Al-Azhar Med. J .(Medicine). *Vol. 50 (1), January, 2021, 719 - 732*
DOI: 10.12816/amj.2021.139866
https://amj.journals.ekb.eg/article_139866.html

IMPACT OF TREATMENT WITH DIRECT ACTING ANTIVIRAL AGENTS (DAAS) ON MIXED CRYOGLOBULINEMIA OF HCV EGYPTIAN PATIENTS

By

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ABSTRACT

Background: Up to 74% of patients affected by HCV have HCV-related extrahepatic manifestation (EHMs) including autoimmune diseases, mixed cryoglobulinemia (MC) and non-Hodgkin lymphomas (NHL).Objective: To study the impact of treatment with direct acting antiviral agents (DAAS) on mixed cryoglobulinemia of HCV Egyptian patients.

Patients and methods: This study was carried out in cooperation between the Hepato-gastroenterology and Infectious Diseases Department, Al-Azhar University, Cairo, and Shebin Elkom Fever Hospital from March 2018 to December 2019. The study included 80 patients with HCV-related EHMs including cutaneous (30 patients), musculoskeletal (30 patients) and renal manifestation (20 patients). All patients were naïve to treatment with DAAs (Sofosbuvir/Daclatasvir ± Ribavirin regimen).

Results: Purpuric papules of the lower extremities were the most frequent cutaneous lesion (80%), and arthralgia was the most frequent in musculoskeletal manifestations (90%). Diffuse membranoproliferative glomerulonephritis was the most frequent lesion in patients, with renal manifestations (90%). Significant increase occurred in the serum cryoglobulin levels and RF, with significant decreasein C3 and C4 serum levels in all groups. There were significant decrease in the mean cryoglobulin levels from positive to negative, with significant decline in RF concentrations, and rise in C3 and C4 serum levels approaching the normal values. There were improvements in the presenting HCV-MC clinical manifestations in variable degrees, rangingfrom 71.42% in patients with glomerulonephritis to 98.4% in patients with purpura. Eighty-seven percent of the included patients showed complete response (clinical, virologicaland immunological recovery), and 13% showed partial response (virological and immunological recovery without clinical improvement of cryoglobulinemia associated manifestations).

Conclusion: DAAs, in the form of Sofosbuvir/Daclatasvir ± Ribavirin regimen, were treatment of choice for HCV infection, and considered in cases of HCV-MC as they were associated with clinical improvement in most patients.

Keywords: Directly acting antiviral drugs, mixed cryoglobulinemia, HCV.

INTRODUCTION

Mixed cryoglobulinemia (MC) vasculitis (Cryovas) is a small vessel vasculitis involving mainly the skin, the joints, the peripheral nerve system and the kidneys. HCV infection is the cause of Cryovas in about 80% of cases. The disease expression is variable, ranging from mild symptoms (purpura, arthralgia)

to fulminant life-threatening complications (glomerulonephritis, widespread vasculitis). Skin is the most frequently involved target organ: palpable purpura, chronic cutaneous ulcers, Raynaud's phenomenon, acrocyanosis, which may evolve to digital ulcerations *(Terrier and Cacoub, 2013)*.

Pegylated interferon alfa (INF-alfa) combined with ribavirin has demonstrated efficacy in patients with cryoglobulinemia associated with HCV infection, and efficacy in patients with chronic myelogenous leukemias and low-grade lymphomas has been reported. The details of therapy and the recommended approach vary based on the clinical setting and expert opinion should be sought *(Mazzaro et al., 2011)*.

Treatment with direct-acting. antivirals not only cures people of hepatitis C, but can also rapidly reduce the severity of one of the most troublesome extra hepatic manifestations of the disease, Although studies of direct-acting antivirals show that newly-licensed combinations can cure hepatitis C in 90 to 95% of people, there is less information about the extent to which curing hepatitis C leads to improvements in the health of the liver or resolution of symptoms such as cryoglobulinemia *(Ramos-Casals et al., 2017)*.

