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Relation of Intima Media Thickening of Carotid Arteries with Parameters of Lipid Profile in Chronic Hepatitis C Patients

Talat Samreen, Asma Aijaz, Shazia Fahmi, Hira Ahmed, Nasreen Naz, Erum Amir

ABSTRACT

Objective: To find out the relation between carotid intima media thickening (CIMT) and lipid profile in HCV seropositive patients.

Study Design & Setting: Descriptive, cross sectional study.

Methodology: Study was conducted in the Institute of Basic Medical Sciences of Dow University of Health Sciences. Duration of study wsd 13 months. After ethical approval, a total of ninety participants including thirty (n=30) control (Group A) and sixty (n=60) HCV sero-positive patients (Group B) were inducted in the study from the hepatitis clinic of Ruth Pfau Civil Hospital, Karachi, Those with history of smoking, hypertension, alcohol, pregnancy, ascities, coinfection with other hepatitis viruses and not willing to participate were excluded. After consent, demographic and anthropometric data were recorded. Blood samples were taken for fasting blood glucose and lipid profile estimation. CIMT was estimated by carotid doppler ultrasound. Data entered and analyzed by SPSS version 20.0. and presented as frequency (n; %) and mean ±SD. Student "t" test and Pearson's correlation test were applied where appropriate. A p-value of <0.05 was considered as statistically significant.

Results: Demographic and anthropometric characteristics of HCV sero-positive participants showed non-significant difference compared to control. FBG and CIMT were found to be significantly raised in HCV infected patients while lipid profile parameters showed insignificant difference. Pearson's correlation test revealed insignificant relation of lipid profile parameters with CIMT ($p > 0.000$).

Conclusion: HCV seropositive patients had significantly raised CIMT with non-significant relation with parameters of lipid profile.

Key Words: HCV: Hepatitis C Virus, CIMT: Carotid Intima Media hickening, FBG: Fasting blood glucose

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INTRODUCTION:

Hepatitis C is slowly progressive hepatotoxic disease which has affected nearly 150 million people globally**,** while about 1.5 million new cases are reported per year. Its prevalence in Pakistan has also reached up to an epidemic proportion with nearly 10 million people in the country living with this virus. According to an estimate of WHO, Pakistan stands at second position after Egypt according to number of people infected with this virus. Data suggested that almost one in every 20 Pakistanis is infected with it.[1]

HCV virus is a hepatotropic RNA virus causing progressive liver damage which might result in fibrosis, cirrhosis and hepatocellular carcinoma. Though hepatocytes are the major site of viral replication, a broad clinical spectrum of extra hepatic complication and disease are associated with HCV infection. Some of these conditions are well documented and more common while others are infrequent. L Kuna et al, revealed that almost 40-74% HCV infected patients present various extra hepatic complications.[2] A meta-analysis, including more than 32,000 patients suggested that only 16% patients with CHC infection developed cirrhosis and only a proportion of these individuals dying from their liver

disease.[3] A meta-analysis of Younnoss et al, (2019) documented that patient with chronic hepatitis C have higher cardiovascular risk compared to never infected people. The prevalence of cardiovascular diseases in hepatitis C infected patients in East Asia is found to be 8.6%.[4] Vascular diseases are now emerged as third leading cause of death in chronic hepatitis C patients. This conclusion was drawn on the basis of published epidemiological studies which analyzed population-based morbidity and mortality data. [5] Presence of atherosclerotic plaque in carotid arteries of HCV patients was first reported by Ishizaka et al, later number of studies confirmed the association of HCV with atherosclerosis.[6] Though earlier it was believed that HCV infection had a protective role for cardiovascular events, due to decreased total cholesterol and circulating low-density lipoproteins, low blood pressure and decreased vascular resistance.[7]

Studies linked this paradigm shift in association of HCV infection with atherosclerosis with several factors like availability of better treatment options, increased life expectancy and use of sophisticated instrument for evaluation of atherosclerotic plaques.

