Catalase and Carbonic Anhydrase Enzyme Activities, and Some Mineral-Heavy Metal Concentrations in Newborns with Congenital Malformations

Anomalili Yenidoğanlarda Katalaz ve Karbonik Anhidraz Enzim Aktiviteleri ve Bazı Mineral-Ağır Metal Seviyeleri

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Geliş Tarihi/*Received:* 15.09.2009
Kabul Tarihi/*Accepted:* 13.04.2010

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ABSTRACT Objective: The aim of this study is to evaluate the factors such as antioxidant condition and mineral-heavy metal level association that may play a role in development of congenital malformations. **Material and Methods:** Fifteen healthy neonates as control group and 15 neonates with various congenital malformations were included study. Erythrocyte cataly (CAT) which is an antioxidant enzyme, erythrocyte carbonic anydrase (CA) enzyme activities which play a important role in acid-base balance, serum copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd), iron (Fe), manganese (Mn) and magnesium (Mg) were measured from venous blood samples of each subject. **Results:** Erythrocyte CAT enzyme activity, serum Zn and Fe levels were statistically significantly decreased ($P<0.05$) in the study group while serum Cu and Cd levels were statistically significantly high ($P<0.05$)in the study group when compared to the control group. A negative correlation between erythrocyte CA activity and serum Pb levels was found in the study group. In the control group, negative correlations were found between erythrocyte CAT activity and serum Pb levels; serum Mg and Cu levels; and serum Cd and Mn levels. In addition, in the control group a positive correlation between serum Zn and Cu levels; erythrocyte CA activity and serum Mn levels was found. **Conclusion:** Our results suggest that erythrocyte CAT enzyme activity and serum Cu, Zn, Fe and Cd levels may play a role in pathogenesis of congenital malformations. The oxidative stress that rise as a result of an imbalance between prooxidant and antioxidant reactions seems to be associated with congenital malformations. Further studies are needed to confirm the association of antioxidant condition and mineral-heavy metal levels in the pathogenesis of congenital malformations.

Key Words: Catalase; carbonic anhydrases; minerals; heavy metals; congenital, hereditary, and neonatal diseases and abnormalities

ÖZET Amaç: Bu çalışmada konjenital anomalilerin patogenezini aydınlatmaya yönelik olarak; anomali gelişiminde rol alabilecek faktörlerden antioksidan durum ve mineral-ağır metal seviyesi ilişkisinin değerlendirilmesi amaçlandı. **Gereç ve Yöntemler:** Çalışmaya çeşitli konjenital anomalileri olan 15 yenidoğan ve kontrol grubu olarak 15 sağlıklı yenidoğan dahil edildi. Her bir olgunun alınan venöz kan örneklerinden bir anti-oksidan enzim olan eritrosit katalaz (CAT) ve asit-baz dengesinde önemli bir rol oynayan eritrosit karbonik anhidraz (CA) enzimlerinin aktiviteleri ve serum bakır (Cu), kurşun (Pb), çinko (Zn), demir (Fe), kadmiyum (Cd), manganez (Mn) ve magnezyum (Mg) seviyeleri ölçüldü. **Bulgular:** Kontrol grubuyla karşılaştırıldığında çalışma grubunda serum Cu ve Cd düzeyleri istatistiksel olarak anlamlı düzeyde yüksek ($P<0.05$) iken eritrosit CAT aktivitesi, serum Zn ve Fe düzeyleri çalışma grubunda istatistiksel olarak anlamlı düzeyde düşüktü ($P<0.05$). Çalışma grubunda eritrosit CA aktivitesi ve serum Pb değeri arasında negatif bir ilişki tespit edildi. Kontrol grubunda eritrosit CAT aktivitesi ve serum Pb düzeyleri; serum Mg ve Cu düzeyleri; serum Cd ve Mn düzeyleri arasında negatif ilişki bulundu. Buna ek olarak kontrol grubunda serum Zn ve Cu değerleri, eritrosit CA aktivitesi ve Mn düzeyleri arasında da pozitif bir ilişki tespit edildi **Sonuç:** Sonuçlarımız eritrosit CAT enzim aktivitesinin ve serum Cu, Zn, Fe ve Cd düzeylerinin konjenital anomali patogenezinde rol oynayabileceğini göstermektedir. Prooksidan ve antioksidan reaksiyonlar arasındaki dengesizliğe bağlı olarak artan oksidatif stres yenidoğanların konjenital anomalileriyle ilişkiliymiş gibi görünmektedir. Ancak, antioksidan durum ve serum mineral-ağır metal seviyesi arasındaki ilişkinin konjenital anomalilerin patogenezindeki etkilerini doğrulayacak ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Katalaz; karbonik anhidrazlar; mineraller; ağır metaller; doğumsal, kalıtsal, yenidoğan hastalıkları ve anomalileri

