BIOCHEMICAL CHANGES ON NONALCOHOLIC STEATOHEPATITIS IN GERIATRIC

Hussein, S.A.; Elsenosy, Y.A. and Hossam S. M. E.

Biochemistry Dept. Faculty of Veterinary Med.Moshtohor, Benha Univ.

ABSTRACT

This study was carried out on 50 patients with nonalcoholic steatohepatitis (age from 50- 69 years), and 10 healthy subjects (age from 50- 69 years) from Mansoura university hospitals and medical clinics. An informed consent from all patients was taken. Patients were subjected to history taking, full clinical examination; general and abdominal examination and abdominal ultrasonography. The aim of this work was to study the biochemical changes in non-alcoholic steatohepatitis in geriatric patients on some inflammatory markers cytokines (IL2, IL6), immunoglobulin, antioxidant (NO, MAD, SOD and GSH), lipid profile and hepatic function enzymes in patient with NASH. The results of the present study showed that Liver functions and lipid metabolism were impaired in geriatric patient with NASH syndrome, Chronic inflammatory state was recorded in geriatric with NASH evidenced by significant elevation in IL2 , IL6 and IgA more a significant increased formation of reactive oxygen species as a response which results in elevation of NO,MDA level and decrease in SOD and GSH.

KEY WORDS: Steatohepatitis, Immunoglobulin, Antioxidant enzymes, Cytokines.

(BVMJ-25(2): 264-270, 2013)

1- INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease and its incidence is rising worldwide). The term NAFLD is used to describe a wide spectrum of fatty liver changes ranging from simple steatosis, to non-alcoholic steatohepatitis (NASH) Although simple steatosis usually follows a benign course, steatohepatitis is prone to progress to hepatic fibrosis and cirrhosis leading to excess morbidity and mortality (Ekstedt *et al.,* 2006). Liver biopsy remains the gold standard for obtaining an accurate diagnosis of NASH, and differentiating NASH from simple steatosis. But, liver biopsy removes only about 1/50,000th of the liver and carries substantial interpretation errors. Liver biopsy is an invasive procedure with certain unavoidable risks and complications. The imaging techniques (Ultrasonography, Computed Tomography, Magnetic Resonance) are less invasive diagnostic tools, but none of them are able to distinguish between liver steatosis and steatohepatitis (Wieckowska *et al.,* 2007).Therefore, the development of noninvasive simple, reproducible, and reliable biomarkers that can allow identifying patients with nonalcoholic steatohepatitis (NASH) among NAFLD patients are greatly needed. Among these biomarkers: Cytokines (IL2, IL6) & antioxidant (NO, MAD, SOD and GSH).

2. MATERIAL AND METHODS:

2.1 Patient:

This study was carried out on 50 patients with NASH (age from 50- 69 years), and 10 healthy subjects (age from 50- 69 years) from Mansoura University Hospitals and Medical Clinics. An informed consent from all patients was taken. Patients were subjected to history taking, full clinical examination; general and abdominal

examination and abdominal ultrasonography.

2.2. Study design:

Subjects were divided into 2 main groups:

- Group I: 10 normal subjects (50- 69 years) served as control group.
- Group II: 50 old patients (50- 69 years) with NASH (diagnosed by ultrasound and liver biopsy) served as study group.

2.3. Blood samples collection and preparation:

Blood samples were collected in vacutainer blood collection tubes (7 ml) from each patient and control case and immediately after clotting of blood, the tubes were centrifuged at 300 rpm for 20 minutes at room temperature to collect the serum, then the sera were divided into aliquots to estimate the following parameterscytokines: (IL2, IL6), Immnoglobilin, Antioxidant (NO, MAD, SOD, GSH), Lipid profile and Hepatic function enzymes in patient with NASH.

3. RESULTS:

Data revealed significant elevations in serum AST, ALT, bilirubin and gamma glutamyl transaminase and significant increase in geriatric with NASH syndrome compared to control group. The significant elevations in liver enzymes were not marked but 2 folds as in AST and 3 folds as in ALT and 2 folds in γGT. Also, the elevation in serum bilirubin and decrease in serum albumin was not marked. This was associated with significant elevation in total cholesterol, triglycerides and LDL, and non significant decrease in HDL in geriatric patient with NASH compared to control persons.

