International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 1231-1236
Review Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

COPPER UPTAKE AND TRANSPORT ACROSS PHYSIOLOGICAL BARRIERS

S Choudhary* and N Sharma

Department of Bioscience and Biotechnology, Banasthali Vidyapith, P.O. Banasthali Vidyapith-304 022, Rajasthan

***Corresponding Author Email:** sangeeta25@gmail.com

ABSTRACT

Copper is an essential multi-functional, micro-nutrient which plays a crucial role in human physiology. This element is found in various proteins and enzymes having significant function in metabolism, development and maintenance of different organs and systems. Diverse organisms, from bacteria to mammals have developed elegant mechanisms of copper uptake, transport and storage. Balance of this micronutrient is maintained by complex homeostatic processes leading to constant and sufficient supply of it and avoids any excess accumulation. Regulation of cellular copper (Cu) homeostasis involves CTR1protein, responsible for Cu uptake across the plasma membrane, the Cu chaperones and Cu transporting ATPase's (Cu-ATPase's), i.e., ATP7A and ATP7B.This manuscript presents a review on the copper transport across the human physiological barriers, delineating its role in metabolism and the multiple steps of metal assimilation. Our attention is focused to copper interaction and trafficking in the gut and to other systems such as blood, brain and placenta.

KEY WORDS

blood-brain barrier, Copper trafficking, Cu transporting ATPase's.

INTRODUCTION

Copper being a vital micro-nutrient is required 1 mg/day, and the permissible limit of copper for human uptake is 2 mg/L [1]. Common source of this element includes food items (animal liver, green vegetables, dried fruits, nuts and chocolate) another important source of copper is pipe water, due to common use of copper pipes in household plumbing [2, 3]. Copper is an integral component of different proteins and metal-enzymes having critical role in human physiological functions and development. Copper speciation study in humans reported high affinity of Cu (I) for thiol group (–SH). It acts as a cofactor for cytochrome c oxidase, superoxide dismutase, lysyl oxidase, monophenol monooxygenase, dopamine-beta-monooxygenase, peptide alpha-amidating mono-oxygenase, coagulation factors V and VIII; tyrosinase/laccase; hephaestin; nitrous oxide reductase and copper amine oxidase. Above mentioned enzymes play significant roles in removal of superoxide radicals, cross-linking of collagen and elastic fibres, synthesis of melanin, dopamine, pituitary hormones and activation of other neuropeptides and hormones and in many other important physiological processes [4]. Advanced bioinformatics tools further suggest high diversity of copper proteins and these cuproproteins represent only a trivial portion of putative Cu binding protein actually found in the eukaryotic genome [4, 5]. Homeostatic balance of cellular Cu is under control of a securely organized set of proteins that include the CTR1 protein for copper transport into the cell, the Cu chaperones, responsible for delivering it to specific target enzymes. Along with these transporter proteins, Copper transporting ATPase's (ATP7A and ATP7B) also have important role in regulation of this ion. Copper accumulation within the cell activates peroxidative cell damage resulting in oxidative injury leading to cell death. Copper dyshomeostasis in the brain has been linked to several neurodegenerative disorders such as Wilsons disease, Menkes disease, Parkinson's disease (IPD), Alzheimer's disease (AD), prion disease, and amyotrophic lateral sclerosis (ALS) [4]. It has been

observed that mutation in genes encoding above Cu transporting P-type ATPase's leads to genetically inherited metabolic disorders such as Menkes disease and Wilson Disease [4, 6]. This manuscript presents a brief review on the copper transport and homeostasis across intestine, placenta, blood, central nervous system (CNS) and brain. Thus, establishing the importance of Cu transporters in various specialized physiological and pathophysiological processes.

Cellular role of copper

In humans, copper absorption occurs in stomach and small intestine. The first step in copper absorption is the uptake of metal available in diet by intestinal epithelial cells [7]. Copper transport across the intestinal epithelium is the channeled through their cytoplasm. As entering to portal blood, two main copper carriers binds the copper atoms in way from gut towards liver [8]. In the physiological conditions, amino acid complexes are not involved in Cu transport in blood [9].

