

ANGIOTENSIN CONVERTING ENZYME INHIBITION: A MODIFYING PLAYER ON ATHEROGENESIS IN HEMODIALYSIS PATIENTS WITH HEPATITIS C INFECTION

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ABSTRACT: Background and Aim: This study aimed to investigate certain non-traditional risk factors for atherosclerosis in CKD hemodialysis patients with and without HCV infection. **Methods:** The eligible hemodialysis patients attending our renal dialysis unit were subjected to demographic assessment, testing for HCV infection, basic biochemical profile, and assaying for serum insulin levels, angiotensin converting enzyme (ACE) levels, and angiotensin II (ANG II) levels. **Results:** 65 patients were included in this study, where 35 of them were HCV negative and 30 patients were HCV positive. Out of the 65 studied patients, 25 patients received treatment with ACE inhibitor (ACEI) for ≥ 6 months. Our data indicated that the HCV positive patients had CIMT values >0.9 mm which is the limiting value for diagnosing atherosclerosis in 63.3% and 66.7% at right and left carotids, respectively, while only 20% and 31.4% of the right and left carotids, respectively of the HCV negative group had CIMT values >0.9 mm with significant difference between the two groups ($p < 0.001$ and $p < 0.005$ respectively for right and left carotids). Furthermore, our results indicated a significant increase in the fasting blood sugar, serum insulin, HOMA-IR, ACE serum levels, ANG II serum levels and CIMT at both the right and the left carotid arteries in HCV positive compared to HCV negative patients, with p values of 0.021, 0.016, 0.002, 0.008, 0.006, 0.001 and 0.003, respectively. **Conclusion:** This study indicated that treatment with ACEI resulted in a significant decrease in the CIMT as well FBG, serum insulin, HOMA-IR, ACE, ANGI in hemodialysis patients.

Keywords:

HCV, Angiotensin, HOMA-IR, atherosclerosis

INTRODUCTION: The prevalence of treated end-stage renal disease (ESRD) patients in the general population shows a high global variation ranging from under 100 to over 2,000 patients per million population ¹.

According to the most recent Egyptian renal registry in 2012, the prevalence of ESRD is 495 per million population, and the total recorded number of ESRD patients on dialysis is 70000. Ninety-eight percent of these patients are on hemodialysis (HD), ².

End-stage renal disease (ESRD) is associated with significant cardiovascular morbidity and mortality due to ischemic heart disease, stroke, peripheral artery disease, and sudden death ³. The increased cardiovascular mortality in patients with CKD is not totally explained by the traditional risk factors such as hypertension, smoking, hyperlipidemia, obesity, diabetes mellitus, or family history of coronary artery disease ⁴. Insulin resistance is increasingly recognized as a 'non-traditional' risk factor contributing to cardiovascular disease through endothelial dysfunction, oxidative stress, dyslipidemia, systemic inflammation, and activation of the renin-angiotensin-aldosterone system ⁵.

Therefore, insulin resistance was proposed to be an important therapeutic target for the reduction of cardiovascular morbidity and mortality in patients with CKD ⁶. Hepatitis C virus (HCV) infection is common among hemodialysis CKD patients and Egypt has the highest prevalence of HCV among the world countries ⁷. Both HCV infections and hemodialysis were reported to contribute to the cardiovascular events that are common in CKD hemodialysis patients ⁸.

This study aimed to investigate the status of certain non-traditional risk factors for atherosclerosis such as serum insulin levels, insulin resistance (IR), angiotensin converting enzyme (ACE) serum levels, and angiotensin II (ANG II) serum levels in relation to carotid intima-media thickness (CIMT) measurements as an indicative index for atherosclerosis in HCV positive and HCV negative CKD hemodialysis patients. Furthermore, we aimed to study the effect of treatment with an ACE inhibitor, Ramipril, on the studied variables and the CIMT progression in those patients.

METHODS: This cross-sectional case-control study included 65 patients with ESRD on regular HD who were recruited from the renal dialysis unit, Minia University hospital, Egypt through the period from December 2015 to September 2016. The study protocol was approved by the Institutional Ethics Research Board of Minia University, Egypt and was consistent with the 1975 Declaration of Helsinki. All included eligible patients signed informed consent to participate in the study.

Exclusion Criteria: Our exclusion criteria included obese patients with BMI >25, diabetic patients, thyroid disease patients, decompensated liver disease, hypertensive patients, Patients > 55 years old, heavy smokers and the use of drugs such as contraceptive pills, corticosteroids, and statins.