All possible correlations between liver enzymes (AST and ALT) and other different studied parameters are mentioned in table 2. There was a strong positive correlation between liver enzymes (ALT and AST) and serum cholesterol, triglycerides, and low density lipoprotein (LDL). However, there was no significant correlation between liver enzymes and high density lipoprotein (HDL).IgA only of immunoglobulins showed significant positive correlation with liver enzymes and other immunoglobulins (IgGandIgM) did not show any significant correlation with liver enzymes. Proinflammatory cytokines (IL2 and IL6) showed positive correlation with liver enzymes.Regarding oxidative stress markers NO, MDA, SOD and SOD, all of them showed significant correlation with liver enzymes (AST and ALT), except GSH which showed significant correlation with only GSH. NO, and MDA showed positive correlation, but SOD showed negative correlation. The NASH is a common syndrome in obese person and increase the morbidity in elderly. In this study we investigated some biochemical changes that occur in geriatric patients with NASH

4. DISCUSSION:

Fatty liver is the most common cause of mildly to moderately elevated liver enzymes both in Sweden (Mathiesen *et al.,* 1999) and elsewhere (Daniel *et al.,* 1999; Skelly *et al.,* 2001). Hypertransaminasaemia, if viral or other causes of liver disease have been excluded, is sometimes used as a surrogate marker for NAFLD (Yu *et al.,* 2003). Using elevated liver enzymes as a marker for NAFLD is simple and cheap, but has several disadvantages. The upper limit of normal for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is not well defined. Recently the upper limit of normal for ALT in Sweden was changed. It was raised from 47 U/L (0.8 μkat/L) to 65 U/L (1.1 μkat/L) in men and from 35 U/L (0.6 μkat/L) to 44 U/L (0.75 μkat/L) in women. These changes were based on the ALT levels in 3,000 adults living in the Nordic countries. There are reasons to believe that the increased ALT levels in the population reflect the increased prevalence of obesity and NAFLD (Kechagias *et al.,* 2004). It has been shown that ALT elevation is seen more frequently in obese (Wejstal *et al.,*

Table (1): serum biochemical changes

Parameter	Normal control group	NASH geriatric group
AST (iU/ml)	27.5 ± 4.88	58.82 ± 16.43*
ALT (iU/ml)	26.4 ± 6.64	74.79 ± 13.3*
GGT (iU/ml)	20.5 ± 6.00	51.2 ±8.15*
Serum bilirubin (mg/dL)	1.132 ± 0.09	1.84 ± 0.39*
Serum albumin (g/dl)	4.49 ± 0.24	3.93 ± 0.39*
Nitric oxide	97.80 ± 8.83	126.31 ± 13.08*
Malondialdehyde (MDA)	4.57 ± 0.91	11.20 ± 3.16*
Superoxide dismutase	1.40 ± 0.16	1.07 ± 0.15*
Reduced glutathione (GSH)	105.29 ± 54.59	100.43 ± 46.32*
IL2	12.17 ± 1.72	38.27 ± 15.11*
IL6	76.02 ± 7.14	148.39 ± 33.26*
Total cholesterol (mg/dl)	129.00 ± 10.21	228.71 ± 35.89*
Triglycerides (mg/dl)	83.30 ± 8.04	221.51 ± 36.72*
HDL (mg/dl)	54.50 ± 8.29	52.00 ±7.45
LDL (mg/dl)	57.84 ± 8.04	132.32 ± 33.29*
Total cholesterol (mg/dl)	129.00 ± 10.21	228.71 ± 35.89*

Table (2): Correlations between liver enzymes (AST and ALT) and all other studied parameters

	AST	ALT
Lipid profile:		
Total Cholesterol		
r =	0.544	0.629
P=	0.000	0.000
Triglycerides		
r =	0.545	0.728
P=	0.000	0.000
HDL		
r=	- 0.014	-0.146
p=	0.914	0.271
LDL		
r=	0.485	0.584
p=	0.000	0.000
Proinflammatory cytokines:		
IL2		
r =	0.443	0.432
P=	0.000	0.001
IL6		
r=	0.410	0.632
p=	0.001	0.000

Immunoglobulins:		
IgA		
r=	0.401	0.517
p=	0.002	0.000
IgM		
r =	0.026	0.141
P=	0.846	0.288
IgG		
r =	0.001	0.005
P=	0.994	0.968
Oxidative stress markers:		
NO		
r=	0.344	0.485
P=	0.008	0.000
MDA		
r=	0.429	0.475
P=	0.001	0.000
GSH		
r=	0.100	0.376
P=	0.450	0.003
SOD		
r=	0.547	0.524
P=	0.000	0.000

