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HEAVY METALS IN SERA OF PATIENTS WITH HEPATITIS B DETERMINATION AND ANALYSIS

Wahran M.Saod*,Tahseen .A. Zaidan *, Abdul Wahab .A. Alfaluji **

*Department of Chemistry, college of Science, University of Anbar, Ramadi, IRAQ

** Fallujah teaching hospital, Fallujah, IRAQ

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Corresponding author Email:rose_wsh@yahoo.com

ABSTRACT

Chronic Hepatitis B is a major global health problem worldwide, However, two Billion People could be infected with this disease Worldwide. Living organisms require varying amounts of heavy metals. Such as manganese(Mn), cobalt(Co) and zinc(Zn). Some heavy metals have bio-importance, but the bio toxic effects of many of them in human biochemistry are of great concern. Heavy metals are essential micronutrients involved in many metabolic processes. One hundred sixty patient samples and thirty healthy individuals were investigated in this study. All patients included in this research are tested positive for the HBSAg test and their specimens were examined by profile test which includes (HBS Ab, HBe Ag, HBe Ab, HBc Ab ,(IgM-IgG), HCV and HIV) and also viral load (HBV PCR) with unit (copies/ml) were carried to test the patients sera. Manganese, chromium, cobalt, and nickel have levels were determined by using inductively Coupled plasma- Mass spectrometry (ICP-MS). Results show a significant increase in the concentration of manganese, chromium, cobalt, and nickel in serum of patients with chronic hepatitis B compared

to healthy individuals. We conclude that hepatitis B may affect the level of studied elements in patients infected with HBV.

INTRODUCTION

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) that affects the liver. The clinical result of infection is related to immune response (8). The disease may develop to liver carcinoma during infection. An estimated 350 million people worldwide are chronically infected with HBV (19). In the United States, there are about 1.25 million hepatitis B carriers and it is estimated that approximately 75% of the hepatitis B carriers live in Asia and Africa (1). Many studies showed that the suffusion of HBV infection has reduced from 4.1% to less than 1% in the period of the form 1970 to 1990 In Iraq (5, 3).

Heavy metals could adversely affect the male reproductive system, either by causing hypothalamic-pituitary axis disruption or by directly affecting spermatogenesis, resulting in impair semen quality (27) . The main problem with heavy metals in our bodies is their ability to bio-accumulate (21). The metals will not be cleared by the system unless some type of intervention is used to chelate the metals and flush them out. There are some heavy metal toxicities that are more prevalent than others, like mercury from dental fillings and lead from lead pipes and paint (13).

Manganese is located mainly in the mitochondria. It is a constituent of many important metalloenzymes like superoxide dismutase, pyruvate carboxylase, arginase, and glycosyltransferase. It is absorbed in small intestine, then bound to albumin in circulation and is transported to the liver and excreted in bile (6). Manganese is an antioxidant nutrient and is important in the breakdown of amino acids and the production of energy. It is essentially required for the metabolism of vitamin B1, C, and E and for the activation of various enzymes which are important for proper

digestion and utilization of foods (25). Chromium is an essential nutrient required by the human body to promote the action of insulin in body tissues, so that the body can use sugars, proteins, and fats (22). In humans, systemic effects have been reported to occur in the airways, cardiovascular system, kidneys and liver (16). Cobalt is required as a constituent of vitamin B12 (22). In addition to its role in vitamin B12, Cobalt is also a cofactor of enzymes involved in DNA biosynthesis and amino acid metabolism (4). Nickel is an essential trace element for many species (2). nickel is found in blood and tissues at consistent levels and is also associated with DNA and RNA in amounts that suggest physiological significance. Nickel is required for normal growth and reproduction in animals, and human beings as well. It has a role in the modulation of the immune system and in development of the brain (10).

The aim of this study is: to compare the level of heavy metals in sera of patients infected with HBV and healthy participants.

MATERIALS AND METHODS

. Materials

A 150 µL aliquot of serum samples were thawed at room temperature for 20 minutes and pipette into pre-cleaned polyethylene tubes. 150 µL of optima grade nitric acid and 100 µL of trace select grade hydrogen peroxide were then added to the samples. The tubes were tightly capped, centrifuged for 10 minutes at a speed of 4400 r/min in a centrifuge. Samples were then placed in a hot block digester and digested at 95 °C for 90 minutes with the tube caps loosened. Following digestion the samples were diluted to 150 µL(12) .

