Carotid Intima Media Thickness in HCV Infected Egyptian Patients and its Relation to Viral Load and Genotype: A Preliminary Study

ABIR ZAKARIA, M.D.*; MERVATE NAGUIB, M.D.*; RAGAI M.F.R. FOUDA, M.D., M.C.R.P.* and LAILA RASHED, M.D.**
The Departments of Internal Medicine-Medical ICU* and Clinical Biochemistry**, Faculty of Medicine, Cairo University

Abstract

Introduction and Aim: Contrasting results were obtained from different studies regarding carotid intima media thickness (IMT) in hepatitis C virus (HCV) infected patients. The current study was done to explore carotid IMT in HCV infected Egyptian patients and to detect possible relation between viral load or genotype and carotid IMT in these patients.

Subjects and Methods: Sixty treatment naive HCV infected patients and 20 healthy control subjects participated in the study. HCV infection was diagnosed in those with a positive polymerase chain reaction to HCV-RNA in serum and testing was done to detect viral genotype. Carotid IMT was measured for all participants using B-mode duplex imaging study. Diabetic, hypertensive, dyslipidaemic patients, or those known to have coronary artery disease, cerebrovascular stroke or HIV infection were excluded from the study.

Results: Mean carotid IMT of HCV infected patients was significantly greater than that of healthy control subjects (0.79 ± 0.28 mm versus 0.56 ± 0.004 mm, p-value 0.00). Carotid IMT did not appear to be significantly correlated to viral load. Mean carotid IMT of HCV genotype 4a infected patients was not significantly different from that of HCV non-4a genotype infected patients (0.08 ± 0.03 mm versus 0.08 ± 0.02), p-value 0.276).

Conclusion: Mean carotid IMT of HCV infected Egyptians of a low cardiovascular risk was significantly greater than that of healthy controls. No significant correlation was detected between HCV viral load and carotid IMT in the studied patients. Mean carotid IMT of HCV genotype 4a infected patients did not differ significantly from those of HCV non-4a genotype infected patients.

Key Words: HCV – Intima media – Viral load – Genotype.

Introduction

DIFFERENT infectious agents had been linked to the development of atherosclerosis. These agents include helicobacter pylori, Chlamydia pneumonia, Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) [1]. HCV infection highest worldwide prevalence was reported in Egypt (15%), HCV is the cause of most cases of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma in this nation. HCV genotype 4a is responsible for around 90% of the infections reported in Egypt [2].

HCV infection can enhance atherosclerosis through a variety of mechanisms that include; induction of an inflammatory state and insulin resistance due to increased concentration of proinflammatory cytokines Tumor necrosis factor TNF-α and interlukin-6, HCV endocytosis through low density lipoprotein LDL receptor facilitating its entrance into atherosclerotic plaques and induction of oxidative stress via HCV core protein resulting in oxidation of lipoprotein in atherosclerotic plaques [3-5].

Carotid intima media thickness (IMT) is an early pointer of atherosclerosis. Increased carotid IMT appears simultaneously with the development of aortic atheromatous plaques [6]. Although HCV RNA was previously detected in carotid plaques obtained from patients with chronic HCV who presented with hemodynamically significant carotid stenosis and underwent carotid end-arterectomy [7], studies regarding the carotid IMT in HCV infected patients showed contrasting results, with little data about its relation with HCV viral load or genotype [8-11].

The current case control study was done to evaluate carotid IMT among HCV infected Egyptian patients compared to that of healthy control subjects and a possible relation between carotid IMT and viral load and genotype in these patients.
Carotid Intima Media Thickness in HCV Infected Egyptian Patients

Subjects and Methods

The protocol of this study was approved by the scientific board of Internal Medicine department and Committee of Research Ethics; Faculty of Medicine, Cairo University, Egypt and Informed consents were obtained from all participants.

The study, it was done over the period from March 2011 till March 2012 included 60 treatment naive HCV infected patients and 20 age matched healthy control subjects. HCV infection, viral load and genotype were diagnosed as follows:

HCV real time PCR Kit (Russia): A real time nucleic acid amplification assay was used for quantitative detection of the HCV RNA in the serum of the studied subjects according to the manufactures instructions. Amplification of HCV RNA was done using specific HCV primers, and then the amplified product was detected using fluorescent dyes. These dyes are attached to oligonucleotide probes which bind to the amplified product during thermo cycling. Test result and HCV load were interpreted from a real time PCR software.