1998). In an Italian study of 3,927 subjects with normal BMI, normal serum cholesterol, triglycerides, and glucose levels, and absence of concurrent medication, the upper limit of ALT was 30 U/L (0.5 μkat/L) in men and 19 U/L (0.32 μkat/L) in women. Moreover, the full spectrum of NAFLD can be found in patients with normal ALT values (Mofrad *et al.,* 2003). When compared to ultrasonography, the sensitivity of elevated ALT for diagnosing NAFLD is 8.2% with a specificity of 98% (Zelber-Sagi *et al.,* 2006). Serum immunoglobulin levels are determined routinely in clinical practice because they provide key information on the humoral immune status. High immunoglobulin levels (polyclonal gammopathy) are observed in liver diseases, chronic inflammatory diseases, hematological disorders, infections and malignancies (Dispenzieri *et al.,* 2001). Moreover, immunoglobulin levels aid in the diagnosis of some disorders, particularly liver diseases (Van de Wiel *et al.,* 1988). Serum immunoglobulin concentrations tend to increase with age. In the present study, there was a significant elevation of IgA only but IgG and IgM did not show any significant elevation in geriatric patients with NASH syndrome compared to normal patients. It is well known that heavy drinkers with advanced liver disease often present with high IgA values (Van de Wiel *et al.,* 1988), but fewer studies have addressed the effects of smoking and alcohol intake (from light to heavy) per se on serum IgA, IgG or IgM (McMillan *et al.,* 1997). Increased IgA concentrations tended to be associated with hyperglycemia, but associations with additional components of metabolic syndrome such as hypertriglyceridemia and abdominal obesity were even stronger. The mechanisms of IgA elevation in individuals with obesity and metabolic syndrome are unknown, but IgA elevation is not surprising because both conditions are chronic inflammatory disorders (Singh *et al.,* 2007). Serum levels of IL-6, an inflammatory marker, were associated with those of serum IgA. However, serum IL-6 levels were not found to be associated with metabolic abnormalities in the present

series. In the present study, there was significant elevation in proinflammatory cytokines IL2 and IL6.The next point in this work that was investigated is the study of oxidative stress markers in geriatric patients. It is now generally accepted that oxidative stress due to increased ROS production has a role in the pathogenesis of NASH. Hepatocytes are continuously exposed to ROS and are protected from oxidative injury by a range of antioxidant pathways (Zhang *et al.,* 2000). The state of oxidative stress exists when there is imbalance between pro-oxidant and antioxidant chemical species. There is insufficient knowledge about antioxidant defense mechanisms, particularly the enzymatic components, in the pathogenesis of NASH. In our study, we observed increased serum MDA, decreased SOD activity and slight but statistically insignificant increases of serum GSH in geriatric NASH patients. These findings may indicate that the hepatic antioxidant enzymatic defense system in NASH is impaired and lipid peroxidations process is enhanced. Increased intrahepatic levels of fatty acids are a source of oxidative stress, which may be responsible for the progression from hepatic steatosis to steatohepatitis and to cirrhosis. Gut-derived endotoxins may play an important role in activating Kupffer cells, causing collateral damage to hepatocytes, and promoting an inflammatory response in the liver acinus (Yang *et al.,* 1997). Lipid peroxidation products alter mitochondrial DNA and also react with mitochondrial proteins to inhibit electron transfer along the respiratory chain, further increasing ROS production, and generating a self-propagating cycle of oxidative stress and lipid peroxidation (Pessayre *et al.,* 2001). Evidence of lipid peroxidation in the form of increased MDA production, a surrogate marker of oxidative stress, has been noted in previous studies, and serum levels of MDA have been correlated with the severity of chronic hepatitis (Paradis *et al.,* 1997; Yadav *et al.,* 2002). In the present study, serum MDA levels were significantly increased in patients with NASH, indicating increased oxidative stress. The defenses against free radical-mediated-injury include enzymatic deactivation and direct reaction with free radicals (DiMascio *et al.,* 1991). Cells have various antioxidant systems (eg, SOD, GSH-Px, GR, and catalase) and non-enzymatic scavengers (eg, vitamins E, A, and C; carotenoids, flavonoids, and thiols) (Michiels *et al.,* 1995). SOD, the first line of defense against oxygen-derived free radicals, converts superoxide anion into H2O2, forming as neutral products O2 and H2O. Reduced glutathione (GSH) is an endogenous non-enzymatic antioxidant that catalyses reductive destruction of hydrogen and lipid hydroperoxides (Harris, 1992). Reports concerning the role of NO in liver damage during inflammatory conditions are contradictory. Zhu and Fung (2000) found that NO protects against liver injury by scavenging lipid radicals and inhibiting the lipid peroxidation chain reaction. On the other hand, Sass *et al.* (2001) reported that iNOS-derived NO regulates proinflammatory genes in vivo, contributing to inflammatory liver injury. Other investigators have reported that, in the pathogenesis of NASH, NO may potentiate cytotoxicity by reaction with superoxide anion to form peroxynitrite, a strong oxidant that promotes nitration of tyrosine to form nitrotyrosine (Clemens, 1999; Garcia-Monzon *et al.,* 2000).The finding that intrahepatic accumulation of nitrotyrosine is associated with the histological severity of NASH strongly suggests that oxide-related oxidative injury may play a significant role in the pathogenesis of NASH (Garcia-Monzon *et al.,* 2000). Although our NASH patients had increased serum levels of NO, we did not find correlations between the histologic severity of the disease and serum NO concentration. This may reflect the early stage of the disease in most of our NASH patients. In addition, NO may have a protective role during an early stage of NASH. At the beginning of hepatic injury, when only a small amount of NO is being produced, NO may protect the liver through vasodilatatory, antioxidative, and