.Procedure

Multi element working standards were prepared containing (Mn ,Cr, Co and Ni) by diluting high purity 1000mg L-1 stock solutions with deionized water and nitric acid. The standard solutions were kept at 4 C^0 in dark room to reduce the risk of contamination. All works were carried out under clear room conditions. Ultra-pure de-ionized water was used for sample preparations. Standard solutions were prepared freshly from the stocks, with dilute nitric acid (5% v/v). In order to obtain appropriate . ICP-MS responses, the experiments were performed using different concentration levels.

. Samples analysis

The digested serum samples were analyzed for (Mn, Cr, Co and Ni) using Inductively Coupled plasma- Mass spectrometry (Agilent Technology, Japan). The analytical calibration method was accomplished with aqueous standards in 0.5% (v/v) HNO_3. Fresh calibrations were made each time before analysis.

.Statistical Analysis

The Pearson correlation test was used to correlate between different variables among the studied groups (26) .

RESULTS AND DISCUSSION

. Prevalence of Chronic Hepatitis B

The record of patients and healthy group shows a difference in disease incidence in rural and urban regions. There is an increase in disease's incidence in rural areas (114 patients) as compared to the urban regions (76 patients) as shown in (Table 1).

This finding can be attributed to lack of the health awareness and because the patients have not been vaccinated with hepatitis B vaccine. details of patients and health groups are shown in (Table 2).

Table (1) List of Symbols and details for study groups .

Symbols	Details	Numbers
1A	Patients HBeAg (+ve)normal liver function PCR= (>100000)	36
2A	Patients, HBeAg (+ve)abnormal liver function PCR= (>100000)	28
1B	Patients HBeAg (-ve) PCR= (>100000)	30
2B	Patients HBeAg (-ve) PCR=4000-100000	36
3B	Patients HBeAg (-ve) PCR=N0N	30
C	healthy individuals	30

Table(2) gender and residence distributions of different groups for HBV patient and healthy individuals

Parameters	**1A**	**2A**	**1B**	**2B**	**3B**	**C**	**Total**
Male	20	15	15	17	17	21	105
Female	16	13	15	19	13	9	85
Urban	9(25%)	8(28.5%)	9(30%)	18(50%)	16(53.3%)	16(53.3%)	76
Rural	27(75%)	20(71.4%)	21(70%)	18(50%)	14(46.6%)	14(46.6%)	114

Manganese

Serum concentration of manganese was significantly higher (0.94±0.12µg\dl) in chronic hepatitis B patients in comparison to controls (0.77±0.08 µg/dl), as in fig.(1A). Serum concentration of manganese was significantly higher (0.94±0.12µg\dl) in chronic hepatitis B patients in comparison to controls (0.77±0.08 µg/dl), as in fig. (1A). study concurs with (23) who reported that serum manganese concentration increased in patients during the active phase of acute hepatitis, chronic

hepatitis and post-hepatitis cirrhosis and the study by (20) who reported that serum manganese may be caused from two reasons, necrosis of liver parenchyma, leading to an important release of hepatic Manganese or decreased hepatobiliary excretion(7).

.Chromium, cobalt and nickel

The results showed that the mean serum chromium level were significantly higher(28.11±2.68 µg\l) in hepatitis patient than in healthy individuals (22.56 +2.54 µg\l) , fig. (1B) This denotes that risk of exposure to chromium was associated with all liver diseases, but fatty liver cases were the ones that were more exposed. These results may be in part due to high initial levels and in another part to liver affection by hepatitis or schistosomiasis. It was claimed to be hepatotoxic (9).

The mean of serum Cobalt level in hepatitis patients was significantly higher(1.81±0.34 µg\l) than in healthy individuals1(11±0.17 µg\l) as in fig. (1C).

Cobalt is a metal that is required by the body for blood formation. It is an integral part of vitamin B12, this vitamin needful for producing red blood cells and maintaining the nervous system. Cobalt activates many metabolic enzymes (17). our study agreement with (15) who studied cobalt in serum patients and control group and found that the mean concentration of serum cobalt in control group was less than patients

The mean of serum nickel level (5.35±0.94 µg\l) showed significantly higher in hepatitis patients than in the healthy individuals (4.18±0.54 µg\l) as in fig.(1D).Our result disagreement with (17) result is very high when compared to our findings. Nickel is a trace element, required in minute quantities by the human body. Enzymes containing Nickel have not been found, though Nickel functions to activate or inhibit enzymes containing other elements. The study (24) found that level of nickel higher in early and advanced stages of hepatic cirrhosis. The Study (18) suggested that the

diminution of serum nickel in hepatic cirrhosis may reflect diminished concentrations of serum nickel oplamin and albumin. nickel oplamin and albumin may play an essential physiological role that includes a Nickel-containing protein ‘‘Nickel oplasmin’’inhuman serum (14).

