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ORIGINAL ARTICLE

## Evaluation of serum angiotensin-II in HCV related glomerulonephritides

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### 1. Introduction

The hepatitis C virus (HCV) is a global health problem. More than 170 million people world wide are chronically infected with HCV, which is responsible for over 1 million deaths resulting from, cirrhosis and liver cancers. The prevalence of HCV infection varies in different parts of the world, an estimated 4 million Americans have been exposed to HCV representing approximately 2% of the US population.<sup>1,2</sup>

Egypt has the highest sero prevalence of HCV reaching up to 20% in some areas.<sup>3,4</sup> About 60–85% of patients infected

with HCV develop chronic hepatitis C, i.e. infection lasting more than 6 months.<sup>5,6</sup>

Although the primary burden of disease that is associated with chronic hepatitis C is liver related, other organ systems may be involved.<sup>2</sup> In the kidney, HCV seems to be strongly associated with membranoproliferative glomerulonephritis (MPGN) usually in the context of cryoglobulinemia, but MPGN can develop without cryoglobulins in addition to other types of glomerulonephritis including membranous GN and focal segmental glomerulosclerosis.<sup>7–9</sup>

The pathogenesis of hepatitis C-associated renal disease remains incompletely defined, but most evidence suggests that it is caused by circulating complexes of antibodies and HCV particles directly causing damage to the kidneys as they are deposited in the glomerulus and tubules of the kidneys.<sup>10</sup>

Cryoglobulins are antibody complexes that precipitate as serum is cooled and that dissolve on rewarming.<sup>11</sup> HCV is associated with mixed cryoglobulinemia (MC) type II which is composed of polyclonal immunoglobulin IgG and monoclonal IgM rheumatoid factor (RF).<sup>12</sup> Renal signs of cryoglobulinemia include proteinuria and microscopic hematuria with mild to moderate renal insufficiency.<sup>13</sup> Other abnormalities include decreased complement levels and the presence of rheumatoid factor and cryoglobulins.<sup>10</sup>

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The long term outcome of HCV-associated nephropathies remains ill defined although it was reported that HCV positive patients had a 40% higher likelihood for developing renal insufficiency.<sup>14</sup> So, some authors have suggested that screening for proteinuria and creatinine clearance may be indicated among patients with chronic HCV.<sup>15,16</sup>

Angiogenesis, the formation of new vessels, has been reported to play a significant role in liver damage-associated hepatitis C virus infection including hepato cellular carcinoma.<sup>17,18</sup> Angiogenesis is regulated by angiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietins. Among the known angiogenic factors, VEGF has emerged as the central regulator of the angiogenic process of physiological and pathological conditions.<sup>19</sup>

VEGF is a member of a family of closely related cytokines, also known as vascular permeability factor. It is highly specific mitogen for vascular endothelial cells by binding to two tyrosine kinase receptors which are selectively expressed on endothelial cells, it induces endothelial cell proliferation, promotes cell migration and inhibits apoptosis.<sup>20-22</sup>

Angiopoietins are a family of vascular growth factors, the best-studied being angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2). They are legends for the tyrosine kinase receptor Tie 2.<sup>23</sup> Angiopoietin 1 (Ang-1) serves as a survival factor for the endothelium and promotes recruitment of pericytes and smooth muscle cells, thereby serving to stabilize vascular networks. Angiopoietin 2 (Ang-2) is a biological antagonist of Ang-1 and is dramatically expressed at sites of vascular remodeling. It reduces vascular stability and facilitates access of VEGF to endothelial cells.<sup>19,24,25</sup>

### 1.1. Subjects

The present study included two groups of patients:

**Group I:** It consisted of 24 patients with chronic hepatitis C virus without nephropathy.

**Group II:** It consisted of 16 patients with chronic hepatitis C virus with nephropathy.

The patients were selected from Medical Research Institute, Alexandria University. Patients with hepatorenal syndrome, hepatitis B virus, DM, autoimmune diseases as SLE or malignancies were excluded from the study.

## 2. Methods

All the studied patients were subjected to the following:

History taking, clinical examination with stress on renal symptoms as edema of lower limbs, haematuria, oliguria and association of hypertension together with any other symptoms related to cryoglobulinemia.

**Table 1** Sex and age of chronic HCV patients groups

	Sex		Age (Years) Mean ± SD
	M	F	
Group I	13	11	42.33 ± 9.092
Group II	8	8	51.062 ± 7.57

M = Male; F = Female.