doi:10.5336/medsci.2009-15429

Turkiye Klinikleri J Med Sci 2011;31(2):405-12

Congenital malformations are structural abnormalities of organs or body parts of newborns. They occur in 3.5% of all live births. Severe malformations may result in very early loss of conceptions that do not survive to clinical recognition and are never detected. Screening during pregnancy and improved medical technology, such as ultrasound and genetic methods, make it possible to detect some birth defects at an early stage, which may lead to induced abortion and affect the prevalence of birth defects.[1] In literature, epidemiological researches have not convincingly established an association between workplace exposure as a human teratogen and birth defects. However, a large body of literature has suggested that organic solvents, pesticides and some heavy metals may be involved in the etiology of birth defects in humans.[2-4] Specific birth defects can also be seen following to the pharmaceutical exposures such as limb defects related to thalidomide, congenital malformations linked to diethylstilbestrol, spina bifida linked to valproic acid, oral clefts related to phenytoin and vitamin A causing neural crest defects.[1]

Free radicals can be defined as any chemical species capable of independent existence that contain one or more unpaired electrons.[5] Therefore, oxygen plays a major role as an oxidant in the form of superoxide (O_2^-), hydroxyl (OH^-), and peroxyl ($R\text{-}OO^-$) radicals, and their derivatives, reactive oxygen species (ROS). There are three main intracellular ROS scavenging enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px).[6-10] CAT is a metalloenzyme containing heme and regarded as one of the most common enzymes in plant and animal tissues. It is a well known plasma antioxidant. It is also an effective scavenger of aqueous peroxide radicals.[11] It has two isoenzymes, one being erythrocytic and the other occurring only in plasma. CAT has a much larger Km (Michaelis-Menten constant) for hydrogen peroxide compared to glutathione peroxidase, which means that it is mainly responsible for the detoxification of hydrogen peroxide when its concentration is high.[12] Various reports over the last 15 years[6-10, 13]have suggested that fetal anomalies in patients are associated with an increase in embryonic ROS or free radicals. ROS, which are produced in large amounts in various metabolic disorders, are involved in the etiology of congenital anomalies and its complications.[14-17] Studies using animal models indicate that oxidative stress may play a causative role. Free oxygen radical-scavenging enzymes and antioxidants aimed at reducing the excess load of radicals also result in a reduced malformation rate.[18] Study of embryonic gene expression has demonstrated that maternal diabetes causes birth defects by disturbing expression of genes that control essential developmental processes, and that oxidative stress is involved. Therefore, a model in which oxidative stress-induced deficient gene expression leads to congenital defects involving p53-dependent apoptosis is discussed.[19] Controlling oxidative stres or free radical damage is a pivotal factor for chronic degenerative diseases of both the eye and the brain or congenital defects. Current science suggests that neurotoxic peptide amyloid-beta (Abeta) can accumulate in the brain and vascular system of those who inherit a specific set of genes.[20]

Deficiencies of trace elements such as zinc (Zn), copper (Cu) and magnesium (Mg) have been implicated in various reproductive events like infertility, pregnancy wastage, congenital malformations, pregnancy induced hypertension, placental abruption, premature rupture of membranes, still births and low birth weight. However, the role of trace elements individually or in combination has not been completely documented in this field.

Carbonic anhydrase (CA) is a Zn-containing metalloenzyme that catalyzes the reversible hydration of carbondioxide. In animals and humans, numerous CA isoenzymes and related proteins have been reported.[21] CA is discovered late in infancy or early in childhood through development of malocclusion, and/or mental subnormality. Typical radiographic features of osteopetrosis are present, and histopathologic study of the iliac crest reveals unresorbed calcified primary spongiosa. The radiographic findings are unusual, however, cerebral

calcification appears by early childhood and the osteosclerosis and skeletal modeling defects may gradually resolve by adulthood.[22]

This study was carried out to compare the CAT and CA enzyme activities in newborns with congenital malformations and healthy newborns. and the concentrations of some trace elements and heavy metals such as Cu, Zn, Cd, Pb, Mn, Mg and Fe.