According to HCV Ab testing and subsequent HCV RNA determination, the recruited patients were classified into two groups:

Group I: including 35 patients negative for HCV (19 males and 16 females) with age range of (16-55ys) and duration of dialysis for 4-15 years and Group II: including 30 patients positive for HCV (11 males and 19 females) with age range of (15-53) and duration of dialysis from (6 months -17 years). BMI was calculated using the following formula:

$$\text{BMI (Kg/m}^2\text{)} = \text{Weight in Kg} / (\text{Height in meters})^2$$

Laboratory Investigations: Complete blood pictures. (CBC) was determined using automated cell counter SYSMEX KX-2iN. Liver function tests [serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphates, and albumin], kidney function tests (urea, creatinine, sodium and potassium), and total cholesterol and triglycerides were performed using a Synchron CX-9 auto-analyser utilizing Beckman reagents (Beckman Instruments; Scientific Instruments Division, Fullerton, CA, United States). Low-Density Lipoprotein (LDL): was calculated according to Friedewald equation (1972) ¹⁰. Parathyroid hormone was measured by using solid-phase enzyme amplified sensitivity immunoabsorbent performed on microtiter plates, using ELA/Kit from sigma aldrich, RAS0412.

Serum insulin was determined by an chemiluminescence immunoassay using commercial Kit (Chemux BioScience INC, china No.10801). C- peptide was determined by ELA/Kit, Chemux BioScience inc, china No.10802). ACE level was determined by immunoassay using ELA/Kit, from Wuhan EIAaBScience Co., LTD, china No.E0004h. ANGII level was measured using ELA/Kit from Wuhan EIAaBScience Co., LTD, china, No.E000Ge. IR was determined by the HOMA method using the following equation: $\text{HOMA-IR} = \text{fasting insulin (mU/ml)} \times \text{fasting plasma glucose (mmol/l)} / 22.5$ ¹¹.

Hepatitis B surface antigen and HCV-antibody were measured using Roche Cobase 411 (Roche Diagnostic GmbH). HCV infection was defined as positive second-generation anti-HCV antibodies and detection of

HCV RNA in serum using quantitative reverse transcription polymerase chain reaction during the study period (Abbott M 2000, United States; -lower limit of detection 12 IU/mL) ¹².

Carotids IMT Assessment: Carotid artery ultrasound scan was the method used to measure the CIMT. High-resolution B-mode system (B-mode imaging was performed using a Toshiba Aplio 500, Japan equipped with a linear array transducer 10-12 MHz with minimal compression (<10:1) and footprint of at least 3 cm. IMT is defined as a double-line pattern visualized by echo 2D on both walls of the common carotid artery (CCA) in a longitudinal view. According the European Society of Cardiology (ESC)/European Society of Hypertension (ESH) guidelines (2013), carotid IMT > 0.9 mm has been reconfirmed as index for atherosclerosis and as a marker of asymptomatic organ damage ¹³.

Statistical Analysis: The collected data were coded, tabulated, and statistically analyzed using the SPSS program (Statistical Package for Social Sciences) software version 20.

Descriptive statistics were done for parametric quantitative data by mean, standard deviation and minimum & maximum of the range, while they were done for categorical data by number and percentage.

Analyses were done for parametric quantitative data between two groups using independent sample t-test, and for non-parametric quantitative data using independent sample t-test after logarithmic transformation.

Analyses were done for qualitative data using the Chi-square test.

Correlation between two quantitative variables was done by using Pearson's correlation coefficient. Correlation coefficient ranges from (0-1):- weak ($r=0-0.24$), fair ($r=0.25-0.49$), moderate ($r=0.5-0.74$), strong ($r=0.75-1$)

The level of significance was taken at (P value < 0.05).

RESULTS: Our data indicated that among the demographic variables, only there was a significant difference in the duration of dialysis (p value = 0.046) between HCV positive and HCV negative HD patients. The age, sex, BMI, blood pressure, hemoglobin level, serum creatinine, blood urea, serum albumin, and the PTH were not statistically different between HCV positive and HCV negative HD patients. Among the indices of lipid profile, only there was a significant increase in LDL in HCV positive compared to HCV negative patients, p -value = 0.036 **Table 1**.

There was a significant increase in FBS, serum insulin, HOMA-IR, ACE, ANGII in group II (HCV positive) compared to group I (HCV negative), with p values=0.021, 0.016, 0.002, 0.008 and 0.006, respectively. At the same time, the results showed a significant increase in CIMT in HCV positive patients compared to HCV negative patients with p values of = 0.001 for CIMT-Right (CIMT-RT), and $P= 0.003$ for CIMT –Left (CIMT-LT), **Table 2**.