HCV genotyping: The amplified HCV RNA mixtures were spun then 10ul of it was removed and added to denaturation solution in test troughs then carefully mixed. INNO-LIPA HCVII strips were numbered and completely submerged into solution in each test trough. Each strip has 3 control lines and 22 parallel DNA lines containing sequences specific for HCV genotypes 1 to 6 and their subtypes. Pattern of the purple brown lines on the strips were compared with interpretation table to determine HCV genotype.

Participants were recruited from patients attending the outpatient clinic and those admitted to Internal Medicine departments in Kasr El Eini Hospital, Cairo University during the period between December 2010 and December 2011. Diabetic, hypertensive, dyslipidaemic, coronary artery disease, cerebrovascular stroke, or HIV infected patients were excluded.

Patients underwent thorough clinical evaluation, chest X-ray, and electrocardiography (ECG). Blood samples were obtained after 12 hours of fasting for assessment of blood hemoglobin, platelet count, prothrombin time, fasting blood glucose, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), albumin and bilirubin. Abdominal ultrasound imaging was done.

In all study participants Carotid artery IMT was evaluated via B-mode duplex ultrasound using a high frequency 7.5MHz linear probe of ATL-HDL 5000 machine. Longitudinal scans of the right and left common carotid arteries were obtained for manual measurement of the IMT using saved images. IMT was measured as the distance from the intima lumen interface to media adventitia interface [12]. Three measurements were obtained from the anterior and posterior walls of the common carotid artery, the carotid bifurcation and the extracranial portion of the internal carotid artery. Measurements from the right and left sides (6 measurements from each side) were averaged. Atheromatous plaque was defined as focal thickening of >1.3mm. Colour or power Doppler was used for better deleniation of the intima-lumen interface. Measurements were performed for all participants by an experienced physician blinded to their clinical condition at the time of assessment.

Statistical analysis:

Statistical Package of Social Science (SPSS) program version 15.0 was used for analysis of data. Data was summarized as Mean±SD. t-test was used for analysis of 2 quantitative data, while Non parametric test (Mann Whitney U-test) was used when data was not symmetrically distributed. One way ANOVA test was used for analysis of more than 2 quantitative data followed by post HOCC test for detection of significance. Pearson’s correlation was also done it \( r \) was considered weak if \( <0.25 \), mild if \( \geq 0.25 <0.5 \), moderate if \( \geq 0.5 <0.75 \) and strong if \( \geq 0.75 \). \( p \)-value was considered significant if \( <0.05 \).

Results

HCV infected patients and healthy control subjects were age and gender matched. Mean values of BMI, cardiometabolic risk factors (total cholesterol, HDL, LDL, triglycerides and fasting plasma glucose levels) of HCV infected patients were not significantly different from the mean values of the studied healthy control subjects. Mean carotid IMT of HCV infected patients was significantly higher than that of the control group (Table 1).

Carotid IMT of HCV infected patients showed a significantly positive correlation to BMI and a significantly negative correlation to serum HDL but no significant correlations were detected between it and cardio metabolic risk factors, markers of liver dysfunction (serum bilirubin, and plasma albumin) or viral load (Table 2).
HCV 4a-genotype patients represented nearly 75% of the studied HCV infected patients. They were significantly older and had a significantly lower viral load compared to non-4a genotype patients. Mean values of BMI, cardiometabolic risk factors, markers of liver dysfunction and carotid IMT did not differ significantly among HCV 4a-genotype patients compared to those of HCV non-4a genotype patients (Table 3).