MATERIAL AND METHODS

BIOCHEMICAL ANALYSIS

The subjects selected were the patients attending the outpatient clinics and those hospitalised in the Pediatric Surgery Department of Yüzüncü Yıl University Hospital. The study included a total of 30 subjects [15 newborns with congenital malformations (Table 1) and 15 healthy newborns]. Venous blood samples of newborns were obtained from the antecubital fossa veins during the first three days after birth in accordance with the guidelines of Declaration of Helsinki. Written informed consent was obtained from the parents. The study was approved by the local ethics committee.

TABLE 1: Disorders in congenital malformation group.

Case no (week)	Gestational age	Disorder
1	30	Esophageal atresia+ Down Syndrome
2	39	Omphalocele
3	40	Duodenal atresia (annuler pancreas) + Down Syndrome
4	32	Pierre Ribin Syndrome + Hydrocephalus
5	35	Diaphragmatic hernia (Bochdaleck hernia)
6	40	Multiple intestinal atresia+ Antral web
7	41	Cleft palate (incomplete)
8	38 (twin)	Ileocecal stenosis + Appendical agenesis + Ladd's bands
9	38 (twin)	Colocecal stenosis + Ladd's bands
10	35	Anal stenosis
11	40	Hirsch sprung's disease (Waardenburg-Shah Syndrome)
12	34	Sacrococcygeal teratoma
13	39	Esophageal atresia
14	38	Cleft palate (Incomplet)
15	36	Meningomyelocele

Serum was separated by centrifugation and the samples were processed immediately. The serum samples were placed in deionised polyethylene tubes and kept in a deepfreeze at -80°C (without thawing) until the study day. Biochemical analysis of CAT activity in erythrocytes was performed with a method described by Aebi[23]in the Biochemistry Laboratory of Chemistry Department, Faculty of Art and Science, Yüzüncü Yıl University. Briefly, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol l^{-1} H_2O_2 solution prepared in potassium phosphate buffer (0.05 M, pH 7.00). The change in absorbance was monitored at 240 nm for 5 min using a spectrophotometer (Shimadzu UV-1201, Japan).

CA activity was assayed by hydration of $C0_2$. Hydration of $C0_2$ was measured by the method of Rickli and Wilbur-Anderson with bromothymol blue as an indicator.[24] Activities were carried out under optimum conditions. Determination of serum concentrations of Cu, Zn, Mg, Mn, Pb, Cd, and Fe was performed by Atomic Absorption Spectrophotometer measurements, in which a UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK) was used.

STATISTICAL ANALYSIS

The results were expressed as the mean ± standard error (SE). Student's t test was used for comparison of mean values of the groups. In addition, Pearson's correlation analysis was carried out to determine the relationship among the variables. A *P*-value < 0.05 were considered statistically significant. Statistical analyses were carried out using the SPSS® statistical software package (SPSS for Windows version 13.0, SPSS Inc., Chicago, Illinois, USA).

RESULTS

Erythrocyte activities of CAT and CA, and the serum levels of Cu, Zn, Fe, Mn, Pb, Mg, Cd of the newborns with congenital malformations and healthy newborns are shown in Table 2. The CAT activity was significantly lower (P< 0.05) and the serum Cu level was significantly higher (P< 0.05) in the erythrocytes of congenital malformations

TABLE 2: Erythrocyte activities of catalase and carbonic anhydrase, serum levels of Cu, Zn, Fe, Mn, Mg, Cd and Pb of the congenital malformation and healthy newborn groups.

	congenital anomaly				healthy newborn				
	Mean	St. Err.	Min.	Max.	Mean	St. Err.	Min.	Max.	p
Catalase EU/(gHb)-1	6.98*	1.507	0.18	17.20	17.91	2.031	8.03	27.52	0.000
Zn (µg/dl)	0.78*	0.082	0.45	1.46	1.28	0.136	0.55	2.12	0.003
Carbonic anhydrase EU/(gHb)-1	0.02	0.003	0.00	0.04	0.02	0.003	0.00	0.04	0.607
Cu (µg/dl)	0.53*	0.084	0.12	1.45	0.25	0.029	0.07	0.48	0.006
Fe (µg/dl)	1.43*	0.307	0.55	4.71	3.08	0.479	0.52	7.42	0.007
Mg (µg/dl)	11.67	1.026	4.23	17.52	11.42	1.006	6.69	16.38	0.867
Mn (µg/dl)	0.03	0.002	0.01	0.04	0.03	0.002	0.02	0.05	0.124
Pb (µg/dl)	0.13	0.012	0.05	0.21	0.14	0.017	0.04	0.25	0.746
Cd (µg/dl)	0.01*	0.001	0.00	0.01	0.01	0.000	0.01	0.01	0.045