Atherosclerosis was traditionally evaluated, by luminographic techniques, such as x-ray Angiography, Magnetic Resonance Angiography (MRA), Computed Tomographic Angiography (CTA). But due to invasive nature of angiography and exposure to radiation and iodinated contrast agent in MRA and CTA, these procedures have less acceptability and low patients compliance. Furthermore, luminographic technique was unable to image vessel wall adequately. It was identified that in early stage of atherosclerosis lumen of vessels are preserved, thus significant atherosclerotic burden could be underestimated or missed entirely with lumnographic technique. B- mode ultrasonography is now recommended by American Heart Association (AHA) for identification of preclinical cardiovascular diseases. It is non-invasive, inexpensive and cost-effective tool and recommended for the measurement of intima media thickening of carotid arteries (CIMT) for assessment of CVD risk.[8]

Changed in assessment methodology of atherosclerosis and recent evidences of propensity of HCV patients for development of atherosclerosis demands to revisit the relation of lipids with atherosclerosis as measured by intima media thickening of carotid arteries. Thus, present study has two-fold scope, first to evaluate the IMT of carotid arteries of CHC patients by B mode ultrasonography and second to find out the relation of lipid profile with intima media thickening of carotid arteries in HCV seropositive patients.

METHODOLOGY:

This descriptive, cross sectional study was conducted in the Institute of Basic Medical Sciences of Dow University of Health Sciences. Patients were selected from the Hepatitis Clinic and medical ward of Ruth Pfau Civil Hospital, Karachi, by nonprobability convenient sampling technique, after ethical approval from the IRB of DUHS. Duration of study was 13 months. With reference to the article[9] sample size was calculated through PASS version II sample size calculator taking confidence interval 99%, power 99%. A total of ninety subjects of both genders with 30-60 years of age were included in the study. Among them 60 participants were HCV related chronic liver disease patients (Group B) while 30 participants were age and sex matched healthy subjects (Group A). History was taken by principal investigator on the pre-approved proforma to avoid person to person bias. Subjects with history of smoking, hypertension, alcohol, pregnancy, ascites, co-infections with other types of hepatitis viruses and not willing to participate in the study were excluded. After taking consent, height and weight of patients were noted to calculate BMI. Blood samples after 12 hours of fasting were collected from patients for blood CP, fasting blood glucose level, lipid profile. Carotid imaging (CIMT) by Doppler ultrasound was performed by Supersonic imaging Aixplorer® multiwave (Paris, France) ultrasound system which is a high-resolution B-mode system equipped with a linear array transducer >7 MHz. Patients were laid down in supine position with hyperextended head, rotated in 45^0 away from side being examined. After applying gel, probe was placed in the mid of neck and lateral probe position was preferred to get best resolution for image acquisition for CIMT measurement. In order to get accurate and successive measurements of CIMT, bifurcation of carotid artery was considered as a land mark in the image plane. Straight, far wall of arterial segment 10 mm in length was measured by auto setting of machine. Three readings of CIMT of both common carotid arteries (right and left) were taken. Statistical analysis was done using SPSS VERSION 23.0. Qualitative and quantitative variables were assessed as frequencies, percentages, and with mean and standard deviation (SD). Chi- square test and independent sample t test were used to know the significance between variable at $p<0.05$ significant level. CIMT values were averaged. Mean CIMT values were preferred.

RESULTS:

A total of ninety participants including thirty (n=30) controls (Group A) and sixty (n=60) HCV related chronic liver disease patients (Group B) were inducted in the study.

More males compared to females were present in patient's group compared to healthy ones. Among study participants, frequency of males was high in HCV positive patients as compared to control. However male- female distribution between groups was found to be non-significant (Table 1).

Fasting blood sugar, BMI and LDL level were increased, while serum cholesterol, TG and HDL were decreased in HCV infected patients as compared to healthy participants. However, no statistically significant difference between patients and control was found as shown in Table 2.

Mean (±SD) CIMT in group A was found to be 0.46 ± 0.06

mm whereas mean (±SD) CIMT value in group B was found to be 0.64 ± 0.17 mm. Intima media thickness of carotid arteries(CIMT) of HCV patients was found to be significantly increased compared to control with p-value = 0.001, as shown in Figure 1.

Table 1: Comparison of cardio metabolic risk factors among study participants

Variables	Group A Healthy (n=30)	Group B patients (n=60)	p- value
Male (n ;%)	14 (30.4%)	33 (69.6%)	0.511
Female (n; %)	16 (37.2%)	27 (62.8%)	
BMI (kg/m^2)	25.5±0.8	26.1±0.6	0.501
FBS mg/dl	88.18±21.6102	102.02±31.2	0.032

p-value < 0.05 is significant

Table 2: Comparison of lipid profile among study participants

Variables	Group A Healthy (n=30)	Group B patients (n=60)	p-value
Serum Cholesterol mg/dl	146.0±29.1	144.1±44.3	0.838
Triglyceride mg/dl	102.46±25.1	100.2±31.7	0.745
LDL mg/dl	83.8±10.4	86.6±32.6	0.641
HDL mg/dl	44.2±9.3	39.1±16.3	0.115

p-value < 0.05 is significant

Figure 1: Comparison of Carotid Intima Media Thickening (mean ±SD) between control and case