Once Cu is within the cell it is characteristically bound by the chaperone protein that carries the metal to various protein targets. The membrane transporter CTR1 transfers copper into the cell, where it typically bound by one of the three chaperone proteins that brings the metal to various protein targets. One of the Cu chaperone Cox17 is responsible for it delivery to mitochondrion for its incorporation into the cytochrome C oxidase. It is transported to Cu superoxide dismutase by CCS chaperone that breaks down the free radical superoxide into hydrogen peroxide, which is then cleared by other antioxidants. Whereas, ATOX1 delivers copper to the endoplasmic reticulum and golgi for modification of several proteins and enzymes before their delivery to specific target (Figure 1). In mammalian system copper transporting ATPase ATP7A is expressed in most cells, while ATP7B has a much more controlled expression [6]. When cellular level of copper is high these proteins move onto vesicles, which traffic to the plasma membrane to release excess copper into the extracellular space. Thus, this copper-induced trafficking, is essential to the maintenance of copper homeostasis [10]. Copper shortage in the cell may inhibit its role in intracellular signaling by modulating transcription via induction of a metal transcription factor (MTF) that activates the expression of a number of genes.

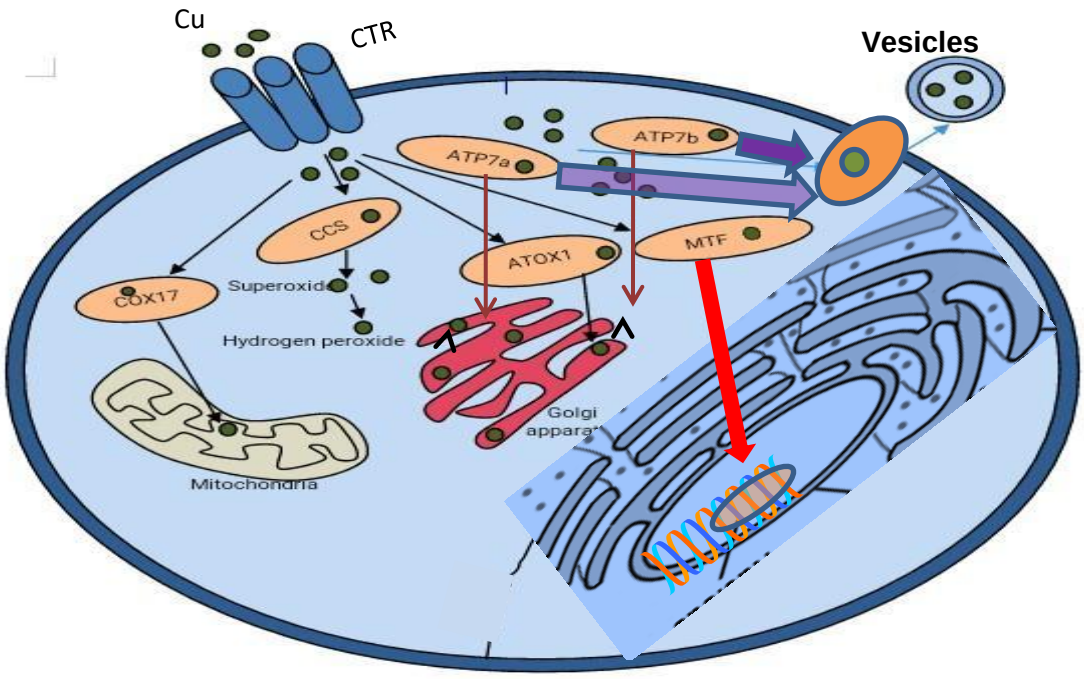

Figure. 1 Copper trafficking and homeostasis within mammalian cell showing principle copper pump CTR1, copper chaperones Cu chaperones (CCS, ATOX1, COX17), and P type ATPase's (ATP7a and ATP7b).

COPPER IN BLOOD

Copper atoms are immediately bound by a plasma protein transcuprein having high- affinity for copper in the way from gut towards liver as they enter into the portal blood. It is a 190-kDa glycosylated protein identified as the macroglobulin alpha (1)-inhibitor III, the most important macroglobulin of rodent blood plasma. Humans do not have transcuprein, but for copper transport other transcuprein-like macroglobulin's, binds nearly 10 % of human plasma copper. The main macroglobulin in human blood plasma [alpha(2)-macroglobulin] which is homologous to alpha (1)-inhibitor III have tight affinity for copper [8]. Apart from macroglobulin like proteins copper atoms may be bound, in portal flow, even to some amino acids such as tryptophan, different peptides, fatty acids despite the fact that amino acide chelation is not having any relevant physiological role in Cu transport [9]. Highly conserved metal (Zn, Ni, Cd) binding sites such as two/three imidazole and one/two carboxylate coordination groups does not play a significant role in copper transport. In comparison to transcuperin albumin in plasma has relatively lower affinity for Cu. It has reported that albumin-bound Cu (II) is rapidly reduced by ascorbate to Cu (I)-albumin that is reoxidized by molecular oxygen. [8]. Thus, it may be suggested that transcuprein or transcuprein-like proteins, some specific peptides as well as transferrin iron-binding blood plasma glycoprotein have higher affinity for copper than albumin [4].