*<0.05 vs healthy newborn.

group when compared controls. The serum Cd level was significantly higher (P< 0.05) in the sera of congenital malformation group. The serum Zn and Fe levels were significantly lower (P< 0.05) in congenital malformation group when compared controls. There was no significant difference (P> 0.05) between the groups regarding other parameters. There was a negative correlation (Figure 1) (P< 0.05) between the CA activity and Pb levels of newborns with congenital malformations (Table 3). There was a negative correlation (Figure 2) (P< 0.05) between CAT activity and Pb level, a positive correlation (Figure 3) (P< 0.05) between Cu and Zn levels, a negative correlation (Figure 4) (P< 0.05) between Mg and Cu levels, a negative correlation (Figure 5) (P< 0.01) between Mn and Cd levels, and a positive correlation (Figure 6) (P< 0.05) between Mn activity and CA levels in healthy newborns (Table 4).

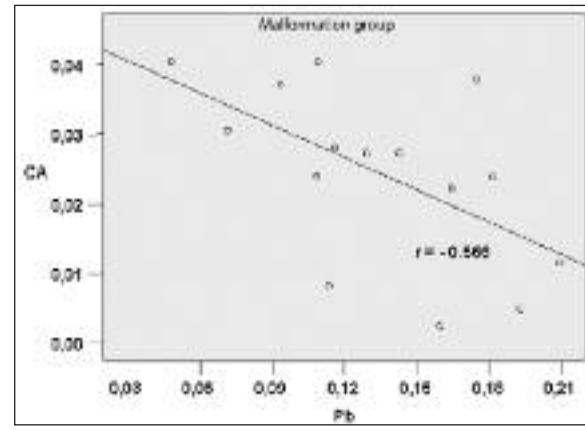

FIGURE 1: Negative correlation between CA and Pb in congenital malformation group.

DISCUSSION

Congenital malformations are significant causes of stillbirths and infant mortality, and the important contributors of childhood morbidity. The precise etiology of most congenital malformations has not been fully understood, however it likely involves both genetic and environmental factors. In the present study, it was found that CAT activity decreased significantly in the erythrocytes of newborns with congenital malformations (Table 2). CAT is an antioxidant enzyme in the body and is predominantly located in cellular peroxisomes. Together with GSH-Px, it catalyzes the conversion of hydrogen peroxide (H_2O_2) to oxygen and water.[11] Similar to our result, individuals with Down syndrome have been reported to have decreased catalase erythrocytic activity.[25] Several different scavengers of free oxygen radicals added to the diet decreased the malformation rate in diabetic rat pregnancy.[9] It has also been shown that oxidative stress induced apoptosis in cultured neurons from rat fetuses. The neural crest cells have been proposed to be a prime target of oxygen radicals in diabetic pregnancy, a hypothesis supported by the facts that tissues displaying malformations are those that are neural crest–derived.[26-28] In literature, erythrocyte catalase activity of patients has been found significantly de-

TABLE 3: Correlations among variables in congenital malformation group (r).

	CAT	Zn	CA	Cu	Fe	Mg	Mn	Pb	Cd
Catalase EU/(gHb)$_{-1}$	1								
Zn (µg/dl)	0.005	1							
Carbonic anhydrase EU/(gHb)$^{-1}$	0.071	0.251	1						
Cu (µg/dl)	-0.158	0.056	-0.307	1					
Fe (µg/dl)	0.459	0.131	0.058	-0.244	1				
Mg (µg/dl)	0.040	0.328	0.030	0.471	0.334	1			
Mn (µg/dl)	0.149	-0.313	0.262	-0.514	0.147	0.156	1		
Pb (µg/dl)	0.128	-0.234	-0.566*	0.216	0.170	0.234	0.211	1	
Cd (µg/dl)	0.009	-0.195	-0.014	0.176	0.194	0.205	0.185	0.370	1

*: P<0.05, **:p<0.01.