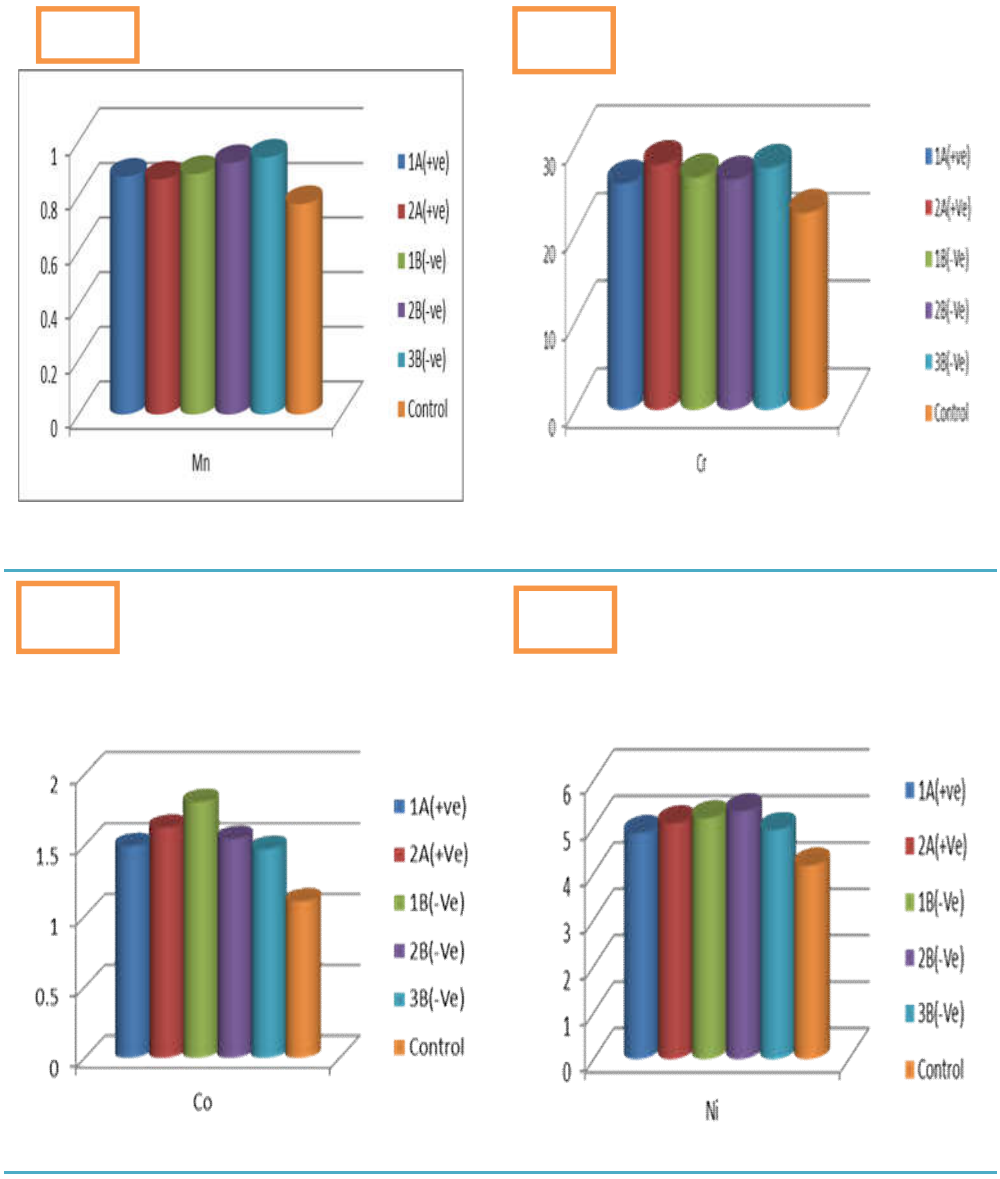

FIGURE 1A. level of manganese concentrations in hepatitis B patients and control group. **1B.** Serum chromium concentration in in hepatitis B patients and control group. **1C.** Serum Cobalt concentration in in hepatitis B patients and control group. **1D.** Serum Nickel concentration in in hepatitis B patients and control group.

CONCLUSIONS|

our study was elucidated that levels of manganese, chromium, cobalt, and nickel were higher in chronic hepatitis B patients as compared to control group. ICP-MS method is an efficient technique in the detection of heavy metals concentration.

