DOI: https://doi.org/10.53350/pjmhs211582487

ORIGINAL ARTICLE

Association of Peripheral Neuropathy in Hepatitis C Infection with and without Cryoglobulineamia

ASMAT ULLAH[1], MIRWAIS KAKAR[2], BILAL AHMED[3], SADIA JABBAR[4], INAYAT ULLAH[5]
[1]*Assistant Professor Gastroenterology, Gambat Institute of Medical Sciences, Khairpur Sindh*
[2]*Senior Registrar Gastroenterology, Bolan Medical Complex Hospital, Quetta*
[3]*Assistant Professor Liver Transplant Unit, Gambat Institute of Medical Sciences Khairpur Sindh*
[4]*Senior Registrar Gastroenterology, Lahore General Hospital, Lahore*
[5]*Senior Registrar Gastroenterology, Bolan Medical Complex Hospital, Quetta*
Corresponding author: Dr Asmat Ullah, Email: asmat.shahwani9@gmail.com, Cell No: 03234998550

ABSTRACT

Objective: The purpose of this study was to find out the association of peripheral neuropathy in hepatitis C infection with and without cryoglobulineamia.

Study Design: Cross sectional study

Place and Duration: Conducted in Liver Transplant Unit, Gambat Institute of Medical Sciences, Gambat Khairpur Mirs, Sindh for the duration of six months from November 2020 to April 2021.

Methods: Total 50 patients who had hepatitis C infection and peripheral neuropathy were included in this study. Patients were aged between 18- 60 years. Detailed demographics of patients including age, sex and body mass index were recorded after taking informed written consent. When symptoms and evidence of peripheral sensory or motor involvement were evident, clinical neuropathy was diagnosed. Sural nerve biopsy was done on patients and the biopsy specimen was evaluated morphologically and morphometrically. Multiple neuropathy, cranial neuropathy, and polyneuropathy are all terms used to describe peripheral nerve involvement. Our research focused on the motor conduction of the median, ulnar, and common peroneal nerves, measuring MCV, CMAP amplitude, and distal latency (DL) in both patients with and without cryoglobulinaemia for each nerve. The SPSS 20.0 version was used to analyze the data.

Results: Mean age of the patients was 46.23±9.87 years with mean BMI 29.16±11.27 kg/m^2. There were 30 (60%) females and 20 (40%) were males. We found that 35 (70%) patients had CG involvement with peripheral neuropathy and 15 (30%) cases were without CG. Prevalence of polyneuropathy was higher 19 (54.3%) in CG patients as compared to non CG 2 (13.3%). Mononeuropathy or multiple neuropathy was higher in HCV CG patients 13 (37.1%) as compared to HCV non CG patients 4 (26.7%). 25 patients underwent nerve biopsy (20 CG patients and 5 non CG). Prevalence of epineurial vasculitis and fascicular loss of axons was higher in non CG patients while demyelination + axonal degeneration were prevalent in CG patients. MCV of the deep peroneal nerve in patients with CG+ was low as compared to CG. Even though no statistically significant differences were detected, the other neurophysiological measures pointed to a more extensive and severe involvement of peripheral nerve in CG+ patients.

Conclusion: We concluded in this study that the association of peripheral neuropathy in HCV patients with cryoglobulinaemia was greater as compared to non-CG HCV patients. It appears that both CG+ and CG patients suffer from peripheral nerve injury via a vasculitic mechanism, as evidenced by clinical and morphological observations. Serum CG levels indicate a more severe and broad neuropathic involvement, however research suggests that cryoglobulins are not the only element in the vasculitic process.