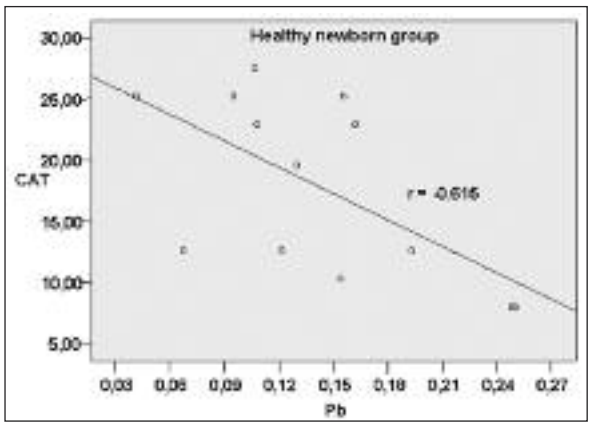

FIGURE 2: Correlation a negative between CAT and Pb in healthy newborn group.
CAT: Catalase

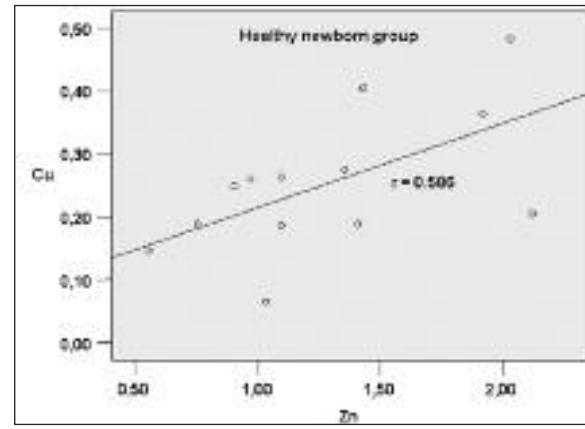

FIGURE 3: Positive correlation between Cu and Zn in healthy newborn group.

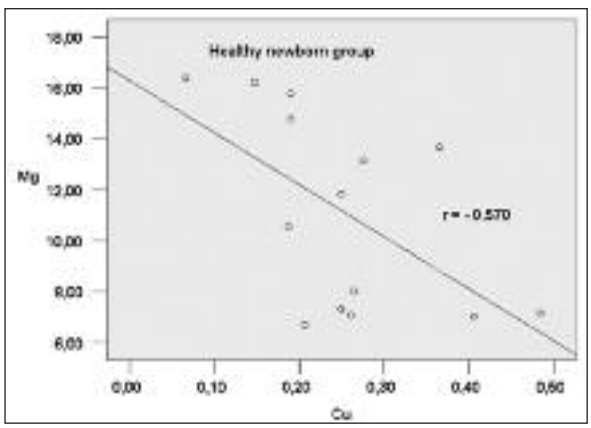

FIGURE 4: Negative correlation between Mg and Cu in healthy newborn group.

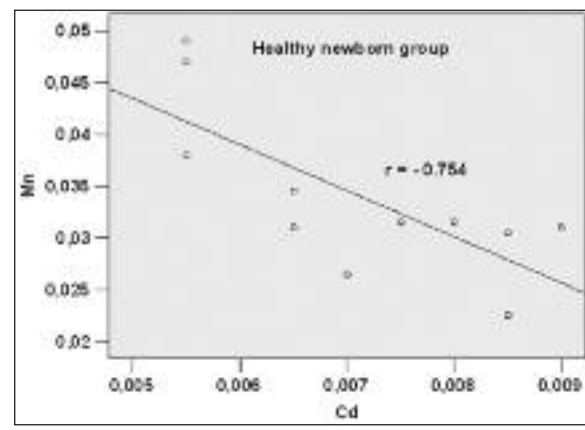

FIGURE 5: Negative correlation between Mn and Cd in healthy newborn group.

creased compared to healty controls. This reduction in catalase activity has been thought to be one of the reasons of the erythrocyte susceptibility to oxidative damage.[29] Earlier studies have shown both increased malformation rate and low catalase activity in malformation-prone U substrain.[30] Catalase enzymes are thus likely to be involved in the protection of embryos against congenital anom-

TABLE 4: Correlation among variables in healthy newborn group (r).

	CAT	Zn	CA	Cu	Fe	Mg	Mn	Pb	Cd
Catalase EU/(gHb)$^{-1}$	1								
Zn (µg/dl)	-0.006	1							
Carbonic anhydrase EU/(gHb)$^{-1}$	0.013	0.044	1						
Cu (µg/dl)	-0.162	0.586*	-0.368	1					
Fe (µg/dl)	0.118	-0.420	0.431	-0.224	1				
Mg (µg/dl)	-0.300	-0.360	0.361	-0.570*	0.109	1			
Mn (µg/dl)	0.295	0.134	0.658*	-0.201	0.488	0.014	1		
Pb (µg/dl)	-0.615*	0.381	0.051	0.433	-0.036	0.139	0.030	1	
Cd (µg/dl)	0.086	-0.199	-0.475	0.377	-0.307	-0.300	-0.754**	-0.261	1

*: p<0.05, **:p<0.01.