Our data indicated that the HCV positive patients had CIMT values >0.9mm which is the limiting value for diagnosing atherosclerosis in 63.3% and 66.7% at right and left carotids, respectively, while only 20% and 31.4% of the right and left carotids, respectively of the HCV negative group had CIMT values >0.9mm with significant difference between the two groups ($p<0.001$ and $p<0.005$ respectively for right and left carotids), **Table 3**.

There was a positive correlation between HOMA-IR, ACE, and ANGII with CIMT-LT and RT with P -value = < 0.001, **Table 4**. At the same time, there was a significant positive correlation between ACE and AGII with HOMA-IR in all patients without ACEI treatment with P -value = < 0.001, **Table 5**.

To clarify the effect of ACE inhibition on the parameters of atherosclerosis, comparisons were done between patients who received ACEI and those who did not receive the ACEI, whether they were HCV positive or HCV negative.

Table 6 presents the results of comparisons between the treated group of patients and the non-treated group as regards the CIMT and the studied risk variables for atherogenesis.

The data indicated a significant decrease of CIMT in the treated group compared to the non-treated group, $p < 0.001$ for the right carotid and $p < 0.003$ for the left carotid. At the same time there was a significant decrease in FBS, serum insulin, ACE, and ANG II in the HD patients who were treated with ACEI compared to those who were not treated with p values of < 0.001 for all variables. Interestingly, the HOMA-IR was found to be significantly lower in the treated group than in the non-treated group, $p < 0.001$.

*: significant difference at p value < 0.05 ; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure ; HB : hemoglobin; S.C: serum creatinine ; PTH: parathyroid hormone ; TG: triglycerides ; TC: total cholesterol

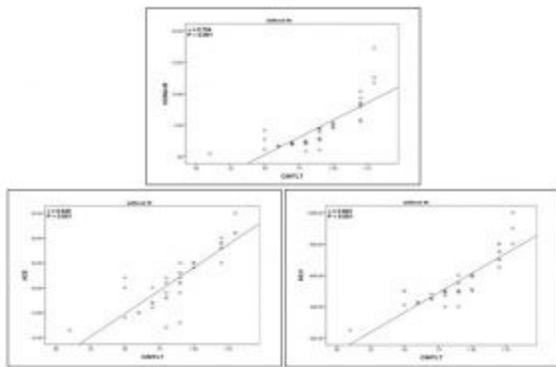


FIG. 1: CORRELATION BETWEEN HOMA-IR, ACE AND AGII WITH CIMT-LT IN ALL PATIENT WITHOUT TREATMENT IN POSITIVE AND NEGATIVE HCV GROUPS

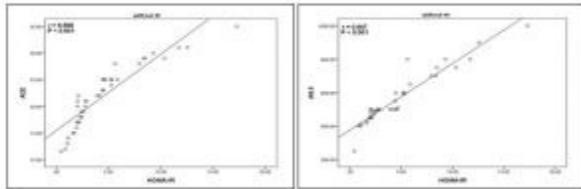


FIG. 2: CORRELATION BETWEEN ACE AND AGII WITH HOMA-IR IN ALL PATIENTS WITHOUT TREATMENT

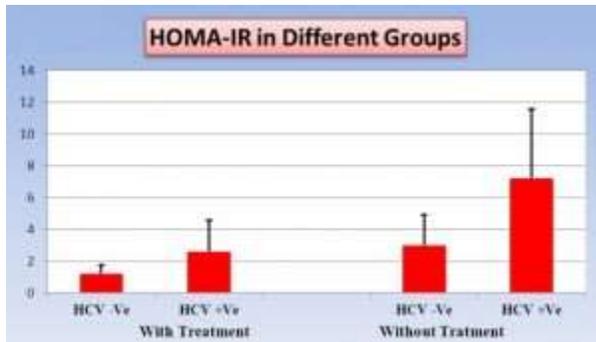


FIG. 3: HISTOGRAM SHOWING HOMA-IR IN THE STUDY GROUPS

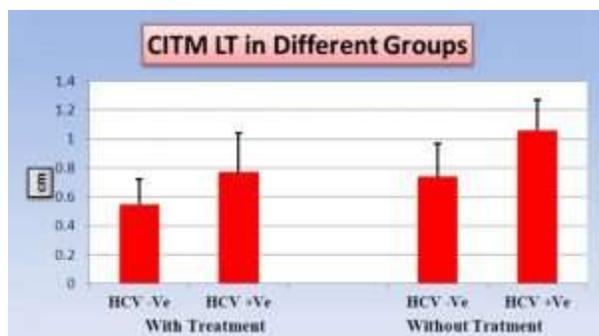


FIG. 4: HISTOGRAM SHOWING CITM-LT IN THE STUDY GROUPS

DISCUSSION: This study confirmed the occurrence of atherosclerosis at a significantly higher frequency in HCV positive patients (63.3% and 66.7% for the right and left carotids respectively) compared to the rate in HCV negative patients (20% and 31.4% for the right and left carotids respectively),