COPPER ACROSS CENTRAL NERVOUS SYSTEM

Copper plays a very important role in development and physiology of human CNS. Copper dependent enzymes (dopamine-b-hydroxylase, peptidyl-α-mono-oxygenase, superoxide dismutase and many others) play a central role in catecholamine production, stimulation of neuropeptides and hormones, protection against reactive oxygen species and in a number of other metaboilc pathways important for regular execution of functions in central nervous system [4]. Dopamine-b-hydroxylase is involved in hydroxylation of the important neurotransmitter dopamine into norepinephrine in the presence of molecular oxygen and reducing co-substrates [11]. Superoxide dismutase I another copper containing enzyme is well characterized in the CNS cells, especially in cortical pyramidal neurons, purkinje cells, deep cerebellar neurons and middle horn cells in the spinal cord. It catalyses the conversion of reactive free radicals to hydrogen peroxide that can be harmful to cells and cause neuronal damage. Thus hydrogen peroxide is further converted to water by catalase or glutathione peroxidase. Superoxide in the intercellular spaces and at the cell surface is neutralized by enzyme SOD3, which maintains cerebral vascular quality and controls neurogenesis. Another copper-dependent enzyme is represented by lysyl oxidase (LOX) protein family which catalyses the oxidation of the side chain of a lysine, giving rise to the cross-linking process that leads to collagen and elastin formation [12].

Ceruloplasmin, a Cu dependent multicopper oxidase is having a lead role in copper transport to peripheral tissue [13]. Copper chaperons to the trans-Golgi network are transported into the Golgi apparatus by ATP7B and then bind to ceruloplasmin. This ceruloplasmin bound copper is transported into the plasma via cytoplasmic vesicles that moves to plasma membrane and discharges this element to cell exterior. Aging leads to loss of ceruloplasmin function in the CNS cells which consequently leads to neural degeneration [14, 4]. Another copper binding protein highly conserved in mammals and expressed predominantly in the brain is prion protein. It is assumed that prion proteins show a superoxide dismutase like activity, involved in the cell response to oxidative stress. In humans, prion disease is characterized by a rapidly [15]. The disbalance of brain metal homeostasis, due to loss normal prion protein might be a significant cause of neurotoxicity in prion disease which may lead to progressive dementia and cerebellar ataxia [16].

Amyloid precursor protein (APP*)* is a multigene family transmembrane glycoprotein having important role in neural growth and repair. Two homologues for this protein is known i.e. amyloid precursor like protein 1 (APLP1) and 2 (APLP2) [17]. This protein binds copper ion within cysteine-rich domain, comprising three histidine residues at positions 147, 149 and 151 [18]. APP has also been shown to reduce Cu (II) to Cu (I) which produces hydroxyl radicals, potentially leading to neurodegeneration and Alzheimer's disease [19].

Copper uptake and trafficking in brain

Adequate amount of Cu is required to maintain the human brain physiology and development [20]. Intricate mechanisms controlling copper uptake and transport across brain is relatively less understood. Previous studies reported greater expression of Cu transporters in brain barriers than in brain parenchyma thus, copper transport through the blood–brain barrier into the brain

