Biochem.,* 2017, **175**, 208.
47. J. C. Rutherford and A. J. Bird, *Eukaryot Cell.,* 2004, **3**, 1.
48. N. Saydam, T. K. Adams, F. Steiner, W. Schaffner and J. H. Freedman, *J. Biol. Chem.,* 2002, **277**, 20438.
49. M. Soinio, J. Marniemi, M. Laakso, K. Pyörälä, S. Lehto and T. Rönnemaa, *Diabetes Care.,* 2007, **30**, 523.
50. A. Ceriello, *Metabolism,* 2000, **49**, 27.
51. L. O. Klotz, K. D. Kröncke, D. P. Buchczyk and H. Sies, *J. Nutr.,* 2003, **133**, 1448.
52. P. Bonaventura, G. Benedetti, F. Albarède and P. Miossec, *Autoimmun. Rev.,* 2015, **14**, 277.
53. S. Bo, M. Durazzo and R. Gambino, *et al., J. Nutr.,* 2008, **138**, 305.
54. B. Schumacher, G. A. Garinis and J. H. Hoeijmakers, *Trends Genet.,* 2008, **24**, 77.
55. A. J. W. Velthuis, S. H. E. van denWorm, A. C. Sims, R. S. Baric, E. J. Snijder and M. J. van Hemert, *PLoS Pathog*., 2010, **6**, e1001176.
56. J. M. Rolain, P. Colson and D. Raoult, *Int. J. Antimicrob. Agents,* 2007, **30**, 297.
57. J. Xue, A. Moyer, B. Peng, J. Wu, B. N. Hannafon and W. Q. Ding, *PLoS One,* 2014, **9**, e109180.
58. M. Guastalegname and A. Vallone, *Clin. Infect. Dis*., 2020.
59. H. Dabbagh Bazarbachi, G. Clergeaud, I. M. Quesada, M. Ortiz, C. K. O'Sullivan and J. B. Fernandez Larrea, *J. Agric. Food Chem.,* 2014, **62***,* 8085.
60. M. H. Lin, D. C. Moses, C. H. Hsieh, S. C. Cheng, Y. H. Chen, C. Y. Sun and C. Y. Chou. *Antiviral Res.,* 2018, **150**, 155.
61. R. Speth, E. Carrera, M. Jean-Baptiste, A. Joachim and A. Linares, *FASEB J*., 2014, **28**, 1067.
62. A. Darma, R. G. Ranuh, W. Merbawani, R. A. Setyoningrum, B. Hidajat, S. N. Hidayati, A. Andaryanto and S. M. Sudarmo, *Indones Biomed. J.,* 2020, **12**, 78.