Group I = patients with chronic HCV without nephropathy.

Group II = patients with chronic HCV with nephropathy.

Preliminary investigations including fasting serum glucose, blood urea, serum creatinine, serum uric acid, ALT, AST, total and direct bilirubin, total proteins, serum albumin, alkaline phosphatase and gamma glutamyl transferase.<sup>26-29</sup> Complete blood picture, prothrombin activity and INR,<sup>30</sup> Rheumatoid factor, cryoglobulins<sup>31-33</sup> and urinary albumin creatinine ratio were estimated.<sup>34,35</sup> Serum levels of vascular endothelial growth factor (VEGF) and angiopoietin 2 were measured by ELISA.<sup>36,37</sup>

## 3. Results

Group I included 13 males and 11 females. Their age ranged from 29 to 60 years with mean 42.33 ± 9.092 years.

Group II included 8 males and 8 females. Their age ranged from 34 to 60 years with mean 51.062 ± 7.57 years (Table 1).

Patients in group II showed several manifestations as oedema of lower limbs in nine patients (56.25%), seven patients

**Table 2** Comparison of liver function tests in both groups of chronic HCV patients.

	Group I (n = 24)	Group II (n = 16)	Z (P)
<i>ALT (u/l)</i>			
Min-max	10.00-216.00	7.00-90.00	0.097
Mean ± SD	36.71 ± 42.27	34.81 ± 25.5	(0.923)
Median	23.00	24.50	
<i>AST (u/l)</i>			
Min-max	14.00-160.00	12.00-160.00	0.193
Mean ± SD	50.00 ± 31.74	55.19 ± 43.43	(0.847)
Median	44.00	38.50	
<i>T. bilirubin</i>			
Min-max	0.50-11.80	0.40-16.80	1.216
Mean ± SD	2.51 ± 2.38	3.79 ± 5.68	(0.224)
Median	1.75	1.15	
<i>D. bilirubin</i>			
Min-max	0.30-8.00	0.10-11.60	0.776
Mean ± SD	1.53 ± 1.70	2.76 ± 4.27	(0.437)
Median	0.90	0.70	
<i>T. proteins</i>			
Min-max	5.50-8.10	4.00-8.30	1.756
Mean ± SD	6.89 ± 0.63	6.39 ± 1.01	(0.079)
Median	6.85	6.15	
<i>S. albumin</i>			
Min-max	2.30-5.10	1.90-4.00	2.242
Mean ± SD	3.34 ± 0.78	2.75 ± 0.58	(0.025)
Median	3.10	2.65	
<i>ALP</i>			
Min-max	58.00-189.00	59.00-624.00	3.010*
Mean ± SD	108.08 ± 36.83	204.62 ± 141.52	(0.003)
Median	104.00	162.00	
<i>GGT</i>			
Min-max	21.00-119.00	16.00-260.00	0.787
Mean ± SD	57.96 ± 28.01	93.12 ± 76.43	(0.431)
Median	51.50	62.00	

ALT = Alanine amino transferase; AST = Aspartate amino transferase; T. bilirubin = Total bilirubin; D. bilirubin = Direct Bilirubin; T. proteins = Total proteins; S. albumin = Serum albumin; ALP = Alkaline phosphatase; GGT = Gamma-glutamyl transferase.

\* Statistically significant at  $P \leq 0.05$ . Z for Mann Whitney test.

**Table 3** Comparison of Some biochemical parameters in both groups of chronic HCV patients.

	Group I (n = 24)	Group II (n = 16)	Z (P)
<i>FBG (mg/dl)</i>			
Min-max	84.00-119.00	78.00-115.00	0.843
Mean ± SD	99.83 ± 10.32	101.56 ± 11.89	(0.399)
Median	97.00	105.50	
<i>B urea (mg/dl)</i>			
Min-max	9.00-48.00	20.00-292.00	2.046*
Mean ± SD	29.916 ± 8.90	78.87 ± 76.56	(0.041)
Median	31.50	45.90	
<i>S Creatinine (mg/dl)</i>			
Min-max	0.70-1.50	0.80-4.30	2.053*
Mean ± SD	1.121 ± 0.23	1.90 ± 1.105	(0.040)
Median	1.10	1.90	
<i>SUA (mg/dl)</i>			
Min-max	2.40-7.60	2.60-16.00	2.251*
Mean ± SD	4.98 ± 1.53	7.04 ± 3.29	(0.024)
Median	4.65	7.10	

FBG = Fasting blood glucose; B urea = Blood urea; S creatinine = Serum creatinine.