المعادن الثقيله في مرضى التهاب الكبد الفايروسي B تقدير وتحليل

وهران منعم سعود* ،تحسين علي زيدان *،عبد الوهاب الفلوجي**

*قسم الكيمياء،كليه العلوم ،جامعة الانبار ، ألانبار ،العراق.

**مستشفى الفلوجه التعليمي ،الانبار ، العراق.

الخلاصة

يمثل مرض التهاب الكبد الفايروسي B المزمن مشكلة صحية عالمية، حيث أن هناك مليارين أنسان عرضة للاصابة . تحتاج الكائنات الحية الى كميات متغيرة من العناصر الثقيلة مثل المنغنيز والكوبالت والزنك. تمتلك بعض الفلزات الثقيلة أهمية حيوية ولكن العديد منها ذات تأثيرات سمية مقلقة جدا على الأنسان . تكون العناصر الثقيلة مغذيات دقيقة تدخل في العديد من العمليات الأيضية. مئة وستون مصابا وثلاثون فردا غير مصابا استخدمت كعينة في هذه الدراسة.

تم اختبار جميع المرضى المشمولين في البحث بإيجابية لفحص (HBSAg) وتم فحص عيناتهم من خلال الاختبارات المتضمنة (HBS Ab , HBe Ag , HBe Ab , HBc Ab , (IgM-IgG) , HCV,HIV) وكذلك الحمل الفايروسي (HBV PCR) بوحدة (نسخ / مل) التي اجريت لجميع المرضى.

تم قياس مستويات المنغنيز والكروم والكوبالت والنيكل بواسطة مطياف الكتلة-البلازما المزدوج. تظهر النتائج زيادة مهمة في تركيز المنغنيز والكروم والكوبالت والنيكل في مصل دم المرضى المصابين بالتهاب الكبد المزمن B مقارنة مع الأفراد الأصحاء. نستنتج أن مرض التهاب الكبد المزمن B ربما يؤثر على مستوى العناصر الثقيلة في المرضى المصابين ب HBV.

REFERENCE

1. **Alavian, SM.(2006).** Immunization: an important strategy to control hepatitis B. *Hepat Mon* . vol. 6 (1), pp.; 3 – 5.

2. **Alexandrovn, R., Costisor, O. and Patron, L. (2006)** . Nickel. *Pathology and parasitology* . vol. 911, pp ; 64 – 74.

3. **Al-Juboury, F., Salih, A., Al-Assadi, K. (2010)** . Seroprevalance of hepatitis B and C among blood donors in Babylon Governorate- Iraq. *Med J Babylon*.vol.7:1–2.

4. **Arinola, OG. (2008)** . Essential Trace Elements and Metal Binding Proteins in Nigerian Consumers of Alcoholic Beverages . *Pak. J. Nutr* . vol, 7(6), pp ; 763 -765.

5. **Ataallah, M., Hanan, A., Maysoun, S. (2011)** . Prevalence of hepatitis B and C among blood donors attending the National Blood Transfusion Center in Baghdad, Iraq from 2006–2009. *Saudi Med*. J; 32:1046–50 .

6. **Berdanier, D. (1998)** .Trace Minerals. In: Wolinsky I and Hickson JF eds. *Advanced Nutrition*. Micronutrients New York. CRC Press, pp ; 224-225.

7. **Chaturvedi, UC., Shrivastava, R. and Upreti, RK . (2004)** .Viral Infections and Trace Elements: Acomplex Interaction .*CurrSci*, vol. 87(11), pp:54 – 1536,.

8. **Das, A. and Maini, K .(2010)** . Innate and Adaptive Iimmune Responses in Hepatitis B Virus Infection. *Dig Dis Sci* , Vol. 28 (1) , p: 32 - 126.

9. **Das, P., Santos, M ., Pereira, L ., Jesus, P. (2005)** . Comparative histological studies on liver of mice exposed to Cr(VI) and Cr(V) compounds. Hum Exp Toxicol; 21(7): 365-9(2002). Public Health Association *(JEPHAss.)*, 80:.3-4.

10. **Dunnick, K., EIwell, R., Eadovsky, E., Benson, .M., Hahn, .F., NikuIa, .J., Barr, B, and Hobbs, H. (1995)** . Comparative Carcinogenic Effects of Nickel

Subsulfide, Nickel Oxide, or Nickel Sulphate Hexahydrate on Chronic Exposures in The Lung. *AACR*. 55: 5251-5256.