antiapoptotic effects. However, in the presence of massive injury (eg, high level of inducers and elevated oxidative stress), greatly increased NO production might induce the hepatocytes to progress to irreversible channel necrosis and cell death.The balance between oxidative stress and antioxidant defense mechanisms may be impaired by depletion of enzymatic antioxidants and increased serum levels of MDA and NO in patients with NASH. We perceive that failure of antioxidant defense mechanisms against oxidative stress may be an important factor in the pathogenesis of NASH. Treatment approaches that address the antioxidant enzymes and the antioxidant vitamins may be helpful in the therapy of patients with NASH.

5. REFERENCES

1. Clemens, M.G. 1996. Nitric oxide and liver injury. Hepatology, 30:1–5. Cobb, J.P. and Danner, R.L.: Nitric oxide and septic shock. JAMA, 275: 15: 43-47.
2. Daniel, S., Ben-Menachem, T., Vasudevan, G., Ma, C. and Blumenkehl, M. 1999. Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. Am. J. Gastroenterol., 94:3010-4.
3. DiMascio, P., Briviba, K., Bechara, E. J., Medeiros, M. H. and Sites, H. (1996): Reaction of peroxynitrite and hydrogen peroxide to produce singlet molecular oxygen Methods Enzymol., 269: 395-400.
4. Dispenzieri, A. Gertz, M.A., Therneau, T.M. and Kyle, R.A. 2001. Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clin. Proc., 76:476–87.
5. Ekstedt, M. 2006. Non alcoholic fatty liver disease: A clinical and histopathological study. Medical Dissertations No. 1081. Faculty Health Sciences Linköping University, Sweden.
6. Garcia-Monzon, C., Majano, P.L., Zubia, I., Sanz, P., Apolinario, A. and Moreno-Otero, R. 2000. Intrahepatic accumulation of nitrotyrosine in chronic viral hepatitis associates with histological severity of liver disease. J. Hepatol., 32:331–338.
7. Harris E.D. 1992. Regulation of antioxidant enzymes. FASEB J. 6:2675–83.34
8. Kechagias, S., Ekstedt, M., Mathiesen, U. and Franzén, L. 2004. New reference interval for ALAT does not identify subclinical liver disease. Lower limit than the proposed should be used for medical considerations. Lakartidningen, 101:2884-7.
9. Mathiesen, U., Franzén, L., Frydén, A., Foberg, U. and Bodemar, G. 1999. The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. Scand. J. Gastroenterol., 34:85-91.
10. McMillan, S.A., Douglas, J.P., Archbold, G.P.R., McCrum, E.E. and Evans, A.E. 1997. Effect of low to moderate levels of smoking and alcohol consumption on serum immunoglobulin concentrations. J. Clin. Pathol., 50:819–22.
11. Michiels, C., Raes, M., Toussaint, O. and Remacle, J. 1994. Importance of Seglutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free RadicBiol. Med., 17:235–240
12. Mofrad, P., Contos, M.J., Haque, M., Sargeant, C., Fisher, R.A., Luketic, V.A., Sterling, R.K., Shiffman, M.L., Stravitz, R.T. and Sanyal, A.J. 2003. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology, 37:1286-1292.
13. Paradis, V., Kollinger, M., Fabre, M., Holstege, A. and Poynard, T. 1997. In situ detection of lipid peroxidation by