SUA = Serum uric acid; Z for Mann Whitney test.

\* Statistically significant at  $P \leq 0.05$ .

**Table 4** Comparison of Haematological parameters in both groups of chronic HCV patients.

	Group I (n = 24)	Group II (n = 16)	Z (P)
<i>Hb</i>			
Min-max	6.30-14.10	4.90-14.20	2.144*
Mean ± SD	11.57 ± 2.03	9.82 ± 2.82	(0.032)
Median	11.20	9.20	
<i>WBCs</i>			
Min-max	2.30-11.70	2.70-18.00	0.700
Mean ± SD	6.01 ± 2.29	6.29 ± 3.99	(0.484)
Median	6.00	5.10	
<i>Platelets</i>			
Min-max	44.00-301.00	52.00-528.00	0.443
Mean ± SD	165.35 ± 67.97	185.94 ± 114.34	(0.658)
Median	173.00	190.00	
<i>Prothrombin</i>			
Min-max	40.50-100.00	51.00-100.00	0.758
Mean ± SD	75.18 ± 20.85	82.99 ± 13.70	(0.449)
Median	78.90	85.10	
<i>INR</i>			
Min-max	1.00-2.40	1.00-2.04	0.444
Mean ± SD	1.46 ± 0.50	1.26 ± 0.25	(0.657)
Median	1.18	1.20	

Hb = Haemoglobin; WBCs = white blood cells; INR = International normalized ratio.

Z for Mann Whitney test.

\* Statistically significant at  $P \leq 0.05$ .

were hypertensive (43.75%), six patients were oliguric (37.5%) and four patients had haematuria (25%).

Four patients of group I showed arthralgia and one patient had peripheral neuropathy while five patients of group II complained of arthralgia.

Renal biopsy was done in five patients in group II and showed membranous glomerulonephritis in two patients while the other three patients had membranoproliferative lesions (Tables 4 and 5).

Serum albumin was significantly lower in group II than group I and UACR was significantly higher in group II than group I (Table 2).

While blood urea, serum creatinine and serum uric acid were significantly higher in group II than group I (Table 3).

Serum levels of both VEGF and Angio-2 were significantly higher in patients with nephropathy than their levels in those without nephropathy (Table 6).

There was a significant positive correlation between VEGF and UACR in group I and group II. Also a significant negative correlation between Angio-2 and serum albumin in group I and a significant positive correlation between Angio-2 and UACR in group II (Tables 7 and 8).

#### 4. Discussion

An estimated 12 million Egyptians have been infected with the hepatitis C virus (HCV), representing approximately 14.7% of the Egyptian population.<sup>38</sup> Although the primary burden of disease that is associated with chronic hepatitis C is liver related (liver fibrosis, cirrhosis, and hepatocellular carcinoma), other organ systems may be involved. In the kidney, HCV seems to be most strongly associated with membranoproliferative glomerulonephritis (MPGN) both with and without cryoglobulins and membranous glomerulonephritis, although other types of glomerulonephritis also have been described.<sup>39-41</sup>

The incidence of nephropathy in HCV-infected patients is unknown, owing to the lack of large-scale field studies. An early study on 188 autopsies with HCV infection<sup>42</sup> reported glomerular lesions in 54.8% of cases, although a cause-and-effect relationship was not confirmed. In a recent study of renal biopsies, obtained at the time of liver transplantation from 30 patients with HCV-associated cirrhosis, 25 had immune complex glomerulonephritis. The majority of these patients were asymptomatic, which suggests that subclinical renal involvement may be highly prevalent among patients with HCV hepatitis. The incidence of clinically overt nephropathy was 40% in our cohort.<sup>43</sup>

While cryoglobulins and HCV antigen-antibody complexes are typically blamed in the pathogenesis of HCV nephropathy, they cannot explain all the reported lesions. Furthermore, many of the histopathological features of HCV nephropathy have certain characteristics that distinguish them from other immune-mediated nephropathies<sup>44</sup>, which suggest that the virus may induce renal injury by other, not necessarily immune-mediated mechanisms.<sup>45</sup> This goes with our observation that cryoglobulins were found in only 75% of nephropathic group.

