3-Nitro-Tyrosine as a Biomarker of Minimal Hepatic Encephalopathy in Patients with Liver Cirrhosis

Thesis

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Mohamed Ahmed Saad Ahmed

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List of Abbreviations

Abb.	Full term
AAA	*Aromatic amino acids*
ALD	*Alcoholic liver disease*
ALT	*Alanine transaminase*
AST	*Aspartate transaminase*
BCAA	*Branched-chain amino acids*
BCAAs	*Branched-chain amino acids*
BDT	*Block design test*
BUN	*Blood urea nitrogen*
CBC	*Complete blood picture*
CFF	*Critical Flicker Frequency*
CHE	*Covert HE*
CLD	*Chronic liver disease*
CRT	*Continuous Reaction Time*
DST	*Digit Symbol test*
DST	*Digital substitution test*
EEG	*Electroencephalographic*
GABA	*Gamma-aminobutyric acid*
GI	*Gastrointestinal*
GPB	*Glyceryl phenylbutyrate*
HCC	*Hepatocellular carcinoma*
HCV	*Hepatitis C virus*
HE	*Hepatic encephalopathy*
HR-QoL	*Health-related quality of life*
ICP	*Intracranial pressure*
ICT	*Inhibitory Control Test*
IL	*Interleukin*
ImPACT	*Immediate Post-Concussion Assessment and Cognitive Testing*
INR	*International normalised ratio*

List of Abbreviations (Cont...)

Abb.	Full term
ISHEN	*International Society for the Study of Hepatic Encephalopathy and Nitrogen Metabolism*
IV L	*Intravenous*
LOLA	*L-Ornithine L-aspartate*
LT	*Liver transplantation*
LTT	*Line-tracing test*
MHE	*Minimal HE*
NCT- A	*MRI Number Connection test A*
NCT- B	*Number Connection test B*
NCTB	*Number Connection test B*
NHPA	*3-nitro-4-hydroxyphenylacetic acid*
OHE	*Overt HE*
PH	*Portal hypertension*
PHES	*Psychometric hepatic encephalopathy score*
PHES	*Psychometric tests*
PSE	*Portosystemic encephalopathy*
PSSs	*Portosystemic shunts*
PT	*Prothrombin time*
PTBR	*Peripheral-type benzodiazepine receptor*
RBANS	*Repeatable battery for assessment of neurological status*
RCTs	*Randomized, controlled trials*
SDOT	*Serial-dotting test*
SDT	*Serial-dotting test*
SPHES	*Simplified psychometric hepatic encephalopathy score*
TIPS	*Transjugular intrahepatic portosystemic shunt*
TMT	*Trail making test*
VMCP	*Visuo-constructive performance*
WM	*Working memory*

ABSTRACT

Background: hepatic encephalopathy (HE) is a common complication in patient with liver cirrhosis. It comprises of a broad spectrum of neuropsychiatric abnormalities of varying severity, and affected patients usually suffer from psychomotor, cognitive, emotional, behavioural, and motor coordination dysfunctions. Patients with minimal HE (MHE), a subclinical form of HE, usually have a normal mental and neurological status upon routine clinical examination. The subtle deficits in patients with MHE can only be elicited by specialized neuropsychological tests. **Aim of the Work:** the aim of this study was to evaluate the role of 3-Nitro-Tyrosine as a biomarker of Minimal Hepatic Encephalopathy in patients with liver cirrhosis. **Patients and Methods:** our conducted study was a prospective case control study carried on 60 adult patients and 30 age matched controls. All were recruited from Internal Medicine and Hepatology and Gastroenterology Department at Ain Shams University Hospitals in the period between September 2016 and June 2018. All patients enrolled in the study were subjected to detailed history taking, full physical examination, laboratory investigations, psychometric tests for detection of MHE using specially digit symbol test (DST), Trail making test A (TMT A), Trail making test B (TMT B), serial dotting test (SDT) and 3-Nitro-Tyrosine level (3NT). **Results:** our study found that the serum levels of 3-nitro-tyrosine are a good predictor of the presence of MHE in patients with liver cirrhosis, with good sensitivity (90%) and specificity (93.33%) and positive and negative predictive values were 93.1% and 90.3% respectively at a cutoff of 14.8 ng. **Conclusion:** determination of 3-nitro-tyrosine in serum is easy and is not time consuming. It only requires taking a serum sample from the patient and determining 3-nitro-tyrosine concentration. This procedure can be therefore easily added to the routine clinical determinations in patients with liver cirrhosis. This would also allow extending the diagnosis of MHE to most clinical settings, helping to identify patients with MHE.