alies. Individuals with Down syndrome and congenital malformations exhibit a significant increase in mitochondrial DNA mutations and a reduction in the genetic expression of adenosine triphosphatase, the enzyme responsible for the degregation of adenosine triphosphate.[31]

Cu is an essential nutrient for all eukaryotic organisms due to its function with enzymes that participate in a broad range of critical processes that include respiration, neuropeptide maturation, protection from oxidative stress, neurotransmitter biogenesis, pigmentation, angiogenesis, Fe absorption, connective tissue maturation, and a lot of other key biological functions. Consistent with the importance of these Cu-dependent processes, genetically programmed or dietary induced Cu deficiency in mammals results in impaired motor function, reduced aortic elasticity, neutropenia, cardiac hypertrophy, anemia, severe cognitive disorders, growth defects, and other pathological states. Generally, younger animals are more susceptible to Cu deficiency compared to mature animals, but the precise mechanisms for this differential sensitivity are not well understood.[32] Reportedly, premature infants receiving Cu-deficient milk diets develop neutropenia, anemia, and scorbutic bone changes, and their growth is impaired along with profoundly low serum Cu and ceruloplasmin.[33-35] Cu concentrations are reported to be physiologically increased due to an induction of Cu-carrying protein by estrogen. In the presence of catalytic amounts of transition metal ions, particularly Fe and Cu, these species can generate the highly reactive hydroxyl radical by Fenton reaction. This radical can initiate the process of lipid peroxidation, which if uncontrolled, may result in endothelial cell damage.[36, 37] However, Cu concentrations were even higher in the congenital malformation group in our study (Table 2). This may be due to higher ceruloplasmin concentrations in these groups, since the method used for copper measurement determines the copper which is bound to ceruloplasmin.[38]

Zinc deficiency in humans is a significant worldwide problem.[39, 40] A deficiency of this essential metal results in a wide spectrum of physiological effects, including disorders of skin as well as neurological, immune, and reproductive systems.[41, 42] These effects reflect the diverse functions of zinc. It serves structural and/or catalytic roles in hundreds of peptides, including the remarkably abundant superfamily of zinc-finger proteins.[43] Therefore, the maintenance of zinc homeostasis is critical, and mechanisms have evolved to modulate the uptake, efflux and storage of this metal in response to zinc availability. Dietary zinc deficiency has been described as a much ignored global health problem.[39, 40] Studies of humans suggest a correlation between serum zinc concentrations, birthweight, and pregnancy outcome; dietary zinc supplementation has been reported to exert therapeutic and preventative effects on low birthweight and childhood diseases in several developing countries. It is also interesting to note that zinc deficiency in humans

has been correlated with abnormal fetal development, diabetes and schizophrenia, decreased cognitive behavior and intellectual development, and increased mortality and disease.[44] Decreased gastrointestinal absorption and tissue-specific absorption of Zn may have contributory effects. The levels of Zn were decreased in the sera of newborns with congenital malformations when compared to the control group (Table 2).

Fe is a redox-active transition metal and can participate in single electron reactions and catalyse formation of free radicals, including the undesirable hydroxyl radicals.[45] Although Fe is an essential nutritional element for all life forms, it is known that excess Fe and Fe deficiency also lead to oxidative DNA damage.[46] Lower serum Fe levels were found in congenital malformation group compared to the control group (Table 2).

There are no studies that evaluated heavy metal levels in congenital malformations in the literature. Cd accumulation have been proved to be very toxic in many organs, such as kidney, liver, lung, testis, brain, bone, blood system etc.[47] The molecular mechanisms of its toxicity are not yet well defined. Recent studies on mammals have shown that Cd stimulates formation of reactive oxygen species (ROS), including oxygen free anion radical, hydrogen peroxide and probably hydroxyl radical. A unique aspect of cadmium deposition is that it has an extremely long half-life in the body, on the order of 10 to 30 years in humans,[48] which is thought to be partially due to the binding to the protein metallothionein.[49, 50] However, the contribution of this toxicokinetic parameter to the toxicity of the compound is unknown. Serum Cd levels were found increased in congenital malformation group compared to the control group (Table 2). As a consequence, enhanced lipid peroxidation, DNA damage, as well as marked disturbances of antioxidant defence system occur.[51]

CONCLUSION

This study is the first one to show the relationships of erythrocyte CAT and CA activities, and the serum levels of Cu, Zn, Fe, Mn, Mg, Cd and Pb in newborns with congenital malformations. Enhanced oxidative stress due to imbalance between prooxidant and antioxidant reactions and disturbed serum mineral levels appear to be associated with congenital malformations in newborns. The assessment of oxidant/antioxidant imbalance and serum mineral status in congenital malformations might be useful in the early determination of congenital anomaly. Supplementation with antioxidants in congenital malformations with low antioxidant status might be helpful in the treatment of congenital anomaly and may prevent recurrent congenital malformation. In conclusion, factors that play role in oxidant/antioxidant balance mechanism may have effects in devolepment of congenital malformations and catalase enzyme as an antioxidant can prevent their devolepment.