Table (1): Clinical, laboratory characteristics and carotid IMT of studied subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCV Infected patients</th>
<th>Healthy controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>60</td>
<td>20</td>
<td>0.33</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.4±9.1</td>
<td>47.2±7.60</td>
<td>0.46</td>
</tr>
<tr>
<td>Men</td>
<td>33 (55%)</td>
<td>8 (40%)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>27 (45%)</td>
<td>12 (60%)</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m(^2))</td>
<td>22.93±1.49</td>
<td>22.67±1.17</td>
<td>0.189</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>135.62±32.8</td>
<td>146.6±29.65</td>
<td>0.692</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41.98±5.28</td>
<td>42.6±5.24</td>
<td>0.649</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>83.33±14.39</td>
<td>79.5±14.58</td>
<td>0.307</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>107.1±34.95</td>
<td>96.2±10.23</td>
<td>0.692</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>86.93±9.01</td>
<td>83.75±8.54</td>
<td>0.17</td>
</tr>
<tr>
<td>2HPPG (mg/dl)</td>
<td>106.03±24.3</td>
<td>101.65±5.17</td>
<td>0.007*</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>3.03±0.8</td>
<td>4.41±0.44</td>
<td>0.00*</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>2.58±1.42</td>
<td>0.93±0.26</td>
<td>0.00*</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.79±0.28</td>
<td>0.56±0.004</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SD.
p-value <0.05 is considered significant*.

HCV: Hepatitis C virus.
C-P: Child-Pugh.
TC: Total cholesterol.
HDL: High density lipoprotein.
LDL: Low density lipoprotein.
FPG: Fasting plasma glucose.
2HPPG: 2 hour post prandial plasma glucose.

Table (2): Correlation of different variables in HCV infected patients with carotid IMT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.7</td>
<td>0.59</td>
</tr>
<tr>
<td>BMI (Kg/m(^2))</td>
<td>0.003</td>
<td>0.003*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>-0.388</td>
<td>0.002*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>-0.142</td>
<td>0.034</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-0.029</td>
<td>0.826</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>-0.028</td>
<td>0.855</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>Viral load</td>
<td>-0.024</td>
<td>0.856</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SD.

Discussion

The current study showed that mean carotid IMT of HCV infected patients was significantly higher than that of control subjects despite no significant difference in age, gender, BMI or cardiometabolic parameters. Carotid IMT showed only significantly positive correlation to patient’s BMI and significantly negative correlation to serum HDL but it did not have a significant correlation with other cardiometabolic risk factors, markers of liver dysfunction or the viral load. It also did not appear to differ significantly among HCV 4a compared to non-4a genotype patients.

Results of studies regarding carotid IMT in HCV infected patients are conflicting. Some studies reported like the results of the current study that HCV infected patients had higher IMT compared to healthy control subjects [8-9] while others reported that HCV infected patients IMT did not differ from that of healthy controls or even that chronic HCV infection over time can reduce progressive increase in carotid IMT [10-11].
Few studies had explored the relation between HCV viral load and carotid atherosclerosis, the results of a recent study showed that there is a positive correlation between viral load and carotid IMT [8] but the results of the current study failed to detect such a correlation which might be due the fact that presence of virus in blood (viraemia) is not responsible for accelerated atherosclerosis in these patients, and that HCV might induce atherosclerosis via direct entry into plaques or by inducing an inflammatory state [5,7].

Similarly studies done to evaluate the relation between viral genotype and carotid IMT were few. Petta et al., 2012 reported that HCV genotype 1 patients had increased carotid IMT compared to healthy control subjects but they did not study carotid IMT in those infected with other HCV genotypes [13]. To the best of our knowledge the current study is the only study in English literature to report that mean carotid IMT in HCV genotype 4a patients did not differ significantly from that of non-4a genotype HCV infected patients.

The study had few limitations: First: The limited number of patients in the study was due to restricting enrollment to those with low cardiovascular risk. Second: The degree of hepatic involvement in HCV patients was not evaluated using liver biopsy, so correlation between the degree of liver fibrosis and carotid IMT was not possible. Third: The current study was not able to compare carotid IMT in genotypes 1, 2 and 3 separately due to the low prevalence of infection with these HCV genotypes in Egypt [2].

In conclusion:
Mean carotid IMT of HCV infected Egyptians was significantly greater than that of healthy controls, that difference cannot be attributed to a difference in age, gender, BMI or cardiometabolic risk factors and carotid IMT in these patients was not correlated to cardio metabolic risk factors, markers of liver dysfunction, HCV viral load or viral genotype.

That data suggests that factors other than conventional risk factors or viral load are responsible for accelerated atherosclerosis in these patients which calls for further studies to clarify the nature of these factors.

References