Keywords: Cryoglobulinemia, Peripheral Neuropathy, HCV

INTRODUCTION

This virus can be transferred via parenteral means and infects the liver as well as the lymph nodes. Cirrhosis, hepatocellular carcinoma, and chronic hepatitis are all directly linked to HCV infection in roughly 170 million persons around the world [1]. Extrahepatic symptoms may include mixed cryoglobulinemia, lymphoproliferation, and membranoproliferative glomerulonephritis, as well as sicca syndrome, porphyria cutanea tarda (a skin disease), thyroiditis, and peripheral neuropathy (PN). [2] In addition to subacute, distal sensory-motor polyneuropathy usually associated with CG, mono- and multiple-neuritis have also been described. [3-5]

Inflammatory vascular lesions linked with HCV are thought to be caused by vascular deposition of HCV RNA carrying Cg[6,7] or by perivascular mononuclear inflammation cells[8,9]. The pathophysiology of HCV-related PN is still largely unclear. Rather than a direct nerve infection and in situ replication, HCV neuropathy is most likely caused by virus-triggered immune mediated mechanisms. [10] Only a few papers have looked at the prevalence of PN in an unselected HCV population to better understand the clinical and electrophysiological spectrum of HCV-associated PN. [11,12]

Many EHMs are immunologic/rheumatologic in nature, as a result of B-cell proliferation with subsequent generation of monoclonal and polyclonal autoantibodies displaying rheumatoid factor activity or cryoglobulin features [13]. This includes a number of neurological disorders. According to findings from quasispecies analysis and the discovery of replicative intermediate forms of the human cytomegalovirus (HCV) RNA and viral proteins in the brain, the central nervous system (CNS) may be a permissive region for viral reproduction. Circulating inflammatory cytokines and chemokines reaching brain tissues via altered blood-brain barrier locations may have

additional pathways contributing to neurological impairment. As many as 50% of HCV-infected patients have cryoglobulinemia, the most common and best-studied EHM of HCV infection, causing symptoms in almost 15% of cases. These cold-precipitable immunoglobulins, known as cryoglobulins (CGs), cause inflammation and blood vessel blockage after vascular deposition.[14]

Chronic infections, autoimmune illnesses, and lymphoproliferative diseases all have CG as a component [15]. Patients with chronic HCV infection or primary Sjögren syndrome often have type I CG, which is monoclonal Ig (10–15 percent of the time). Patients with lymphoproliferative disorders, chronic infections, and autoimmune diseases, on the other hand, are more likely to have type II CG, which is a mixture of monoclonal Ig rheumatoid factor and polyclonal IgG (50–60 percent). Type II and III CG, also known as "mixed CG" (MC), are nearly always the result of long-term HCV/HIV infection. Lymphocytic microvasculitis and/or necrotizing arteritis are secondary mechanisms that cause CG-induced ischemic tissue damage. This results in transmural fibrinoid necrosis and polymorphonuclear cell infiltration. Skin purpura, arthritis, peripheral neuropathy, and membranous proliferative glomerulonephritis are common symptoms of CG [15, 17].

Most individuals with CG develop peripheral neuropathy within the first few months after diagnosis, with CNS involvement occurring in just about 6% of cases. Carotid plaque development, hepatic fibrosis, and liver steatosis are also possible side effects of CGs in addition to vascular damage. NHL, diabetes, thyroid disorders, and rheumatological diseases are less common EHMs of HCV infection that provide a risk of brain involvement. Importantly, antiviral treatment can improve or even resolve most EHMs, particularly in those who have had a persistent virological response to the virus.

We looked at a group of 50 HCV neuropathic patients in order to identify the prevalence of CG and to better understand the pathogenetic process by which HCV causes PN.

MATERIAL AND METHODS

This cross sectional study was conducted at Liver Transplant Unit, Gambat Institute of Medical Sciences, Gambat Khairpur Mirs, Sindh for the duration of six months from November 2020 to April 2021. The study consisted of 50 HCV patients. Demographical baseline details of patients including age, sex and body mass index were recorded after taking informed written consent. Patients who had diabetes mellitus, renal failure, thyroid disorder, alcohol abuse, neoplasm including hepatocellular carcinoma, toxicity and those who did not give any written consent were excluded from this study.