is mainly achieved as a free copper ion [21]. The blood–brain barrier has a highly significant role in copper trafficking from general circulation into the brain (Fig 2). Copper radio isotope based tracking studies confirmed prominent differences in copper content between blood and the cerebrospinal fluid. Major site of copper accumulation is the choroid plexus (where the blood-cerebrospinal fluid barrier (BCB) is primarily located) but copper accumulation in cerebrospinal fluid was insignificant [21]. However, copper carriers such as diethylthiocarbamate – copper combination therapy on the macular mouse, an animal model of Menkes disease revealed the passage of copper ions across the blood–brain barrier [22]. Molecular mechanisms behind brain cell copper metabolism show involvement of gene family SLC 31 (solute-linked carrier 31), the human cation transporter 1 gene (hCtr1) and hCtr2 (23 Crichton, 2008). Amongst these, hCtr1gene product CTR1 a copper permease is responsible for uptake of Cu(I) from the extracellular milieu after reduction of Cu(II) by a cell surface metallic reductase [24]. Two methionine-rich domains of this protein regulates copper-stimulated endocytosis. The amino-terminal methionine cluster MMMMPM regulates endocytosis in response to lower copper concentrations, whereas the transmembrane MXXXM motif does the same during high copper concentrations [25]. In vitro studies suggests that during excess of copper CTR1 level is controlled by rapid endocytosis leading to ubiquitination and degradation by vacuolar proteases [26]. In mammalian brain, CTR1 is highly expressed in the whole brain parenchymal cells, reaching the highest levels in the choroid plexus cells. Thus movement of Cu into the brain is essentially through the blood brain barrier as a free Cu ion. On the other hand, blood-cerebrospinal fluid barrier is the main regulatory site of Cu homeostasis in the cerebro spinal fluid [21].

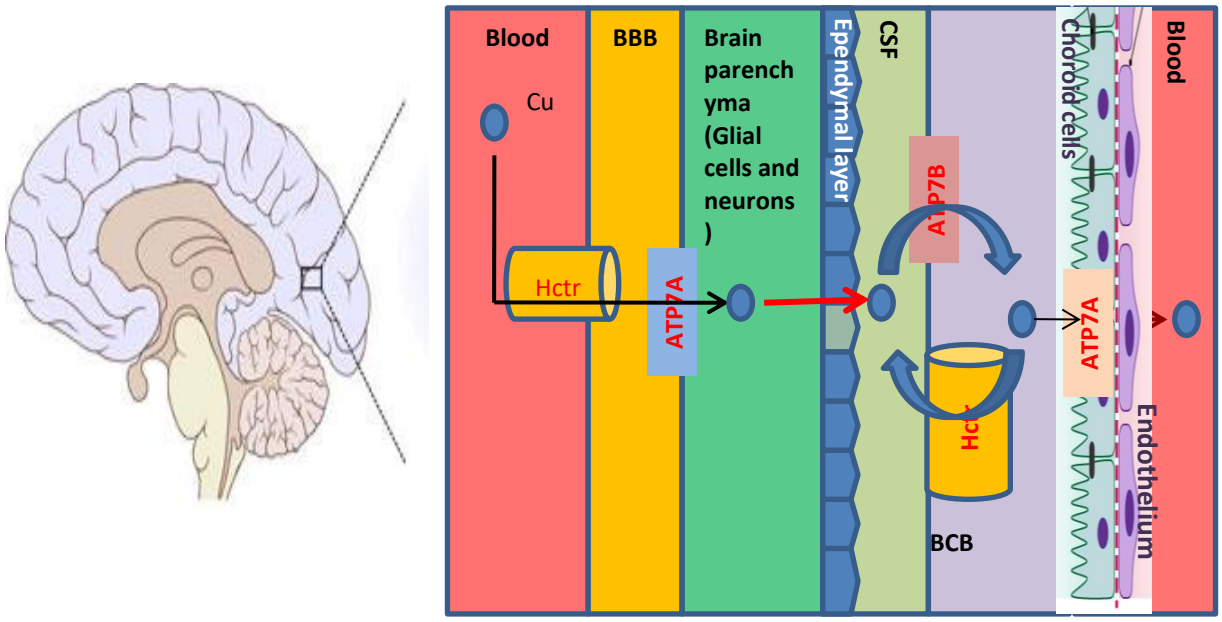

Figure. 2 Copper uptake and trafficking in brain, showing blood brain barrier (BBB), cerebro spinal fluid (CSF) and blood cerebrospinal fluid barrier (BCB)

COPPER ACROSS PLACENTA

Copper deficiency during pregnancy either dietary or due to inactivation of copper transporters such as CTR1 may lead to insufficient supply of this ion to growing fetus and further lead to various abnormalities including embryonic mortality, neonatal growth retardation, pulmonary and cardiovascular defects [27]. Previous studies have reported placental Cu accumulation in both Menkes disease and Wilson disease patients and role of ATP7A and ATP7B in the transfer of copper across the placenta during gestation was established by expression and localization studies [28]. It has been observed that in human placenta both transporters are expressed throughout gestation; however, their localization varies [6, 29]. In maternal to fetal transfer of nutrients both syncytiotrophoblasts and embryonic endothelial cells surrounding the maternal blood vessels within the placenta plays an important role. The copper transporting protein ATP7A and CTR1 is located on the fetal wall of the human placenta (mainly in the