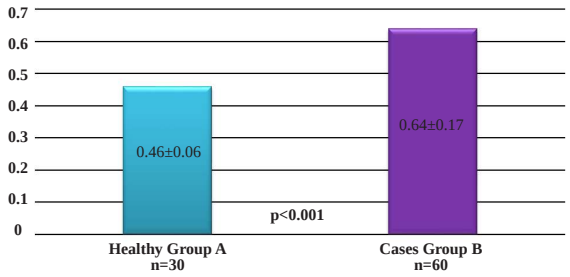

Figure 2. Age wise categorization and comparison of mean (±SD) CIMT values between control and patients

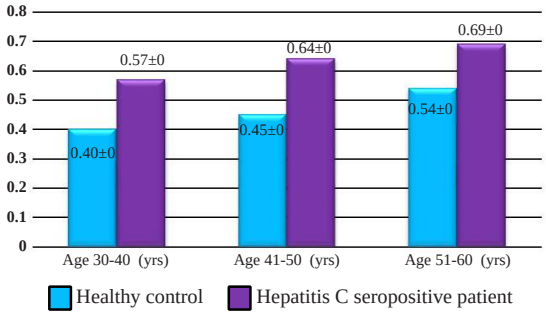

Table 3: Correlation of CIMT with parameters of lipid profile in hepatitis C patients

Lipid profile	CIMT (mm) r^{2}value	p-value
LDL(mg/dl)	-0.067	0.533
HDL(mg/dl)	-0.119	0.264
Cholesterol(mg/dl)	-0.085	0.426
Triglyceride(mg/dl)	-0.093	0.383

Even, participants of both groups were stratified into three groups on the basis of their age 30-40years, 40-50years and 50-60years. Comparison of mean (±SD) CIMT values between controls and HCV infected patients revealed that patients with Hepatitis C had higher mean (±SD) CIMT values when compared with age matched healthy individuals as shown in Figure 2.

When correlation between fragment of lipid profile like serum cholesterol, TG, HDL and LDL and CIMT were analyzed by Pearson's correlation, it showed negative weak relation with insignificant p-value as shown in Table 3

DISCUSSION:

Hepatitis C is a chronic, progressive hepatic disease, associated with increased risk of atherosclerosis eventually triggering cardiovascular events. Atherosclerosis is one of the leading cause of mortality and morbidity throughout the world. It is slowly progressive disease with long asymptomatic course. Atherosclerosis passes two phase pre-clinical (early stage) and clinical stage. A variety of imaging modalities have been used to assess subclinical atherosclerosis. Studies identified CIMT as simple, inexpensive and noninvasive tool to predict early phase of atherosclerosis. Now ultrasound based measurement of CIMT has become a standard for assessing atherosclerosis and also recommended by British Heart Association for noninvasive evaluation of cardiovascular risk.[9]

Present study revealed significantly increased intima media thickening of carotid arteries in HCV infected patients with non-significant relation with lipid profile. Role of lipids in pathogenesis of atherosclerosis was suggested more than 100 years ago after discovery of presence of yellow substance in atheroma by Virchow and later on this substance was identified as cholesterol by Windaus. Early observations suggested that cholesterol is a key component of arterial plaques which gave rise to the cholesterol hypothesis for the pathogenesis of atherosclerosis. Later on, after several decades multiple studies revealed relation between elevated blood cholesterol levels and increased risk of cardiovascular events. [10, 11]

Although some of the earlier studies did not identify an association between HCV and CVD morbidity, it was suggested that HCV viron circulates in the blood stream as hybrid lipoviral particle that consists of lipoprotein tightly adherent with HCV viral particle. These complex lipovral particles resemble with low density lipoprotein (LDL) and very low density lipoprotein (VLDL). It promotes the entrance of HCV in to hepatocyte by binding it with LDL receptor and prevents it from antibody neutralization. HCV infection interferes the intrahepatic cholesterol synthesis due to its utilization in viral replication and compromise the

delivery of available cholesterol in peripheral circulation. This ultimately results in up regulation of LDL receptors and more uptake of LDL in hepatocyte. Besides, up regulation of LDL receptor expression, and interference by HCV in Mevalonate pathway of cholesterol synthesis, inactivation of genes essential for fatty acid and lipid biosynthesis was the other suggested mechanisms in HCV infected patients.[12]

Study conducted in CHC infected Nigerian patients reported significantly lower level of all variables of lipid profile as compared to healthy control subjects.[13] Similarly, significant reduction in TC and LDL level were observed when pre and post infected acute HCV patients were analyzed.[14] Moreover, the observed hypolipidemia resolved with HCV viral eradication; either with spontaneous or treatment-induced sustained virological response and LDL and TC levels had rebound in to levels at or above their pre-infection baseline.[15] Thus, owing to favorable lipid profile, it was assumed that patients with chronic hepatitis C are protected from atherosclerotic cardiovascular diseases.