Furthermore, our results indicated that the HCV positive hemodialysis patients had significantly higher values of serum insulin, fasting blood glucose level, HOMA-IR, ACE, AGII and CIMT-RT and LT side, when compared to HCV negative hemodialysis patients with P values of: $p < 0.016$, $p < 0.021$, $p < 0.002$, $p < 0.008$, $p < 0.006$, $p < 0.001$, $p < 0.003$, respectively. This finding was relevant whether those patients received ACEI or not. This finding indicates that HCV infection in CKD with ESRD who are on hemodialysis not only induces metabolic disorders as evidenced by the increased values of FBS, Fasting insulin and HOMA-IR, but also it displays a significant role in the development of cardiovascular complications as evidenced by the increased values of CIMT in both sides together with the higher values of ACE and ANG II in HCV positive patients.

This finding can support the assumption that HCV infection contributes to further deterioration in insulin signaling and insulin-resistant state independent of uremia, since both HCV (-) and HCV(+) groups were under hemodialysis condition. Hyperinsulinemia is a frequent finding in chronic renal failure and hemodialysis patients¹⁴.

Ozdemir *et al.*,¹⁵ mentioned that insulin resistance is an independent predictor of cardiovascular mortality in uremic and non-uremic patients.

Many but not all studies in the general population reported a positive association of indices of insulin resistance with arterial wall changes, coronary artery disease and cardiovascular mortality¹⁶.

Negro¹⁷, in an observational cohort of HCV patients including 82,083 HCV-infected and 89,582 uninfected subjects, reported that HCV increased the risk of coronary artery disease. By multivariate analysis, and despite their favorable lipid profile, HCV-infected veterans had a higher risk of coronary artery disease (HR, 1.25; 95% CI, 1.20–1.30) on top of classical risk factors such as age, arterial hypertension, chronic obstructive pulmonary disease, diabetes, and hyperlipidemia.

Our finding of the significant increase in carotid intimal thickness (CIMT) in the right and left side in HCV positive patients compared to HCV negative patients may reflect the interaction between HCV and its associated insulin resistance with the increased ACE and ANGII in inducing/or accelerating the atherosclerotic process in CKD hemodialysis patients.

Yelken *et al.*,¹⁸ measured coronary flow reserve (CFR) by transthoracic Doppler echocardiography in non-diabetic anti-HCV positive (n=26) and -negative (n=26) patients undergoing regular dialysis (hemodialysis or peritoneal dialysis) after kidney transplant failure. Clinical and demographic characteristics were similar between the two groups. CFR was significantly lower in HCV-infected than uninfected individuals

(1.5 ± 1.1 vs. 1.63 ± 0.26 , $P=0.03$). Kaplan-Meier survival curves for cerebrovascular and cardiovascular event-free rates indicated a significant difference between HCV-RNA positive and negative patients on dialysis (log-rank test, $P<0.05$). The authors pointed to an atherogenic role of HCV infection through the aggravation of metabolic syndrome and dyslipidemia¹⁹ presented data that suggested a positive association between HCV and carotid atherosclerosis irrespective of positivity of HCV RNA or not in HCV antibody-positive patients²⁰. Reported increased risk of coronary artery disease, in HCV infected individuals with CKD whether on dialysis or not. However, there is evidence that serum HCV RNA levels are independently associated with carotid atherosclerosis. HCV RNA was isolated inside the atherosclerotic plaques, suggesting that HCV replicates within the plaques. Due to the damage exerted directly on the arteries, HCV might stimulate the synthesis of pro-inflammatory cytokines to cause a pro-atherogenic activity²¹.

Our data indicated a significant increase in the serum levels of ACE, ANG II and HOMA-IR in HCV positive hemodialysis patients compared to those who are negative to HCV infection. These data may indicate the role displayed by HCV infection in inducing activation of the renin-angiotensin system which is a step in development of cardiovascular disorders and atherosclerosis.

In correspondence with this assumption, our data furthermore presented significant positive correlation between ACE, ANG II and HOMA-IR with the CIMT in HCV positive patients ($p<0.001$ for all variables) and also in HCV negative hemodialysis patients, ($p<0.001$ for all variables), a finding which indicates that CKD in dialysis even without HCV infection has also its impact on activation of the renin-angiotensin system that in turn may participate in development of cardiovascular complications.