REFERENCES

1. Thulstrup AM, Bonde JP. Maternal occupational exposure and risk of specific birth defects. Occupational Medicine 2006;56(8):532-43.
2. Sever LE. Congenital malformations related to occupational reproductive hazards. Occup Med 1994;9(3):471-94.
3. Garcia AM. Occupational exposure to pesticides and congenital malformations: a review of mechanisms, methods, and results. Am J Ind Med 1998;33(3):232-40.
4. Burm AG. Occupational hazards of inhalational anaesthetics. Best Pract Res Clin Anaesthesiol 2003;17(1):147-61.
5. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40(4):405-12.
6. Eriksson UJ, Borg LA. Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformations in vitro. Diabetologia 1991;34(5):325-31.
7. Eriksson UJ, Borg LA. Diabetes and embryonic malformations. Role of substrate induced free-oxygen radical production for dysmorphogenesis in cultures rat embryos. Diabetes 1993; 42(3):411-9.
8. Sivan E, Lee YC, Wu YK, Reece EA. Free radical scavenging enzymes in fetal dysmorphogenesis among offspring of diabetic rats. Teratology 1997;56(6):343-9.
9. Cederberg J, Galli J, Luthman H, Eriksson UJ. Increased mRNA levels of Mn-SOD and catalase in embryos of diabetic rats from a malformation- resistant strain. Diabetes 2000; 49(1):101-7.
10. Zaken V, Kohen R, Ornoy A. Vitamins C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. Teratology 2001;64(1):33-44.
11. Kolusari A, Kurdoglu M, Bugdayci G, Adali E, Yildizhan R, Cebi A, et al. Relationship between erythrocyte catalase and serum adenosine deaminase activities in eclampsia. J Matern Fetal Neonatal Med 2009;22(4): 321-4.
12. Garcez M, Peres W, Salvador M. Oxidative stress and hematologic and biochemical parameters in individuals with Down syndrome. Mayo Clin Proc 2005;80(12):1607-11.