Keywords: 3-Nitro-Tyrosine, Hepatic Encephalopathy, Liver Cirrhosis

INTRODUCTION

Liver disease is a major cause of mortality and morbidity worldwide. In most cases, liver-related mortality results from complications of chronic liver disease (CLD) including advanced cirrhosis and hepatocellular carcinoma (HCC) ***(El-Serag and Rudolph, 2007).***

Chronic Liver Disease and complications of cirrhosis including ascites, bleeding tendency, Hepatocellular Carcinoma, minimal and overt hepatic encephalopathy also are associated with severe impairments in health-related quality of life ***(Afendy et al., 2009).***

Furthermore, Chronic Liver Disease influences resource use, negatively contributing to well-being of the individual patient and society ***(Davis, 2010).***

The burden of liver disease in Egypt is exceptionally high, maintaining the highest prevalence of hepatitis C virus (HCV) worldwide ***(Lavanchy, 2011).***

Hepatic encephalopathy (HE) is a frequent complication and one of the most debilitating manifestations of liver disease, severely affecting the lives of patients and their caregivers. Furthermore, cognitive impairment associated with cirrhosis results in utilization of more health care resources in adults than other manifestations of liver disease. Progress in the area has

been hindered by the complex pathogenesis that is not yet fully elucidated.

Apart from such biological factors, there remains the larger obstacle that there are no universally accepted standards for the definition, diagnosis, classification, or treatment of HE, mostly as a result of insufficient clinical studies and standardized definitions. Clinical management tends to be dependent on local standards and personal views. This is an unfavorable situation for patients and contrasts with the severity of the condition and the high level of standardization in other complications of cirrhosis.

The lack of consistency in the nomenclature and general standards renders comparisons among studies and patient populations difficult, introduces bias, and hinders progress in clinical research for HE ***(Rakoski et al., 2012).***

Between 30 and 50 % of the patients with liver cirrhosis who do not show evident symptoms of clinical hepatic encephalopathy (HE) have minimal HE (MHE). MHE cannot be detected in routine analysis but can be unveiled using psychometric tests or neurophysiological assessment ***(Amodio et al., 2004; Ferenci et al., 2002; Montoliu et al., 2007 Montoliu et al., 2009).***

MHE has a negative impact on daily life activities and working capacity ***(Goroeneweg et al., 1998)***, affects health-

related quality of life (HR-QoL) ***(Marcheseni et al., 2001; Wang et al., 2013; Mina et al., 2014; Schomerus et al., 2001)*** impairs fitness to drive ***(Watanabe et al., 1995; Wein et al., 2004; Felipo et al., 2013).***

Currently, the "gold standard" for diagnosis of MHE is the psychometric hepatic encephalopathy score (PHES), a battery of five psychometric tests ***(Ferenchi et al., 2002)***. However, PHES is time consuming and needs adjusting for age and educational level. As aconsequence, MHE is not routinely diagnosed in most clinical settings because of lack of simple procedures, and most patients with MHE remain undiagnosed and untreated. Hence, there is a need for a simple diagnostic test that can be performed routinely in the laboratory to detect MHE in patients with liver cirrhosis ***(Montoliu et al., 2007).***

It would be very useful in clinical practice to have some peripheral biomarker that could be measured in blood samples and reflect the presence of MHE in cirrhotic patients. We have been looking for such possible peripheral indicator during the last few years. We showed that the presence of MHE correlates with increased activation of soluble guanylate cyclase by nitric oxide in freshly isolated lymphocytes ***(Montoliu et al., 2007)***, and that increased serum levels of pro inflammatory cytokines, interleukin (IL)-6 and IL-18 also correlate with MHE and would be useful to detect MHE ***(Montoliu et al., 2009).***