Assessment of Serum Zinc Level in Relation to Different Presentation of Wilson Disease in a Tertiary Care Hospital

MS ALAM[a], N HAQ[b], M BEGUM[c], F AFROOZ[d], NL DAS[e], MW MAZUMDER[f]

Abstract:

Background: Wilson disease (WD) is an autosomal recessive disorder of copper metabolism with diverse clinical manifestations. Zinc (Zn) has been used for treatment of WD. Recent studies showed low serum zinc level in patients suffering from WD than the normal.

Objective: This cross sectional observational study has been designed to compare the serum Zinc level in children suffering from different presentation of WD before started treatment. Methods: This work was carried out at the department of Pediatric Gastroenterology and nutrition, BSMMU, Dhaka between July' 2018 to June2019. Total 27 children diagnosed as WD disease, aged between three to eighteen years were included in this study. The patients of WD were divided into four groups according to their presentation as acute hepatitis, decompensated liver disease, acute liver failure and Wilson disease with neurological manifestation. Informed written consent were obtained from all patients for participation in this study. Along with other physical findings and laboratory investigations 3 ml of venous blood were collected for estimation of serum Zinc level. After estimation of serum Zinc level results were analyzed statistically. The difference in serum zinc levels were compared between the groups.

Results: Serum Zinc level was founded low in all Wilson disease patients (43.8 ±19.7 [13-83] µg /dl) compared to normal value (64-124µgm. /dl). Among the patients, Serum Zinc level was significantly lower in those who presented as CLD (18 cases, 38.4±17.4µg/dl) and acute liver failure (4 cases, 33.1±3.7µg/dl) compared to those presented as acute hepatitis (4cases, 71.8±4.3 µg/dl) and p value was 0.001 and <0.001 respectively. Mean serum Zinc level was low in 4 patients suffering from acute liver failure (33.1±3.7µg/dl) but was not significant compared to those (23) who presented as Wilson disease non-ALF (45.7±20.8 µg/dl) (P= 0.013) and as Wilson disease CLD (38.4±17.4µg/dl) (p=.25).

Conclusion: Serum Zinc level was lowered in patients of Wilson disease. Serum level of zinc was significantly low in patients presented as CLD and acute liver failure in comparison to patients with acute hepatitis.

Key words: Serum zinc, Wilson disease.

(J Bangladesh Coll Phys Surg 2023; 41: 126-131)
DOI: https://doi.org/10.3329/jbcps.v41i2.64604

Introduction:

Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism. Due to mutation of ATP7B gene located in the chromosome 13, ATP 7B protein a p type protease is deficient. In the absence of ATP7B protein copper cannot be incorporated in apoceruloplasmin leading to deficient ceruloplasmin, a copper containing protein that transports copper to the different parts of the body and excretion of copper into bile is also reduced. As a result copper starts to accumulate progressively in the hepatocytes, excess copper causing hepatic toxicity. Subsequently, copper accumulates in the other tissues like the brain, cornea, and skeleton & rarely in the heart. The failure to excrete biliary copper is present since birth but symptom do not develop until approximately 3 years of age [1]. It affects I in 30,000 peoples, occurs all over the world with slight male predominance [2]. Without treatment outcome of WD is fatal.

In recent decades another metal zinc has also been linked with Wilson disease especially in the field of treatment. Zinc induces copper binding metallothioneins (MTs) both in enterocytes and hepatocytes. In the gut zinc reduces intestinal absorption of copper into portal

a. Dr. Md. Shafiul Alam, Ju. Con. Department of Pediatrics, Shaheed Suhrawardy Medical College Hospital, Dhaka, Bangladesh

b. Dr. Nadia Haq, Consultant, Department of Pediatric, Bangladesh Specialized Hospital (BSH), Dhaka, Bangladesh

c. Dr. Morsheda Begum, Registrar, Department of Gynecology & Obstetrics, Ad Din Women Medical College Hospital, Mogbazar, Dhaka, Bangladesh

d. Dr. Farzana Afrooz, Assistant Professor, Department of pediatrics, Shaheed Suhrawardi Medical College Hospital, Dhaka, Bangladesh

e. Dr. Nanda Lal Das, Assistant Professor, Department of pediatrics, Shaheed Suhrawardi Medical College Hospital, Dhaka, Bangladesh

f. Dr. Md Wahiduzzaman Mazumder, Associate Professor, Department of Pediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh

Address of Correspondence: Dr. Md. Shafiul Alam, Ju. Con. Department of Pediatrics, Shaheed Suhrawardy Medical College hospital, Dhaka, Bangladesh. E-mail: alam_nizam@yahoo.com, Mobile: 01673551828

Received: 28, April, 2022 **Accepted:** 02 January, 2023

circulation and in the hepatocytes it reduces the damaging effects of free liver copper [3]. It is well known that within the body, copper and Zinc have competing roles in a way that Cu overload leads to Zn deficiency [1]. Cu and Zn interact at the intestinal mucosal level, influencing each other's absorption. MTs bind Cu within intestinal cells and thereby prevent the systemic absorption of Zn. Cu overload could also negatively influence the expression of Zn transporters and reduce absorption of Zn in intestine leading to low Zn level [4]. The behavior of serum Zn levels remains unclear in clinical conditions such as inflammatory states and malignancy in which serum Cu levels are elevated [5].

It is widely accepted that zinc is a strong inducer of intestinal MTs that block copper absorption however there are doubts that an excess copper not clearly documented in the intestinal lumen of patient's with Wilson disease can impair the absorption of zinc. [6]

Recent studies showed discrepancy in serum level of Zinc in patients suffering from Wilson disease. Most of the study showed low serum Zinc level in Wilson disease children. A study conducted by Palittiya Sintusek et al. found serum Zn level was significantly lower in WD with acute liver failure patients compared to those with WD-non-ALF and ALF from indeterminate causes even when corrected serum Zn for low albumin & regardless of presentation, Zn levels showed a negative correlation with the disease severity [7].

The mechanism of Zinc as a decoppering agent has been known for a long time; however, there are few studies focused on the level of serum Zn in untreated WD patients. There is no study in Bangladesh regarding serum Zinc level in patients suffering from WD so far. So, this study was done to determine serum Zinc level in patient's suffering from various grades of WD before starting treatment.

Methods:

Study protocol and subject

This cross sectional observational study was conducted at department of Pediatric Gastroenterology and Nutrition, BSMMU, Dhaka, Bangladesh, from July' 2018 to June 2019.This study was approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University, Bangladesh. (IRB No. BSMMU/2018/1839). Every ethical issue was discussed with the parents regarding the study. Parents were clearly informed about the nature and purpose of the study, procedures followed, risk associated with it and benefits from the study in easily understandable local language. Then a written consent was obtained from parents. Every precaution was taken, so that study would not cause any harm to participants.

The standardized study protocol was made and set to be applied to all study subjects. The subjects were, children of 3 to 18 years, suffering from WD having hepatic with or without neurological manifestation and was not treated before. Sample size was calculated following the methodology of Two -Sample T-Test, allowing Unequal variance alternative hypothesis $\mu 1 \neq \mu 2$.

Total 27 diagnosed case of Wilson disease were selected purposively from inpatient department. The patients of WD were divided into four groups according to their presentation as acute hepatitis, chronic liver disease, acute liver failure & neuro-psychiatric manifestation.

Along with clinical data including demographic data, clinical features, and laboratory investigations (Hb% ,Serum ALT ,Serum Albumin, Serum Ceruloplasmin, 24 hr Urinary copper , INR. S.bilirubin,KF ring)for establishing WD, 3 ml of venous blood were collected for estimation of serum zinc level. The measurement of serum zinc was done at the analytical Chemistry laboratory of Atomic Energy Center Dhaka by a method that has been optimized and validated as per ISO guideline. Sample was collected in zinc free containers. The collected Serum samples were acidified with concentrated Nitric acid (E. Merck, Germany) for mineralization at least 30 minutes prior to analysis. Then, concentration of Zn in Serum was determined using a Varian AA240 FS Atomic Absorption Spectrometer. Acetylene gas with 99.99% purity was used for ûame. A hollow cathode lamp having a wavelength of 213.9 nm and a slit of 0.7 nm was used and operated according to the conditions recommended by the manufacturer. The working standard solutions were prepared daily by the appropriate dilution of the stock Zn standard solution. After estimation of serum zinc level results were analyzed statistically. The difference in serum zinc levels were compared between the groups.