syncytiotrophoblast, the cytotrophoblast and in the endothelial cells) which suggests that copper movement occurs from the placenta to the fetal circulation. On the other hand, excess copper accumulation in placenta is controlled by its efflux to mother via ATP7B transporter located on the apical surface of the placenta mostly in microvilli of syncytiotrophoblast [29]. This hypothesis was further tested using Polarized Jeg-3 cells derived from placental trophoblasts which express both ATP7A and ATP7B. in vitro studies on choriocarcinoma cell line, Bewo also confirmed the role of ATP7A transported in copper efflux [30]. Above studies suggest the proposed role of ATP7A and ATP7B in copper homeostasis in placenta [6, 29].

CONCLUSION

Copper being an essential micronutrient, optimum cellular level is essential for normal functioning while excess of it may toxic to cells. Complex homeostatic mechanisms control its transportation, distribution, storage and excretion thus maintaining requisite supply of the micronutrient while simultaneously avoiding its overload. This homeostatic balance of copper in the cell is under strict control of a group of proteins that include the membrane bound transporter CTR1, which mediates Cu uptake across the plasma membrane, and the Cu chaperones (CCS, ATOX1, COX17) and P type ATPase's (ATP7A and ATP7B) that deliver Cu to specific target enzymes. Research activities from different groups highlight the mechanism of Cu transport across different physiological barriers such as gut blood brain barrier and placenta. The significance of copper regulation in human system is evident from the two most well studied diseases of Cu metabolism, Menkes disease and Wilson disease, which are caused by mutations in ATP7A and ATP7B respectively.

Deeper insight into cu transporters will bring more significant understanding of regulatory mechanism which can lead further development of methods to track the cu availability in mammalian systems. Specific biomarkers may be developed to trace total body copper status, thus preventing potentially detrimental health effect due to imbalance of this element. Several enzymes may be developed as biomarkers to monitor copper deficiency. During copper deficiency, a cupro-enzyme dopamine-β-hydroxylase (DBH), level may decrease resulting in high proportion of dopamine to norepinephrine. Ceruloplasmin contains 95% of the copper found in plasma, and levels decrease during severe copper deficiency. Copper/zinc superoxide dismutase, cytochrome c oxidase, peptidyl glycine amidating mono-oxygenase, and lysyl-oxidase are all cuproenzymes whose activity or gene expression may be influenced by copper deficiency. Excess copper is deposited in the liver, here a few of nonspecific enzymes such as the hepatic aminotransferase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and -glutamyl-transferase, may serve as biomarkers for liver damage due to elevated copper. In order to ascertain importance of Cu in fundamental metabolic processes and its contribution to various diseases and physiological conditions, further cellular and biochemical studies are required in physiological contexts.

REFERENCES:

1. Marsh, M.N., Riley, S.A., Digestion and absorption of nutrients and vitamins. In: Feldman M, Sleisenger MH, Scharschmidt BF (eds). Sleisenger and Fordtran's gastrointestinal and liver disease, WB Saunders Co., Philadelphia, PA, 1998.
2. Delves, H.T., Dietary sources of copper. In: Biological roles of copper, Ciba Foundation Symposium 79, Excerpta Medica, 1980, 5–22pp.
3. Linder M.C., Azam H.M., Copper biochemistry and molecular biology. *Am J Clin Nutr, 63*:797S, (1996).
4. Crisponi G., Nurchi V.M., Gerosa C., et al. Copper uptake and trafficking in the brain. In: Linert W, Kozlowski H (eds.) Metal Ions in Neurological Systems, Springer-Vienna, 2014, 47-63pp.
5. Andreini C., Banci L., Bertini I., et al. Occurrence of copper proteins through the three domains of life: a bioinformatic approach. J Proteome Res, 7:209–16, 2008.
6. Lutsenko S., Barnes N.L., Bartee M.E., et al., Function and Regulation of copper-transporting ATPase. *Physiol Rev, 87*:1011-46, 2007.
7. Ledoux D.R., Pott E.B., Henry P.R., et al. Estimation of the relative bioavailability of inorganic copper sources for sheep. *Nutr Res, 15*:1803–13, 1995.
8. Linder M.C., Lomeli N.A., Donley S., et al., Copper transport in mammals. In: Leone A, Mercer JMC (eds) Copper transport and its disorders. Kluwer/Plenum, New York, 1999, 1–16pp.
9. Carter D.C., Ho J.X., Structure of serum albumin. *Adv Protein Chem*, *45*:153–203, 1994.
10. La Fontaine S., Mercer J.F., Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. *Arch Biochem Biophys, 463(2):*149-67, 2007.
11. Slama P., Boucher J.L., Re`glier, M., N-hydroxyguanidines oxidation by N3S copper complex mimicking the