Angiogenesis has been reported to play a significant pathogenic role to liver damage during CHC.<sup>17,18</sup> CHC is associated with elevated serum levels of angiogenesis markers namely vascular endothelial growth factor (VEGF), angiopoietin 2 (Ang-2).<sup>18</sup> Abnormal angiogenesis also occurs in diabetic nephropa-

**Table 5** Comparison of distribution of rheumatoid factor, complement 3, cryoglobulins and values of urinary albumin creatinine ratio between the two groups of chronic HCV patients.

	Group I (n = 24)		Group II (n = 16)		Test of sig.
	No	%	No	%	
<b>RF</b>					
-ve	14	58.3	7	43.8	$X^2 = 0.819$
+ve	10	41.7	9	56.3	$P = 0.366$
<b>C3</b>					
Low	7	29.2	8	50	$X^2 = 1.778$
Normal	17	70.8	8	50	$P = 0.182$
<b>Cryoglobulins</b>					
-ve	20	83.3	12	75.0	FET = 0.690
+ve	4	16.7	4	25.0	
<b>UACR</b>					
Min-max	3.90-28.0		90.0-3480.0		$Z = 5.304^* (<0.001)$
Mean $\pm$ SD	18.23 $\pm$ 5.73		716.94 $\pm$ 931.91		
Median	18.0		350.0		

RF = rheumatoid factor; C3 = complement 3; UACR = urinary albumin creatinine ratio. Z for Mann Whitney test.  $X^2$  Chi Square test.

FET = Fischer Exact test.

\* Statistically significant at  $p \leq 0.05$ .

**Table 6** Comparison of Angiopoietin-2 and Vascular endothelial growth factor between both groups of chronic HCV patients.

	Group I (n = 24)	Group II (n = 16)	Z (P)
<b>Angio-2</b>			
Min-max	500.00-8805.00	3705.00-150000.00	3.728* (<0.001)
Mean $\pm$ SD	4963.96 $\pm$ 1881.43	49099.06 $\pm$ 41554.27	
Median	5047.50	56375.00	
<b>VEGF</b>			
Min-max	1000.00-6800.00	1060.00-9000.00	2.708* (0.007)
Mean $\pm$ SD	2016.75 $\pm$ 1446.90	3785.31 $\pm$ 2734.19	
Median	1430.00	2107.50	

Angio-2 = Angiopoietin 2; VEGF = Vascular endothelial growth factor. Z for Mann Whitney test.

\* Statistically significant at  $P < 0.01$ .

**Table 7** Correlation between Angiopoietin-2, Vascular endothelial growth factor, albumin and urinary albumin creatinine ratio in Group I of chronic HCV patients.

	Angio-2	VEGF
Albumin	$r = -0.488^*$ $p = 0.016$	$r = 0.363$ $p = 0.081$
UACR	$r = 0.288$ $p = 0.172$	$r = 0.454^*$ $p = 0.026$

Angio-2 = Angiopoietin 2; VEGF = Vascular endothelial growth factor;  $r$  = correlation coefficient;  $P$  = significance.

\* Statistically significant at  $P < 0.05$ .

**Table 8** Correlation between Angiopoietin-2, Vascular endothelial growth factor, albumin and urinary albumin creatinine ratio in Group II of chronic HCV patients.

	Angio-2	VEGF
Albumin	$r = -0.492$ $p = 0.053$	$r = 0.041$ $p = 0.881$
UACR	$r = 0.829^*$ $p = < 0.001$	$r = 0.552^*$ $p = 0.027$

Angio-2 = Angiopoietin 2; VEGF = Vascular endothelial growth factor;  $r$  = correlation coefficient;  $P$  = significance.

\* Statistically significant at  $P < 0.05$ .

thy, and associated with elevated level of both VEGF and Ang-2.<sup>19</sup>

To the best of our knowledge this is the first work investigating the role of VEGF and Ang-2 in pathogenesis of HCV related nephropathy. We can show that: (1) serum level of both VEGF and Ang-2 was significantly higher in patients with

nephropathy (Table 6), (2) there was a significant correlation between serum level of VEGF and UACR, (3) also a significant correlation was found between serum levels of Ang-2 and UACR (Tables 7 and 8).