Data analysis and statistics:

Questionnaire was filled up for each group, which contained the detailed information obtained from the

history, clinical examination, findings of laboratory reports and diagnosis. Collected data was entered in a data sheet (MS Excel in Windows PC). Data was checked manually with the questionnaire responses, and data was edited whenever required. Afterwards data was analyzed by computer-based program SPSS for Windows (using version 24.0 Standard Edition). Univariate analyses were performed for analyzing different variables, their dispersions and distributions. Data were expressed in percentage, mean with standard deviation. Independent t-test was done to compare mean values and for all statistical tests p value less than 0.05 was considered as significant.

Results:

Twenty seven patients were enrolled in this study. Among them 16 (59.3 %) were male and 11 (40.7 %) were female and 26 (98.30 %) were Muslim and 1 (3.70%) was Hindu. Mean age of the studied population was 10.2±2.2 yrs and all were above 5 years of age. (Table-I).

Table-I

Age distribution of the studied children with mean age

Age in yrs.	Wilsonian children(27)
0-5	0(00)
6-10	15(55.6%)
11-18	12(44.4%)
Mean age	10.22±2.2

In the study population 18 patients (66.7 %) presented as chronic liver disease. Among the rest, acute liver failure and acute hepatitis compriseds 4 patients (14.8 %) each and 1 patients presented with neuropsychiatric manifestation (3.7 %).

Table II shows the presenting complains of the studied population. It shows that 96.3% patients presented with jaundice, 81.5% with ascites, and 22.2% having hepatomegaly. Splenomegaly was present in 25.9% cases, encephalopathy in 14.8% cases, and coagulopathy in 33.3% cases. K-F ring was found in 63% cases and both K-F ring and sunflower cataract in 7.4% patients.

Table III shows that in the studied population, mean Hb% was 9.1±1.7 gm. /dl, mean Serum ALT was 109±20 u/L, Serum Albumin was 21.7±5.1gm/L, Serum Ceruloplasmin was 8.2±4.5gm/L, mean 24 hr Urinary copper was1199±128 µg/day, INR was 2.6± 1.1 denotes presence of significant coagulopathy and S.bilirubin was 6.025±5.9 mg/dl .S.zinc level was 43.8±19.7 (µgm/dl)

Table-II

Clinical parameters of children with WD (n=27)

History/Clinical parameter	No (%)
Consanguinity	11(40.7)
Family history of liver disease	11(40.7)
Family history of death from liver disease	3(11.1)
History of sib death from liver disease	4(14.8)
Jaundice	26(96.3)
Ascites	22(81.5)
Hepatomegaly	6(22.2)
Splenomegaly	7(25.9))
Hepatosplenomegaly	6(22.2)
Encephalopathy	4(14.8)
Coagulopathy	9(33.3)
K-F ring	17(63)
K-F ring+sunflower cataract	2(7.4)

Table-III

Biochemical parameter of the children with WD (n=27)

Biochemical parameter	Minimum	Maximum	Mean	Std. deviation
Hb(gm/dl)	5.50	11.7	9.1	1.7
S.ALT(u/l)	10	1095	109	20
INR	1.1	5.5	2.6	1.1
S.albumin (gm/dl)	10	35	21.6	5.1
S.ceruloplasmin(gm/dl)	3	21	8.2	4.5
24hr.u.copper(µgm/d)	120	4362.8	1199	128
S.bilirubin(mg/dl)	.90	23.4	6.02	5.9
S.Zinc (µgm/dl)	13	83	43.8	19.7

Table IV shows that mean Hb was low in all cases but normal in neuropsychiatric Wilson disease. ALT was normal in neuropsychiatric WD but higher in WD with CLD, Serum Albumin was low in all cases. Serum Ceruloplasmin was also low in all cases. Mean 24 hr. Urinary copper was high in acute hepatitis group .INR was more than normal in all groups but highest in acute liver failure group which denotes presence of significant coagulopathy and bilirubin was highest in WD presented as CLD.

Table V shows normal level of serum zinc (64.8±11.8 µgm/dl) and lower than normal (43.9±19.7µgm/dl) in children with Wilson disease. Among the children with WD low serum zinc level was found in those who presented as chronic liver disease and acute liver failure but found normal in acute hepatitis and in neuropsychiatric manifestation.