The present study aimed to study the impact of treatment with direct acting antiviral agents (DAAS) on mixed cryoglobulinemia of HCV Egyptian patients.

PATIENTS AND METHODS

This study was carried out in cooperation between the Hepato-Gastroenterology and Infectious Diseases Department, Al-Azhar University, Cairo, and Shebin Elkom Fever Hospital after the approval of the ethical committee and obtaining consent from all the enrolled patients. The study period was from March 2018 to December 2019. Six hundred thirty patients with chronic hepatitis C (CHC) were clinically examined and investigated for variable manifestations of mixed cryoglobulinemia related HCV. CHC was diagnosed by laboratory test (PCR quantitative for HCV-RNA). The inclusion criteria was evidence of HCV infection in the form of a positive test for anti-HCV antibodies and confirmed by PCR for HCV RNA, age from 18-75 years and systemic disorders in which cutaneous vasculitis, arthralgia or glomerulonephritis associated HCV infection. All indications of treatment of HCV patients according to the Egyptian protocol of therapy. We excluded in this study conditions that might be associated with secondary cryoglobulinemia such as hematologic tumors, autoimmune disorders, and acute or chronic infectious disease not related to HCV, serious medical illness other than liver disease that might preclude completion of the study as hepatic failure, characterized by a history of ascites, hepatic encephalopathy and bleeding esophageal varices and pregnancy or lactating women. Only 230 patients had one or more than manifestation of MC, and were tested for cryoglobulin levels in their serum. Eighty patients were positive for cryoglobulin levels, and were enrolled to complete our study. Full history taking and thorough clinical examination was done with stress on the manifestations of chronic liver illness and special emphasis

on musculoskeletal, renal symptoms and skin lesions.

Eighty patients enrolled in the study were classified into three groups:

Group I: Thirty patients with cutaneous manifestations (ulcer, erythematous papules, purpura, hemorrhagic crusts, oral lichen planus and chronic urticaria). Skin punch biopsy was done for all patients.

Group II: Thirty patients with musculoskeletal manifestations. Only arthralgia and myalgia were observed in our study.

Group III: Twenty patients with renal manifestations as hematuria and proteinuria were put in this group. Renal biopsy for histopathological and immunohistochemical examination was done for all patients.

After taking written consents from all included patients, full history taking and thorough clinical examination was done with stress on the manifestations of chronic liver illness and special emphasis on musculoskeletal, renal symptoms and skin lesions.

- Eight milliliter of venous blood was collected from each patient and control under aseptic precautions, of which 2 mL of blood was placed in EDTA containing a vacutainer tube to obtain a complete blood picture in the automated hematology analyzer cell dyne-1800 (Abbott Diagnostics, Lake Forest, IL, USA). One milliliter was used for ESR test and five milliliters of blood was taken in a plain glass tube, and after clotting, the tube was centrifuged at 3,000 rpm for 5 min. Then, the serum was separated for use in estimating liver function (including liver enzymes, albumin, bilirubin-total and direct, prothrombin time, prothrombin concentration, and INR), serum urea, serum creatinine, serum alfa fetoprotein, serum cholesterol, fasting blood sugar and RF using Cobas c311 automated chemistry analyzer (Roche Diagnostics, Mannheim, Germany). HBs Ag, HCV-RNA levels, cryoglobulins, serum ANA and complement 3 (C3) and complement 4 (C4) serum assays were done. eGFR was calculated using the Mayo Clinic Quadratic Equation and urine analysis was done for the included patients.
- RF was measured using an immunoturbidimetric assay kit based on the immunological agglutination principle with enhancement of the reaction by latex, supplied by Roche Diagnostics, catalog No. c501/502. The normal reference range is 0–20 IU/mL.
- HBs Ag testing were performed using commercially available assays (Abbott Laboratories, Abbot Park, IL, USA).
- HCV-RNA was detected by a PCR assay (Cobas Amplicor Roche Molecular Systems Inc., Branchburg, NJ, USA), and serum HCV-RNA levels were quantified by the Cobas Ampli Prep/Cobas TaqMan HCV-RNA assay (Roche Diagnostics) with a lower detection limit at 15 IU/mL.
- The biochemical assessments of ANA, C3 and C4 were done using commercially available enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol; these kits were supplied by Chongqing Biospes Co., Ltd., Chongqing, People's Republic of