The mechanism of stimulation of VEGF by HCV infection is through the stabilization of hypoxia-inducible factor  $1\alpha$

(HIF-1 $\alpha$ ). This stabilization is mediated via oxidative stress induced by HCV gene expression. HIF-1 $\alpha$  induction in turn led to the stimulation of vascular endothelial growth factor. It was showed that HCV-infected cells released angiogenic cytokines, leading to neovascularization in vivo.<sup>46</sup> The significantly higher level of VEGF in patients with nephropathy denotes that the source of VEGF in this group is not merely hepatic and suggests a renal contribution. It is noteworthy that many of the ingredients required for HCV attachment (e.g. glycosaminoglycans), endocytosis e.g. (LDLR), entry (e.g. SR-B1 and TLR-3) are abundantly expressed in the renal parenchyma. In addition, it may be speculated, though yet unproven, that the virus has the potential of entry and replication in renal tissue if conveyed by infected B-lymphocytes<sup>47</sup> or exosomes.<sup>48</sup>

Previous reports have shown that increased plasma levels of VEGF may be associated with proteinuria in patients with diabetes.<sup>49</sup> Our results showed positive correlation between VEGF and UACR (Tables 7 and 8). Increased VEGF in the glomeruli may result in proteinuria through two possible different yet closely overlapping or related mechanisms. First, glomerular VEGF derived from podocytes is involved in the maintenance of the glomerular endothelium (including maintaining fenestration) and/or selective permeability to macromolecules. High levels of VEGF derived from podocytes can strongly bind to capillary endothelial cells through specific VEGF receptors, which may result in increased permeability or glomerular hyperfiltration by altering capillary fenestration or basement membrane components or indirectly through the induction of nitric oxide and prostacyclin. Second it has been reported that VEGF stimulates increased synthesis of collagenase by endothelial cells, which result in the proteolytic disruption of the basement membrane may participate in the enhancement of proteinuria.<sup>50</sup>

Elevated expression of Ang-2 was observed in CHC and its expression is modulated by viral structural proteins.<sup>51</sup> Also the kidney endothelium itself has been identified as a rich source of Ang-2, so that chronic organ impairment might directly result in increased Ang-2 release from the kidney.<sup>51,52</sup>

Alterations in the expression of the angiopoietins have been implicated in the progression of diabetic nephropathy.<sup>53</sup> In addition, transgenic mice with inducible overexpression of angiopoietin-2 in podocytes in otherwise normal healthy adult animals develop significant increases in albuminuria.<sup>54</sup> These observations agree with the positive correlation we found between circulating angiopoietin level and proteinuria (Tables 7 and 8). These observations raise an important question that warrants further discussion: How does angiopoietin-2 cause proteinuria?

Electron microscopic studies in these angiopoietin-2 overexpressing mice demonstrate glomerular endothelial apoptosis, but there is no evidence of glomerular capillary collapse or foot

process effacement. These findings are consistent with the role of angiopoietin-2 in destabilizing endothelial cell integrity<sup>55</sup> but raise questions about the mechanism of proteinuria (Table 9).

The authors provide evidence that the slit diaphragm protein nephrin, an essential component of the glomerular permselectivity barrier, is downregulated in angiopoietin-2 overexpressing mice. On the basis of the observation that proteinuria has been described in the absence of foot process effacement, the authors argue that these changes in the expression of nephrin may give rise to a defect in slit diaphragm function without inducing a structural abnormality in podocytes.<sup>54</sup> This is certainly a possibility that might be confirmed by more detailed ultrastructural analysis of the slit diaphragm. However, an alternative possibility is that the primary defect in these mice results from loss of glomerular endothelial integrity.<sup>56</sup>

Importantly, the biological effects of angiopoietin-2 are context dependent and, in vivo, depend on ambient levels of VEGF-A, such that vessel regression occurs if VEGF-A is lacking, whereas vessel destabilization followed by angiogenesis occurs if the local milieu is rich in VEGF-A.<sup>53</sup> It could be postulated that the increased levels of angiopoietin-2 alongside a VEGF-A rich milieu in glomeruli will lead to the destabilization of blood vessels and hence excessive angiogenesis as what happens during the initial phases of diabetes.<sup>57</sup>

## 5. Conclusions

Circulating levels of the assayed angiogenic factors; VEGF and Ang-2, are significantly higher in patient with HCV nephropathy than those without nephropathy. The correlation between circulating levels of VEGF, Ang-2 and UACR a marker of renal injury points to the potential role of these angiogenic factors in the pathogenesis of HCV nephropathy.

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**Table 9** Correlation between Angiopoietin-2 and Vascular endothelial growth factor in both groups.

	Group I	Group II
Angio-2 and VEGF	$r = 0.279$ $p = 0.187$	$r = 0.390$ $p = 0.136$

Angio-2 = Angiopoietin 2; VEGF = Vascular endothelial growth factor;  $r$  = correlation coefficient;  $P$  = significance.

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