Patients were aged between 18-60 years. Median, ulnar, peroneal, and sural nerves were explored in all patients and distal symmetric polyneuropathy was diagnosed when all explored nerves or both lower limb nerves were affected. When symptoms and evidence of peripheral sensory or motor involvement were evident, clinical neuropathy was diagnosed. Sural nerve biopsy was done on patients, and the biopsy specimen was evaluated morphologically and morphometrically. Multiple neuropathy, cranial neuropathy, and polyneuropathy are all terms used to describe peripheral nerve involvement. Using an enzyme linked immunosorbent assay and a more specific recombinant immunoblot assay, all of the patients tested positive for anti-HCV serum antibodies. Cryoglobulins were detected and characterized in laboratory studies that included regular blood testing, immunological assays (the latex test for IgM rheumatoid factor, C4 values, and autoantibodies). 1 Cryoglobulin determined by a minimum of three measurements were made during the course of the investigation.

Standard electromyographic equipment was used by a single operator to conduct electrodiagnostic examinations. All patients had their sensory conduction along the median and ulnar nerves examined. Scanners measured the speed of sensory conduction (SCV) and the magnitude of sensory action potentials (SAPs) in each nerve. The magnitude of low-amplitude sensory responses was calculated via computer averaging. A motor conduction study was conducted on nerves of the median and ulnar, as well as the common peroneal, to determine the speed at which the nerves conduct electrical impulses (DL). F waves are used to detect changes in the proximal conduction. We'll go with one with the shortest F wave latency. SPSS 20.0 version was used to analyze the complete data.

RESULTS

We found that 35 (70%) patients had CG involvement with peripheral neuropathy and 15 (30%) cases were without CG. Mean age of the patients was 46.23±9.87 years with mean BMI 29.16±11.27 kg/m^2. There were 30 (60%) females and 20 (40%) were males. There was no any significant difference in age and BMI in CG and non CG patients. Prevalence of polyneuropathy was higher 19 (54.3%) in CG patients as compared to non CG 2 (13.3%). Mononeuropathy or multiple neuropathy was higher in HCV CG patients 13 (37.1%) as compared to HCV non CG patients 4 (26.7%) while cranial neuropathy and polyneuropathy + cranial neuropathy was prevalent in non CG patients (40% and 20%) and in CG patients (5.7% and 2.9%). HCV CG+ patients showed significantly higher proportion of rheumatoid factor positivity and low C4 levels (p=0.003).(Table 1)

Table 1: Demographically details with clinical and laboratory data

Variables	CG patients	Non- CG patients
Frequency	35 (70%)	15 (30%)
Mean age (years)	46.23±9.87	46.02±7.23
Mean BMI (kg/m^2)	29.16±11.27	29.13±8.22
Gender		
Male	15 (30%)	5 (10%)
Female	20 (40%)	10 (20%)
Clinical data		
polyneuropathy	19 (54.3%)	2 (13.3%)
multiple neuropathy	13 (37.1%)	4 (26.7%)
cranial neuropathy	2 (5.7%)	6 (40%)
polyneuropathy + cranial neuropathy	1 (2.9%)	3 (20%)
Laboratory data		
Low C4 levels	32 (91.4%)	3 (20%)
Rheumatoid factor positive	30 (85.7%)	8 (53.3%)
Transaminase activity increase	24 (68.6%)	7 (46.7%)

25 patients had undergone nerve biopsy (20 CG patients and 5 non CG). Prevalence of epineurial vasculitis and fascicular loss of axons was higher in non CG patients while demyelination + axonal degeneration were prevalent in CG patients. (Table 2)

Table 2: Association of morphological data (frequency of biopsies)

Variables	CG patients (n=35)	Non- CG patients (n=15)
Biopsy		
Yes	20 (57.1%)	5 (33.3%)
No	15 (42.9%)	10 (66.7%)
Morphological data		
Epineurial vasculitis	15 (42.8%)	9 (53.3%)
Differential fascicular loss of axons	12 (34.3%)	6 (40%)
Demyelination + axonal degeneration	8 (22.9%)	1 (6.7%)

MCV of the deep peroneal nerve in patients with CG+ was low as compared to non CG. Even though no statistically significant differences were detected, the other neurophysiological measures pointed to a more extensive and severe involvement of peripheral nerve in CG+ patients. (Table 3)