products in chronic liver disease. Hepatology, 20(1): 135-142.
14. Pessayre, D., Berson, A., Fromenty, B. *et al.* 2001. Mitochondria in steatohepatitis. Semin Liver Dis., 21:57–69.
15. Sass, G.K., Koerber, R., Bang, R. *et al.* 2001. Inducible nitric oxide synthase is critical for immune-mediated liver injury in mice. J. Clin. Invest., 107:439–447.
16. Singh, B., Mallika, V., Goswami, B. 2007. Metabolic syndrome: diagnosis, potential markers and management − an update. Clin. Chim. Acta, 380:4–12.
17. Van de Wiel, A., Van Hattum, J., Schuurman, H.J., Kater, L. 1988. Immunoglobulin A in the diagnosis of alcoholic liver disease. Gastroenterol., 94:457–62.
18. Wejstål, R., Hansson, G., Lindholm, A., Norkrans, G. 1988. Persistent alanine aminotransferase elevation in healthy Swedish blood donors--mainly caused by obesity. Vox Sang, 55:152-6.
19. Wieckowska, Zein, N.N., Yerian, L.M., Lopez, A.R., McCullough, A.J. and Feldstein, A.E. 2007. Department of Gastroenterology and Hepatology, Clevel and Clinic Foundation, Cleveland, OH 44195, USA.
20. Yang, S.Q., Lin, H.Z., Lane, M.D. *et al.* 1997. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. PNAS USA, 94:2557–2562.
21. Yu, A. and Keeffe, E. 2003. Elevated AST or ALT to nonalcoholic fatty liver disease: accurate predictor of disease prevalence? Am. J. Gastroenterol., 98:955-6.
22. Zelber-Sagi, S., Nitzan-Kaluski, D., Halpern, Z. and Oren, R. 2006. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. Liver Int., 26:856-63.
23. Zhang, C., Walker, L.M., Hinson, J.A. *et al.* 2000. Oxidant stress in rat liver after lipopolysaccaride administration: effect of inducible nitric-oxide synthase inhibition. J. Pharmacol. Exp. Ther., 293:968–972.

التغيرات الحيوية على الكبد الدهني غير الكحولي في الشيخوخة

سامي علي حسين، ياقوت عبد الفتاح السنوسي، حسام سعد محمد الشربينى
قسم الكيمياء الحيوية-جامعة بنها – كلية الطب البيطري بمشتهر

الملخص العربي

يعتبر متلازمة الالتهاب الكبد الدهني الغير كحولي (NASH) شائعة في الاشخاص الذين يعانون من السمنة المفرطة ويزيد معدلات الاعتلال في كبار السن.وقد أجريت هذه الدراسة علي 60 مريض من كبار السن بين 50 و 69 عام وتم تقسيمهم إلى مجموعتين المجموعة الأولى تتكون من 10مرضى (المجموعة الضابطة)وهم من كبار السن الغير مصابين ب NASH والمجموعة الثانية تتكون من 50مريض من كبار السن المصابين ب NASH بعد تشخيصهم بالموجات الفوق صوتية والعينة الكبدية .و قد تم جمع عينات دم وذلك لمقارنة التغيرات الكيميائية الحيوية للسيرم من خلال قياس بعض المؤشرات مثل وظائف الكبد حيث تم قياس كلا من انزيمات الكبد AST، ALT، وGGT، ونسبة الزلال او albumin ونسبة الصفراء فى الدم ،قياس مستوى الدهون فى الدم وتشمل الدهون الثلاثية (TG)و الكوليسترول الكلي (TC)،والدهون المفيدة HDL، والدهون الضارة LDL ،قياس مستوى السيتوكينات IL2و IL6فى المصل ،قياس الاجسام المضادة فى المصل (IgA, IgG, IgM))،قياس دلالات الاكسدة NOو MDAكمنتج نهائي من بيروكسيد دهني وقياس مضادات الاكسدة الأنزيمية بما في ذلك (SOD)والمواد المضادة للأكسدة غير الأنزيمية (GSH) .من هذه الدراسة يتضح اختلال وظائف الكبد ونقص فى الايض للدهون في المرضى المسنين الذين يعانون من متلازمة NASH، بالإضافة الى حالة التهاب مزمن في الشيخوخة مع NASHيتضح من الارتفاع الكبير في IL2و IgA, IL6 كما يوجد زيادة كبيرة فى شوارد الاكسجين الحرة و ذلك يتضح من خلال ارتفاع كبير في MDA، وانخفاض في NO و SOD وGSH.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):264−270, ديسمبر 2013)