Conversely, literatures pronounced contradictory results despite findings of protective levels of lipid in HCV infected patients with atherosclerosis. First time a study conducted by Ishizaka et al, in 2002, found a link between HCV infection and carotid atherosclerosis.[16] Later on multiple studies proved the evidence that HCV infection is independently related with carotid plaques and an independent predictor of increased carotid intimal medial thickness (IMT). A study by Butt et al, reported higher risk of CAD in HCV infected patients despite favorable risk factors and low lipid levels.[17] In a treatment based study conducted by MA Mohamed et al, in Egyptian population reported significantly raised CIMT in HCV infected patients and negative correlation with lipid profile was observed. [18] In another study similar relation between lipid profile and CIMT was also reported. Abir Zakaria et al, compared 60 treatment naïve HCV patients with 20 healthy control and found that CIMT of HCV infected patients were significantly higher with insignificant difference in lipid profile.[19] Findings of our study with significantly raised CIMT in chronic hepatitis C patients and non-significant difference in lipid profile compared to controls bolsters the findings of previous studies.

Like others, our study findings also supported that atheroma formation in CHC patients is not per se the role of lipids that via classical cholesterol hypothesis promote atheroma formation. But rather other mechanisms are involved in this process. Studies reported various mechanisms through which atherosclerosis in CHC patients is promoted. It has been reported that HCV directly colonizes and replicates in the arterial walls. Apart from this direct effect, Arvind and his colleagues found increased HCV endocytosis by LDL receptors, induction of oxidative stress, systemic vasculitides and presence of anti-endothelial antibodies in CHC patients, all are promoters of atherosclerosis. Besides, liver steatosis and fibrosis also through insulin resistance indirectly accelerate atherosclerosis. Molecular mechanisms that causes metabolic derangement is associated with alteration in apoliprotein metabolism which triggers the level of apolipoprotein B, C reactive protein, and phospholipase A2 but decreases the level of apolipoprotein A1,which ultimately results in progression of increased CIMT leading to the atherosclerosis. Similarly, it is also assumed that inflammation linked with the hyper activation of cytokines, such as IL-1â, IL-6, IL-10, and TNF-á are responsible for pathogenesis of coronary heart disease and atherosclerosis.[20]

Furthermore, in the past, atherosclerosis was considered as disease of aging, such that advancing age is independent risk factor for development of atherosclerosis. A large Chinese population based cross sectional study found that age is also positively related with hyperlipidemia.[21] Contrary to this, our study revealed increased CIMT in HCV infected patients even when age wise stratified HCV patients suffering from hypolipidemia were compared with match control. Therefore, growing wealth of knowledge now favor to consider HCV infection as a risk factor for atherosclerosis despite promising lipid profile.

But these findings of lipid profile were in contrast to published study by Bozdar K (2020), who found significant difference of lipid profile variables (serum cholesterol, TG, HDL and LDL) associated with raised CIMT.[2] Possible reason of insignificant findings is, inclusion of all chronic hepatitis C patients in the present study regardless of selection of patients on the basis of severity of liver disease.

Thus, after availability of new and efficacious drugs for treatment, those with advance fibrosis, and suffering with HCV related liver disease, morbidity and mortality due to extra hepatic complications of HCV is gaining paramount significance. This may be particularly true for life expectancy of HCV treated patients that may scrum victims to extra hepatic complications. Therefore, it is suggested that ultrasonography screening for carotid atherosclerotic disease should be carried out in HCV patients even in the absence of cardiometabolic risk factors.

CONCLUSION:

Increased intima media thickening of carotid arteries with no significant relation with parameters of lipid profile in HCV infected patients was observed. Findings demand careful evaluation of atherosclerosis in HCV infected patients inspite of presence of normal lipid profile.

Authors Contribution:
Talat Samreen: Study Design
Asma Aijaz: Study Design and Setting
Shazia Fahmi: Drafting of Work
Hira Ahmed: Data Collection
Nasreen Naz: Analysis of Data
Erum Amir: Data collection

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