reactivity of dopamine beta-hydroxylase. *J Inorg Biochem*, *103*:455–62, 2009

12. Li P.A., He Q., Cao T., et al., Up-regulation and altered distribution of lysyul oxidase in the central nervous system of mutant SOD1 transgenic mouse model of amyoprophic lateral sclerosis. *Mol Brain Res, 120*:115–22, 2004.
13. Martin F., Linden T., Katskinski D.M., et al., Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation. *Blood,105*:4613–19, 2005.
14. Miyajima H., Kono S., Takahashi Y., et al., Cerebellar ataxia associated with heteroallelic ceruloplasmin gene mutation. *Neurology,* 57:2205–10, 2006.
15. Waggoner D.J., Bartnikas T.B., Gitlin J.D., The role of copper in neurodegenerative disease. *Neurobiol Dis, 6*:221–30, 1996.
16. Singh N., Das D., Singh A., et al., Prion protein and metal interaction: physiological and pathological implications. *Curr Issues Mol Biol, 12*:99–107, 2010.
17. Bayer T.A., Cappai R., Masters C.L., et al., It all sticks together–the APP-related family of proteins and Alzheimer's disease. *Mol Psychiatry, 4*:524–31, 1999.
18. Hesse L., Beher D., Masters C.L., Multhaup G., The beta A4 amyloid precursor protein binding to copper. *FEBS Lett, 349*:109–16, 1994.
19. Multhaup G., Schlicksupp A., Hesse L., et al. The amyloid precursor protein of Alzheimer's disease in the reduction of copper (II) to copper (I). *Science, 271*:1406–9, 1996.
20. Madsen E., Gitlin J.D., Copper and iron disorders of the brain. *Ann Rev Neurosci, 30*:317, 2007.
21. Choi B.S., Zheng. W., Copper transport to the brain by the blood-brain barrier and blood-CSF barrier. *Brain Res, 1248*:14–21, 2009.
22. Kodama H., Sato E., Gu Y.H., et al., Effect of copper and diethylthiocarbamate combination therapy on the macular mouse, an animal model of Menkes disease. *J Inherit Metab Dis, 28*: 971–78, 2005.
23. Crichton R.R., Biological inorganic chemistry. In: Metal assimilation pathways. Elsevier Science, Amsterdam, 2008, 117–29pp.
24. Rees E.M., Thiele D.J., Identification of a vacuole-associated metalloreductase and its role in Ctr2-mediated intracellular copper mobilization. *J Biol Chem*, *282*: 21629–38, 2007.
25. Guo Y., Smith K., Lee J., et al., Identification of methionine-rich clusters that regulate copper-stimulated endocytosis of the human Ctr1 copper transporter. *J Biol Chem*, *279:*17428–33, 2004.
26. Liu J., Sitaram A., Burd C.G., Regulation of copper-dependent endocytosis and vacuolar degradation of the yeast copper transporter, Ctr1p, by the Rsp5 ubiquitin ligase. *Traffic,8*:1375–84, 2007.
27. Lee J., Prohaska J.R., Thiele D.J., Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. *Proc Natl Acad Sci USA*, *98*: 6842–47, 2001.
28. Oga M., Matsui N., Anai T., et al., Copper disposition of the fetus and placenta in a patient with untreated Wilson's disease. *Am J Obstet Gynecol, 169*: 196–98, 1993.
29. Hardman B., Manuelpillai U., Wallace E.M., et al., Expression and localization of menkes and Wilson copper transporting ATPases in human placenta. *Placenta, 25*: 512–17, 2004.
30. Qian Y., Majumdar S., Reddy M.C., Harris E.D., Coincident expression of Menkes gene with copper efflux in human placental cells. *Am J Physiol Cell Physiol*, *270*: C1880–84, 1996.

Received: 06.08.18, Accepted: 08.09.18, Published: 01.10.2018

***Corresponding Author:**
S Choudhary* and N Sharma
Email: sangeeta25@gmail.com