11. **Farell, J. (2010)**. Digestion and Absorption of Nutrients and Vitamins. *Gastrointestinal and Liver Disease.* 9:7 - 423.

12. **Gang L., John. B., Shih,. L., Christian A., Lance S., David R. J**. (2012) .Measurement of the Trace Elements Cu, Zn, Fe, and Mg and the Ultratrace Elements Cd, Co, Mn, and Pb in Limited Quantity Human Plasma and Serum Samples by Inductively Coupled Plasma-Mass Spectrometry. *American Journal of Analytical Chemistry*. 3, 646-650.

13. **Janine, B. (2005)** . Heavy Metal Toxicity-An Unsuspected Illness. http://www.janinebowring.com.

14. **Himmelhoch, R ., Sober, A., Vallee, L.,Peterson, A .and Fuwa, K.(1966).** Spectrographic and Chromatographic Resolution of Metalloproteins in Human Serum . *Biochemistry*.5: 2523.

15. **Khan, H. and Qayyum, K .(2002)** .Determination of Trace Amounts of Iron, Copper ,Nickel ,Cadmium and Lead in Human Blood by Atomic Absorption Spectrometry .*PakJBiol Sci* . 5(10): 7- 1104.

16. **Langard, S., Friberg, L. and Norseth, T. (1986)** . Chromium. In: ed. Handbook on The Toxicology of Metals, 2nd ed . *Elsevier/North-Holland Biomedical Press* . 2:185–210,

17. **Lin.1., Huang, F., Tsai, Y. and Huang, L. (2006).** Selenium, Iron, Copper, and Zinc Levels and Copper-to-Zinc Ratios in Serum of Patients at Different Stages of Viral Hepatic Diseases . *Biol Trace Elem Res*. 109(1):15-24.

18. **Mcneely, D., Sunderman, W., Nechay, W. and Levine, H . (1971).** Abnormal Concentrations of Nickel in Serum in Case of My Ocardialinfection, Stroke, Burns, Hepatic Cirrhosis an Duremia . *ClinChem*. 17(11) : 8 - 1123.

19. **McQuillan, M., Coleman, J., Kruszon, D., Moyer, A. and Lambert, B. Margolis, S.(1999)**.Prevalence of Hepatitis B Virus Infection in The United States. *Am J Public Health*.89:14-18.

20. **Rashed, N., Mohamed, A., Farouk , A. and Mahmoud, S. (2010)** . Trends in Speciation Analysis of Some Heavy Metals in Serum of Patients With Chronic Hepatitis C and Chronic Hepatitis B Using Differential Pulse Adsorptive Tripping Voltammetric Measurement and Atomic Absorption Spectrophotometry . *J Trace Elem Med Biol* . 24: 45 -138.

21. **Sharma, K . and Agrawal, M . (2005)** . Biological Effects of Heavy Metals : An Overview . *J. Environ Biol*. 26: 301-13.

22. **Shrivastava, R., Upreti, K., Seth, K. and Chaturvedi, C . (2002)**. Effects of Chromium on The Immune System . *Med. Microbiol*, 34:1-7.

23. **Versieck, J ., Barbier F., Speeke, A . and Hoste J. Mangaese .(1974)** .Copper and Zinc Concentrationsin Serum and Packed Blood Cells During Acute Hepatitis, Chronic Hepatitis and Posthepatitic Cirrhosis .*ClinChem* . vol. 20(9), pp : 5 – 1141,.

24. **Volini, F ., dalaHuerga, J ., Kent, G . (1968)** . Trace Metal Studies in Liver Disease Using Atomic Absorption Spectrometry in Laboratory Diagnosis of Liver Diseases In: Sunderman ,W., SundermanJr, W. *Laboratory diagnosis of liver diseases.* St. Louis, MO: Warren H. Green, Inc.; p. 199.

25. **Wang, M., Howell ,J. M., Libbey,E., Tainer, A.and Fujinami,S. (2003).** Manganese Superoxide Dismutase Induction During Measles Virus Infection . *J. Med. Virol*. vol. 70, pp ; 470–474.

26. **Williams, B. (1993) .**Biostatistics Concepts and Applications for Biologists. first ed., Chapman & Hall/CRC, London.

27. **Wyrobek, J .,Schrader, M., Perreault , D., Fenster, L., Huszar, G ., Katz, F. , Osorio, M ., Sublet,V . and Evenson . (2010).** Assessment of Reproductive Disorders and Birth Defects in Communities Near Hazardous Chemical Sites. *Fertil Steril*. 93:130-140.