Table 3: Sensory and motor nerve conduction in all patients

Variables	CG patients	Non- CG patients
Deep peroneal nerve		
MCV (m/sec)	44.23±9.87	49.18±8.78
DL (m/sec)	7.5±3.8	7.2±3.8
Amp (mV)	6.4±3.4	8.6±3.2
F wave (m/sec)	45.12±7.66	41.16±7.33
Median nerve		
MCV (m/sec)	54.21±5.43	54.20±5.20
DL (m/sec)	5.3±1.2	5.11±1.6
Amp (mV)	19.3±7.34	13.6±4.71
Ulnar nerve		
MCV (m/sec)	56.9±8.45	57.17±3.44
DL (m/sec)	3.2±4.5	3.0±5.4
Amp (mV)	15.12±6.25	15.97±6.31
F wave (m/sec)	25.32±3.22	24.88±3.45
Sural nerve		
SCV (m/sec	44.1±8.11	45.3±5.32
SAP ampl (mcV)	7.13±3.17	11.23±2.43
Median nerve		
W-F SCV (m/sec	52.4±8.4	51.6±9.5
SAP ampl. (mcV)	30.7±4.8	35.2±6.2
E-W SCV (m/sec)	56.3±3.7	59.3±2.9
SAP ampl (mcV)	11.1±4.8	15.3±6.4
Ulnar nerve		
SCV (m/sec)	54.1±4.7	53.2±6.2
SAP ampl (mcV)	31.4±9.3	39.3±8.5

We observed a substantial fibre loss in CG+ compared with CG− patients ($p < 0.005$, t test), as well as in percentage of big myelinated fibres ($p < 0.005$, t test). Total number of clusters, signifying regenerated fibres, was significantly enhanced in CG− compared with CG+ individuals. (Table 4)

Table 4: Association of sural nerve biopsy specimens (histometry)

Variables	CG patients	Non- CG patients
Fibre density (n/mm2)	3.322±4.721	5.265±3.876
Fibres >8 µm (%)	10.8±3.12	22.4±6.14
number of clusters	20.5±3.65	87.9±3.33

DISCUSSION

In HCV-infected patients, the PNS is damaged in a variety of ways, mostly because of the presence and type of CG, as well as other co-morbidities and iatrogenic variables that are present. Since type I CG with HCV involvement is extremely rare, the pathogenesis is still a mystery. However, our observations are in line with axonal polyneuropathy, which is characterized by perivascular infiltrates, endoneurial purpura, and microangiopathy. These findings suggest an ischemic pathogenesis linked to endoneurial microcirculation obstruction [18].

In this cross-sectional study 50 patients of both genders were presented. Mean age of the patients was 46.23±9.87 years with mean BMI 29.16±11.27 kg/m^2. Majority of the patients 30 (60%) were females and 20 (40%) were males. We found that 35 (70%) patients had CG involvement with peripheral neuropathy and 15 (30%) cases were without CG. These findings were comparable to the previous studies.[19,20] Prevalence of polyneuropathy was higher 19 (54.3%) in CG patients as compared to non CG 2 (13.3%). Mononeuropathy or multiple neuropathy was higher in HCV CG patients 13 (37.1%) as compared to HCV non CG patients 4 (26.7%) while cranial neuropathy and polyneuropathy + cranial neuropathy was prevalent in non CG patients (40% and 20%) and in CG patients (5.7% and 2.9%). HCV CG+ patients showed significantly higher proportion of rheumatoid factor positivity and low C4 levels ($p=0.003$).[19,21] In addition, Lidove found that a large percentage of HCV CG patients had mono- or multiple-system neuropathy in his small series (three of four patients). Even in HCV CG patients with clinical mono or multiple neuropathy, electrophysiological study revealed a greater than predicted involvement of the peripheral nervous system. [22] These findings imply that the same pathogenic process of nerve destruction causes variable degrees of damage depending on whether the patient has HCV CG or HCV CG+. According to laboratory findings, HCV CG+ patients had higher RF+ and lower C4 levels than HCV CG patients. These findings confirm prior literature and indicate that complement activation and consumption are likely to occur as a result of immunocomplex formation.[23] When compared to these patients, the vast majority of HCV-CG ones showed no evidence of RF positivity and normal C4 readings, including the four in Lidove's study.