Table VI shows serum zinc level was significantly lower in WD patients presented as CLD (38.4±17.4µgm /dl) than who presented as acute hepatitis (71.8±4.8µgm / dl) (p=0.001).and in acute liver failure patients (33.10±3.7) µgm/dl compared to patients presented as acute hepatitis (71.8±4.8µgm/dl) (p=<0.001). This table also shows significant difference of serum zinc level between

Table-IV

Biochemical parameters of different presentation of Wilson disease patients (n=27)

Lab parameter	Acute hepatitis	Chronic liver disease	Acute liver failure	Neuropsychiatric Wilson disease
	(4)	(18)	(4)	(1)
Hb(gm/dl)	9.2	9.1	8.2	11
S.ALT(u/L)	57.3	127.5	101.7	18
INR	1.9	2.7	3.2	1.4
S.albumin (gm/l)	23	20.2	23.8	32
S.ceruloplasmin(gm/L)	5.3	8.9	6.3	16
24 hrucopper (µg/day)	3075	1234	2069	665
S.bilirubin(mg/dl)	1.7	7.1	6.9	1.02

Table-V

Serum Zinc level of the studied children (n=27)

Presentation of patients	Number of patients	S.Zinc level µgm /dl (Normal)	S.Zinc level µgm /dl (Measured)
Wilson disease	27	64-125	43.8±19.7
Wilson disease as AH	4	64-125	71.8±4.8
Wilson disease as CLD	18	64-125	38.4±17.4
Wilson disease as ALF	4	64-125	33.1±3.7
Neuropsychiatric WD	1	64-125	71

Table-VI

Serum Zinc level in different presentation of WD in the studied population

	WD (CLD) (18)	WD (AH) (4)	WD (ALF) (4)	WD non ALF (23)	P Value Wd (CLD) and WDas AH	WD (ALF) and WDas AH	WD (ALF) and WD as NonALF	WD (ALF) and WDas CLD
S.Zinc level µgm /dl	38.4±17.4	71.8±4.8	33.1±3.7	45.65±20.8	.001	<0.001	.013	.249

patients of acute liver failure and non-acute liver failure (33.1±3.7µgm/dl vs 45.7±20.8µgm/dl, p=0.013 and non-significant difference between acute liver failure and CLD (33.1±3.7µgm/dl vs 38.4±17.4 µgm/dl, p =0.249)

Discussion

In this study, 27 Bangladeshi children aged 3-18 yr. diagnosed as WD were included before starting treatment. Among them 16 (59.3 %) were male and 11 (40.7 %) were female and 26 (98.30 %) were Muslim and 1 (3.70%) was Hindu. Median age at diagnosis was 10.2 years with minimum7 years and maximum 15 years. The studied children (27) were subdivided according to their presentation as acute hepatitis (4) (14.81%), chronic liver disease (18) (66.7%), acute liver failure (4) (14.81%) and neuropsychiatric WD (1) (14.81%). Presenting features in the studied patients were: jaundice 26 (96.3.3%), ascites 22 (81.5%), hepatomegaly 6 (22.2%), splenomegaly 7 (25.9%), hepatosplenomegaly 6 (22.2%), coagulopathy 9 (33.3%), Encephalopathy 4(14.8%) and deterioration of school performance in 5 (18.5%) cases. There was male preponderance over female 16(59.3%) v 11(40.7%). Males have a slightly higher risk of developing WD than females, possibly because of differences in estrogen level and iron metabolism [2]. Mean age was similar to Li et al, who found mean age as 8.3 years, Deverabhavi et al found the mean age was 9.7 years with male preponderance in a large paediatric WD associated liver failure cohort[8, 9].Iacob et al.and Deguti et al. also found male predominance (51.4% and 65.2%) [10,11]. Consistent result was also observed in another study done in Bangladesh by Karim et al [12]. In that study 32 patients with WD were studied and the mean (±SD) age was 9 ± 2.9 years, and the youngest age at diagnosis was 3.5 year. In contrast, Wang et al. found mean age 15±9 years in their study and Taly et al in Bangalore, India, found female preponderance[13, 14]. Present study demonstrated parental consan- guinity in 11(40.7%) of cases which is almost similar to the study done in Bangladesh by Rukunuzzaman, who found consanguinity in 30% cases but not similar to Taly et al, who found parental consan- guinity in 55 % patients[14,15]. Wilson disease more commonly expressed in consanguineous family as it is an autosomal recessive genetic disorder. History of sib death was found in 4(14.3%) cases. K-F ring was present in 18 (64.3%) patients. It is higher than the findings of Iacob et al. (39.5%), Hua et al. (50%) and Li et al. (43%) however KF ring is usually present in 50 to 60% cases of hepatic manifestation of WD [8,10,16]. Mean serum Hb level in studied population was 9.08gm/l but the lowest in patients who presented as WD with ALF. Albumin level in was also low (21.7 gm/l).