ANG II, however, has many actions beyond its effects on blood pressure and has been shown to inhibit insulin signaling. Indeed, insulin and ANG II are two important hormones in the control of the metabolic and hemodynamic homeostasis, respectively. Functional interaction between ANG II and insulin is operative in insulin-sensitive tissues as well as the CV system. This interaction, in turn, participates in the regulation of metabolism and cardiovascular function¹⁶. ANG II has been shown to inhibit the insulin –PI3K signaling pathway in both vascular and skeletal muscle cells²². In our study, we tested the possible beneficial effect of ACE inhibition on the mediators and occurrence of atherosclerosis in ESRD hemo-dialysis patients.

When we tried a collective comparison between patients without treatment and those with treatment irrespective of hepatitis C virus infection, we found significant decrease in CIMT in the right and left sides in patients who received Ramipril (number = 25), compared to those patients who were not treated with Ramipril (number = 40), with p -value = 0.019 and $p = 0.003$ for both the right and the left carotids respectively. This finding was associated with a significant decrease of HOMA-IR, ACE, and ANGII, ($P<0.001$, $P<0.001$ and $P<0.001$, respectively in the treated group than the non-treated group).

These findings not only provide evidence for the concept of possible reversibility of the atherosclerotic lesions but also presented an explanation to mechanisms that may participate in this regression of the atherosclerotic lesions. A growing number of experimental and clinical studies provide data indicating that pharmacological blockade of the RAS by either angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type 1 receptor blockers (ARBs) not only reduces cardiovascular injury but also improves insulin sensitivity and reduces the incidence of new-onset T2DM in subjects with hypertension and/or cardiovascular disease, independently of blood pressure reducing effects¹⁶.

Cacciatore *et al.*,²³ stated that ACE inhibitors are documented to slow down plaque formation in the progression of atherosclerosis with hyperlipidemia. They decreased plaque cholesterol content and cellularity.

Our study showed a non-significant decrease in LDL and total cholesterol in the treated groups. This was also associated with a non-significant increase in HDL levels in all treated groups. These findings may suggest that the decrease in CIMT that was detected in the ACE inhibitor group was not related to changes in the lipid variables but rather due to the improvement in the insulin resistance and the amelioration of activities of the renin-angiotensin system as evidenced by the significant decrease of HOMA-IR, ACE, and ANGII in the Ramipril treated group in which CIMT was also decreased compared to values in the non-treated group.

Highly activated pro-inflammatory monocytes in the circulation may enhance plaque growth and progression of vascular disease²⁴. Angiotensin II facilitates recruitment of monocytes and macrophages into the vessel wall by stimulating smooth muscle cell production of monocyte attractant protein-1 and expression of vascular cell adhesion molecule-1²⁵.

In addition, angiotensin II is a well-characterized mitogen for vascular smooth muscle cells and may stimulate the accumulation of extracellular matrix either directly or indirectly through the production of the transforming growth factor- β ²⁶.

In addition to the aforementioned effects of ACE inhibitors on the reduction of levels of angiotensin II and the increase of bradykinins, emerging evidence suggests that ACE inhibitors have important implications on the vascular oxidative stress²⁷. This may be another target for ACE inhibition through which it can display retardation of the atherosclerotic process.

CONCLUSION: It is concluded that ESRD patients on hemodialysis are prone to atherosclerosis events, particularly when infected with HCV infections which through multiple mediators can accelerate the atherosclerotic process. These risk mediators include FBS, serum insulin, HOMA-IR, serum ACE and serum ANG II which were found to be significantly increased in the HCV infected patients than in the non-infected group. The CIMT as a measure of atherosclerosis and the mediators including HOMA-IR, ACE, ANG II, FBG and serum insulin were significantly lower in patients treated with ACE inhibitor than in the non-treated group.

Limitations of the Study: The first limitation of our study is the small sample size; however this may be understood by the limited number of patients attending our hemodialysis unit together with exclusion of the non-eligible patients. The second limitation is the lack of a longitudinal pattern of the study with follow up for the studied variables in the treated group of patients to assess the impact of long term ACE inhibition in the same patients.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

ETHICAL APPROVAL: The institutional review board of faculty of pharmacy, Minia University, Egypt is constituted and operated according to the 1975 declaration of Helsinki and related international and local regulations and guidelines which govern IRB operation. It comprised 7 professional members and a non-institutional society representative.

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