In our study 25 patients underwent nerve biopsy (20 CG patients and 5 non CG). Prevalence of epineurial vasculitis and fascicular loss of axons was higher in non CG patients while demyelination + axonal degeneration were prevalent in CG patients. HCV CG+ and CG patients had pathological signs of a vasculitic process, according to our findings. Neuropathy can be caused by ischaemia if there is either epineurial vasculitis or fascicular axonal loss.[22,24] MCV of the deep peroneal nerve in patients with CG+ was low as compared to non CG. Even though no statistically significant differences were detected, the other neurophysiological measures pointed to a more extensive and severe involvement of peripheral nerve in CG+ patients. We observed a substantial fibre loss in CG+ compared with CG− patients ($p < 0.005$, t test), as well as in percentage of big myelinated fibres ($p < 0.005$, t test). Total number of clusters, signifying regenerated fibres, was significantly enhanced in CG− compared with CG+ individuals. Antibodies called cryoglobulins can activate the complement system, resulting in vasculitis. With regards to

the pathophysiology of HCV CG neuropathy's vasculitic process, there are three possible mechanisms for activating the complement: one is caused by the virus' ability to activate the complement pathway; another is based on the reactivity of natural killer cells against viral proteins; and the third is based on the existence of an interaction between HCV and anti-HCV antibodies. The presence of CG in the serum isn't necessary for the development of vasculitis due to immunocomplex deposition. Natural killer cells are known to be inhibited by the primary HCV envelope protein.[25,26]

To summarize, we found that individuals with HCV CG+ had more severe impairments than those with CG, as measured by clinical, electrophysiological and histometrical studies. Clinical and anatomical evidence suggests that the cause of peripheral nerve injury is vasculitic in CG+ and CG patients. When CG is found in the blood, it indicates a more serious and extensive neuropathy, but there is some evidence to suggest that cryoglobulins are not the only cause in the vasculitic reaction.

CONCLUSION

We concluded in this study that the association of peripheral neuropathy in HCV patients with cryoglobulinaemia was greater as compared to non-CG HCV patients. It appears that both CG+ and CG patients suffer from peripheral nerve injury via a vasculitic mechanism, as evidenced by clinical and morphological observations. Serum CG levels indicate a more severe and broad neuropathic involvement, however research suggests that cryoglobulins are not the only element in the vasculitic process.