Among the studied population mean serum ceruloplasmin level was 6.2 mg/dl who presented as ALF and 8.6 mg/dl in without ALF. This values are lower than normal. Whereas up to 35% of WD patients with hepatic presentation and 60% of patients with fulminant hepatic failure maintain normal or raised levels at the time of diagnosis as ceruloplasmin is an acute phase reactant[17, 18]. Mean 24 hours urinary copper excretion was 1187 ìg /day and the level was much higher in those who presented as liver failure (2069 mcg/day) than who presented without liver failure (1040 ìg /day, normal =<40ìg /day). Basal 24-hour urinary copper excretion >100 ìg /day is considered positive for WD[19].

Mean serum Zinc level in the studied patients was significantly lower (45.1 ±20.1 µg /dl) than normal (64-124µg /dl). Similar findings was described by Geetha et al in a cohort of 18 children with Wilson disease versus 20 age and sex matched healthy volunteers 60.6 ±7.3 µg/dl Versus 131.9±15.8 µg /dl, P<0.001 [24].In our study, we found mean serum Zinc level in patients with WD who presented as ALF was lower (33.1±1.8µg/dl) compared to non-ALF patients (45.6±20.8µg/dl) however p value was non-significant (P>0.005) This findings is not similar to the findings observed by Palittiya Sintusek et al [7].They found serum Zn was significantly lower in WD-ALF patients compared to those with WD-non-ALF. This may be due to the small number of patients of WD with ALF we could include in our study.

Serum Zinc level was significantly lower in WD patients who presented as chronic liver disease (38.43±17.4 µgm/dl) and in acute liver failure (33.10±3.7) µgm/dl compared to acute hepatitis (71.8±4.8 µgm/dl) (p=.001 and <0.001 respectively). In our study we found serum Zinc level was normal in acute hepatitis group (71.8±4.8 µgm /dl , normal=64-124 µgm /dl), this findings is similar to the findings of a study conducted by Iorio and Ranucci, where they found normal or high serum Zinc at diagnosis in patients with a mild liver disease (163±32.6µgm /dl)[6].

Conclusion:

The Serum Zinc level was lower in Wilson disease children in this study. Moreover among the children with WD Zinc level was also found significantly lower

in CLD and acute liver failure group in comparison to who were presented as acute hepatitis.

Limitation of the study

1. Time and resources were limited.

2. Small sample size.

3. Single center study.

Recommendations:

Further studies with larger sample size of children with WD before starting treatment is necessary to find out the better correlation of serum Zinc level with clinical phenotype and the severity of disease.

Acknowledgement:
All authors thank the all stuff of Department of Paediatric Gasreoenterology & Nutrition Bangabandhu Sheikh Mujib Medical University, Bangladesh. This study was supported by a research fund from the Bangabandhu Sheikh Mujib Medical University, Bangladesh.

References:

1. Ranucci G, Socha P, Iorio R. Wilson disease: what is still unclear in pediatric patients? Clinics and research in hepatology and gastroenterology. 2014 Jun 1; 38(3):268-72.DOI: 10.1016/j.clinre.2014.03.002.
2. Chen C, Shen B, Xiao JJ, Wu R, Canning SJ, Wang XP. Currently clinical views on genetics of Wilson's disease. Chinese medical journal. 2015 Jul 5; 128(13):1826. DOI: 10.4103/0366-6999.159361. PMID: 26112727.
3. Huster D, Finegold MJ, Morgan CT, Burkhead JL, Nixon R, Vanderwerf SM et al S. Consequences of copper accumulation in the livers of the Atp7b"/"(Wilson disease gene) knockout mice. The American journal of pathology. 2006 Feb 1; 168(2):423-34.DOI: 10.2353/ajpath.2006.050312.
4. Van Biervliet S, Küry S, De Bruyne R, Vanakker OM, Schmitt S, Velde SV et al. Clinical zinc deficiency as early presentation of Wilson disease. Journal of Pediatric Gastroenterology and Nutrition. 2015 Apr 1;60(4):457-9.DOI:10.1097/MPG.0000000000000628.
5. Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. Advances in nutrition. 2013 Mar; 4(2):176-90. https://DOI:org/10.3945/an.112.003210.
6. Iorio R, Ranucci G. Wilson disease: a matter of copper, but also of zinc. Journal of Pediatric Gastroenterology and Nutrition. 2015 Apr 1;60(4):423-4.
7. Sintusek P, Kyrana E, Dhawan A. Value of serum zinc in diagnosing and assessing severity of liver disease in children with wilson disease. Journal of pediatric gastroenterology and nutrition. 2018 Sep 1; 67(3):377-82.DOI: 10.1097/MPG.0000000000002007.
8. Li XH, Lu Y, Ling Y, Fu QC, Xu J, Zang GQ et al. Clinical and molecular characterization of Wilson's disease in China: identification of 14 novel mutations. BMC medical genetics. 2011 Dec 1; 12(1):6.DOI: 10.1186/1471-2350-12-6.
9. Devarbhavi H, Patil M, Sheth KA, Krishnamurthy AC. A review and current perspective on Wilson disease. Journal of clinical and experimental hepatology. 2013 Dec 1;3(4):321-36.DOI: 10.1016/j.jceh.2013.06.002.
10. Iacob R, Iacob S, Nastase A, Vagu C, Ene AM, Constantinescu A et al. The His1069Gln mutation in the ATP7B gene in Romanian patients with Wilson's disease referred to a tertiary gastroenterology center. Journal of Gastrointestinal & Liver Diseases. 2012 Jun 1;21(2).PMID: 22720308
11. Deguti MM, Genschel J, Cancado EL, Barbosa ER, Bochow B, Mucenic M et al. Wilson disease: novel mutations in the ATP7B gene and clinical correlation in Brazilian patients. Human mutation. 2004 Apr;23(4):398-.DOI: 10.1002/humu.9227.
12. Karim MB, Rahman MM, Islam MS. Wilson's disease with hepatic presentation in childhood. Mymensingh medical journal: MMJ. 2007 Jan; 16(1):29-32.DOI: 10.3329/mmj.v16i1.244.
13. Wang JS, Lu Y, Wang XH, Zhu QR. Urinary copper/zinc ratio: a promising parameter for replacement of 24-hour urinary copper excretion for diagnosis of Wilson's disease in children. World Journal of Pediatrics. 2010 May 1; 6(2):148-53.DOI: 10.1007/s12519-010-0023-4.
14. Taly AB, Meenakshi- SS, Sinha S, SwamyH S,Arunodaya GR. Wilson disease: description of 282 patients evaluated over 3 decades. Medicine. 2007; 82:112–121.

DOI: 10.1097/MD.0b013e318045a00e

15. Rukunuzzaman, M. Wilson's Disease in Bangladeshi Children: Analysis of 100 Cases', Pediatric Gastroenterology Hepatology&Nutrition.2015; 18:121-127. https://DOI.org/10.5223/pghn.2015.18.2.121
16. Hua R, Hua F, Jiao Y, Pan Y, Yang X, Peng S et al. Mutational analysis of ATP7B in Chinese Wilson disease patients. American Journal of Translational Research. 2016;8(6):2851.Pubmed .PMC4931180
17. Sternlier I, Morell AG, Bauer CD, Combes B, De Bobes-Sternberg S, Schein-Berg IH. Detection of the heterozygous carrier of the Wilson's disease gene. The Journal of Clinical Investigation. 1961 Apr 1; 40(4):707-15.DOI: 10.1172/JCI104304.
18. Yang X, Tong DJ, Liang J, Zhang YH, Lei JH, He XE et al. Ceruloplasmin level of patients with liver disease in China. ZhonghuaNeiKeZaZhi. 2005 Jan 1; 44(1):13-5. PMID: 15769390.
19. Geetha A, Jeyachristy SA, Selvamathy SM, Ilavarasi S, Surendran R. A study on the concentrations of serum zinc, non-ceruloplasmin copper, reactive oxygen and nitrogen species in children with Wilson's disease. Clinicachimicaacta; international journal of clinical chemistry. 2007 Aug; 383(1-2):165.DOI: 10.1016/j.cca.2007.05.005.