13. Zangen SW, Ryu S, Ornoy A. Alterations in the expression of antioxidant genes and the levels of transcription factor NF-kappa B in relation to diabetic embryopathy in the Cohen diabetic rat model. Birth Defects Res A Clin Mol Teratol 2006;76(2):107-14.
14. Oberley LW. Free radicals and diabetes. Free Radic Biol Med 1988;5(2):113-24.
15. Sakurai T, Tsuchiya S. Superoxide production from nonenzymatically glycated protein. FEBS Lett 1988;236(2):406-10.
16. Van Dam PS, Van Asbeck BS, Erkelens DW, Marx JJ, Gispen WH, Bravenboer B. The role of oxidative stress in neuropathy and other diabetic complications. Diabetes Metab Rev 1995;11(3):181-92.
17. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404(6779):787-90.
18. Reece EA, Homko CJ, Wu YK, Wiznitzer A. The role of free radicals and membrane lipids in diabetes-induced congenital malformations. J Soc Gynecol Investig 1998;5(4):178-87.
19. Loeken MR. Free radicals and birth defects. J Matern Fetal Neonatal Med 2004;15(1):6-14.
20. Dhitavat S, Ortiz D, Rogers E, Rivera E, Shea TB. Folate, vitamin E, and acetyl-L-carnitine provide synergistic protection against oxidative stress resulting from exposure of human neuroblastoma cells to amyloid-beta. Brain Res 2005; 1061(2):114-7.
21. Tarun AS, Bryant B, Zhai W, Solomon C, Shusterman D. Gene Expression for Carbonic Anhydrase Isoenzymes in Human Nasal Mucosa. Chem Senses 2003;28(7):621-9.
22. Whyte MP. Carbonic anhydrase II deficiency. Clin Orthop Relat Res 1993;294:52-63.
23. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-6.
24. Rickli EE, Ghazanfar SA, Gibbons BH, Edsall JT. Carbonic anhydrase from human erytrocytes. Preparation and properties of two enzymes. J Biol Chem 1964;239:1065-78.
25. Teksen F, Sayli BS, Aydin A, Sayal A, Isimer A. Antioxidative metabolism in Down syndrome. Biol Trace Elem Res 1998;63(2):123-7.
26. Ratan RR, Murphy TH, Baraban JM. Oxidative stress induces apoptosis in embryonic cortical neurons. J Neurochem 1994;62(1): 376-9.
27. Sadler LS, Robinson LK, Msall ME. Diabetic embryopathy: possible pathogenesis. Am J Med Genet 1995;55(3):363-6.
28. Simán M. Congenital malformations in experimental diabetic pregnancy: aetiology and antioxidative treatment. Minireview based on a doctoral thesis Ups J Med Sci 1997;102(2):61-98.
29. Kalak S, Akkus I, Caglayan O, Aksoy NH, Can UG, Menekse Zeren E, et al. [Erythrocyte catalase activity in type II diabetes mellitus patients]. Turkiye Klinikleri J Med Sci 1996;16(3): 212-5.
30. Cederberg J, Eriksson UJ. Decreased catalase activity in malformation-prone embryos of diabetic rats. Te r a t o l o g y 1997;56(6): 350-7.
31. Lee SH, Lee S, Jun HS, Leong HJ, Cha WT, Cho YS, et al. Expression of the mitochondrial ATPase6 gene and Tfam in Down syndrome. Mol Cells 2003;30;15(2):181-5.
32. Nose Y, Kim B-E, Thiele DJ. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. Cell Metab 2006;4(3):235-44.
33. al-Rashid RA, Spangler J. Neonatal copper deficiency. N Engl J Med 1971;285(15):841-3.
34. Seely JR, Humphrey GB, Matter BJ. Copper deficiency in a premature infant fedon iron-fortifieddiet. N Engl J Med 1972;286(2):109-10.
35. Ashkenazi A, Levin S, Djaldetti M, Fishel E, Benvenisti D. The syndrome of neonatal copper deficiency. Pediatrics 1973;52(4):525-33.
36. Arosio P, Levi S. Ferritin, iron homeostasis, and oxidative damage. Free Radic Biol Med 2002;33(4):457-63.
37. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002; 18(10):872-9.
38. Ilhan N, Ilhan N, Simsek M. The changes of trace elements, malondialdehyde levels and superoxide dismutase activities in pregnancy with or without preeclampsia. Clin Biochem 2002; 35(5):393-7.
39. Prasad AS. Zinc deficiency in humans: a neglected problem. J Am Coll Nutr 1998;17(6): 542-3.
40. Prasad AS. Zinc in human health: an update. J Trace Elem Exp Med 1998;11(2-3):63-87.
41. Apgar J. Zinc and reproduction. Ann Rev Nutr 1985;5:43-68.
42. Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc: health effects and research priorities for the 1990s. Environ Health Perspect 1994;102(Suppl 2):5-46.
43. Ravasi T, Huber T, Zavolan M, Forrest A, Gaasterland T, Grimmond S, et al; RIKEN GER Group; GSL Members. Systematic characterization of the zinc-finger-containing proteins in the mouse transcriptome. Genome Res 2003;13(6B):1430-42.
44. Dufner-Beattie J, Huang ZL, Geiser J, Xu W, Andrews GK. Mouse ZIP1 and ZIP3 Genes Together Are Essential for Adaptation to Dietary Zinc Deficiency During Pregnancy. Genesis 2006;44(5):239-51.
45. Gurzau ES, Neagu C, Gurzau AE. Essential metals-case study on iron. Ecotoxicol Environ Saf 2003;56(1):190-200.
46. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 2001;475(1-2):7-20.
47. Liu J, Qu W, Kadiiska MB. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 2009;238(3):209-14.
48. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposea review of the literature and a risk estimate. Scand J Work Environ Health 1998; 24(Suppl 1):1-51.
49. Liu J, Liu Y, Michalska AE, Choo KH, Klaassen CD. Distribution and retention of cadmium in metallothonein I and II null mice. Toxicol Appl Pharmacol 1996;136(2):260-8.
50. Tohyama C, Satoh M, Kodama N, Nishimura H, Choo A, Michalska A, et al. Reduced retention of cadmium in the liver of metallonein null mice. Environ Toxicol Pharmacol 1996;1(3): 213-6.
51. Demir H, Kanter M, Coskun O, Uz YH, Koc A, Yildiz A. Effectc of black cumin (Nigella Sativa) on heart rate, some hematological values, and pancreatic ß-cell damage in cadmium-treated rats. Biol Trace Elem Res 2006;110(2):151-62.