REFERENCE

1. Lauer G M, Walker B D. Hepatitis C virus infection. *N Engl J Med* 200134541–52.
2. Gumber S C, Chopra S. Hepatitis C: a multifaceted disease. Review of extrahepatic manifestations. *Ann Intern Med* 1995123615–620.
3. Apartis E, Leger J M, Musset L.*et al* Peripheral neuropathy associated with essential mixed cryoglobulinaemia: a role for hepatitis C virus infection? *J Neurol Neurosurg Psychiatry* 199660661–666.
4. Lidove O, Cacoub P, Maisonobe T.*et al* Hepatitis C virus infection with peripheral neuropathy is not always associated with cryoglobulinaemia. *Ann Rheum Dis* 200160290–292.
5. Nemni R, Sanvito L, Quattrini A.*et al* Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. *J Neurol Neurosurg Psychiatry* 2003741267–1271.
6. Agnello V, Chung R T, Kaplan L M. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 19923271490–1495.
7. Agnello V, Abel G. Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. *Arthritis Rheum* 1997402007–2015.
8. Bonetti B, Scardoni M, Monaco S.*et al* Hepatitis C virus infection of peripheral nerves in type II cryoglobulinaemia. *Virchows Arch* 1999434533–535.
9. Bonetti B, Invernizzi F, Rizzuto N.*et al* T-cell-mediated epineurial vasculitis and humoral-mediated microangiopathy in cryoglobulinemic neuropathy. *J Neuroimmunol* 199773145–154.
10. Authier F J, Bassez G, Payan C.*et al* Detection of genomic viral RNA in nerve and muscle of patients with HCV neuropathy. *Neurology* 200360808–812.
11. Ripault M P, Borderie C, Dumas P.*et al* Peripheral neuropathies and chronic hepatitis C: a frequent association? *Gastroenterol Clin Biol* 199822891–896.
12. Cacoub P, Renou C, Rosenthal E.*et al* Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. *Medicine (Baltimore)* 20007947–56.
13. F. Dammacco, D. Sansonno, C. Piccoli, V. Racanelli, F. P. D'Amore, and G. Lauletta, “The lymphoid system in hepatitis C virus infection: autoimmunity, mixed cryoglobulinemia, and overt B-cell malignancy,” *Seminars in Liver Disease*, vol. 20, no. 2, pp. 143–157, 2000.
14. N. F. Fletcher and J. A. McKeating, “Hepatitis C virus and the brain,” *Journal of Viral Hepatitis*, vol. 19, no. 5, pp. 301–306, 2012.
15. D. Sansonno and F. Dammacco, “Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations,” *Lancet Infectious Diseases*, vol. 5, no. 4, pp. 227–236, 2005.
16. R. Sargur, P. White, and W. Egner, “Cryoglobulin evaluation: best practice?” *Annals of Clinical Biochemistry*, vol. 47, no. 1, pp. 8–16, 2010.
17. S. J. Robbins, A. A. M. Shaheen, and R. P. Myers, “Immunologic complications of the hepatitis C virus,” *Current Hepatitis Reports*, vol. 6, no. 4, pp. 138–144, 2007.
18. alvatore Monaco, Sergio Ferrari, Alberto Gajofatto, Gianluigi Zanusso, Sara Mariotto, "HCV-Related Nervous System Disorders", *Journal of Immunology Research*, vol. 2012, Article ID 236148, 9 pages, 2012.
19. R Nemni, L Sanvito, A Quattrini, G Santuccio, M Camerlingo, N Canal. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. J Neurol Neurosurg Psychiatry 2003;74:1267–1271
20. Santoro L, Manganelli F, Briani C, et al. Prevalence and characteristics of peripheral neuropathy in hepatitis C virus population. *J Neurol Neurosurg Psychiatry*. 2006;77(5):626-629. doi:10.1136/jnnp.2005.081570
21. Nemni R, Sanvito L, Quattrini A*, et al* Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia *Journal of Neurology, Neurosurgery & Psychiatry* 2003;74:1267-1271.
22. Lidove O, Cacoub P, Maisonobe T, et al. Hepatitis C virus infection with peripheral neuropathy is not always associated with cryoglobulinaemia. Ann Rheum Dis 2001;60:290–2.
23. Trejo O, Ramos-Casals M, Garcìa-Carrasco M, et al. Cryoglobulinemia. Study of etiologic factors and clinical and immunologic features in 443 patients from a single center. Medicine 2001;80:252–62
24. Paoletti V, Donnarumma L, De Matteis A, Mammarella A, Labbadia G, Musca A, Francia A. Peripheral neuropathy without cryoglobulinemia in patients with hepatitis C virus infection. Panminerva Med. 2000 Sep;42(3):175-8.
25. P. Vermeersch, K. Gijbels, G. Mariën, R. Lunn, W. Egner, P. White, *et al.* A critical appraisal of current practice in the detection, analysis, and reporting of cryoglobulins. Clin Chem, 54 (2008), pp. 39-43
26. ManalAly Abdel Khalek[a]Amal MohamadEl-barbary[a]Ferial SalahElkalla[b]Salwa Abdel-MoneimEssa[c]. Prevalence of peripheral neuropathy in Egyptian hepatitis C virus patients: Correlation to some clinical and laboratory parameters. Received 24 January 2012, Accepted 2 April 2012, Available online 19 May 2012.