China, catalog no. (BYEK2304, BYEK1247, BYEK1247), respectively, using ELISA Multiskan EX Microplate Photometer [STAT FAX-2100; Thermo Scientific, Waltham, MA, USA]. The normal reference ranges for ANA, C3 and C4 are 0-1.5 U/ml, 90–180 and 10–40 mg/dL, respectively.

- Abdominal ultrasound was done for all enrolled patients.
- All the previously mentioned investigatory workups, in addition to the evaluation of the clinical manifestations of MC, were done to the included patients before starting therapy and 12 weeks after end of treatment.

The severity of the skin involvement was determined as follows: a score of 0 indicated the absence of skin lesions. A score of 1, the presence of less than 10 purpura spots on the lower legs. A score of 2, the presence of more than 10 spots on the lower legs. A score of 3, the extension of the spots to the upper leg and/or the abdomen and a score of 4, the presence of skin ulcers and/or gangrene.

To assess the severity of the arthralgia a clinical score was used: 0 indicated no arthralgia, 1 for occasional arthralgia, 2 for continuous arthralgia and 3 for intense arthralgia with impairment of movement.

In patients with renal involvement, the complete response was defined as the return to the normal level of the serum creatinine associated with the disappearance of proteinuria. A partial response was defined as the decrease of creatinine and proteinuria by more than 50% and a minimal response as the decrease of creatinine and of proteinuria by less than 50%. The decrease of proteinuria by less than 10% was considered as no response.

All patients were diagnosed and treated with sofosbuvir 400 mg and daclatasvir 60 mg ± ribavirin (800–1,200 mg) once daily for 3 months. Patients were treated according to the Egyptian protocol for treatment of chronic HCV virus infection, which depends on the European Association for the Study of the Liver 2018 guidelines for HCV infection treatment.

The judging points regarding the efficacy of sofosbuvir and daclatasvir in improving the condition of HCV-MC patients were as follows:

i. Clinical improvement of the presenting cryglobulinemic related manifestations,
ii. SVR, which is defined as undetectable HCV-RNA levels 12 weeks after end of treatment and,
iii. Disappearance of cryoglobulins in serum. According to the presence or absence of the 3 previously mentioned criteria, the effect of the used DAAs can be classified into 3 types: complete response if all 3 parameters have been achieved, partial response if SVR with either of the remaining 2, and no response if none of the 3 parameters have been achieved.

Statistical methods:

Data Management and Analysis: The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for the Social Sciences (SPSS 20). Data was presented and suitable analysis was done according to

the type of data obtained for each parameter.

Descriptive statistics:

Mean, Standard deviation (± SD) for numerical data, frequency and percentage of non-numerical data. Analytical statistics:

1 Paired t-test was used to assess the statistical significance of the difference between two means measured twice for the same study group.

2. McNemar test was used assess the statistical significance of the difference between a qualitative variable measured twice for the same study group.

3. Wilcoxon Signed Rank test: non-parametric test was used to compare repeated measurements for the name group when the data are non-parametric.

4. One-way ANOVA should be used to compare more than 2 measurements.

5. Chi-square test was used to compare quantitative data. $P \leq 0.05$ was considered significant.

RESULTS

The study was completed on 80 patients who proved to have MC by testing positive for serum cryoglobulins. The mean age of the included HCV-MC patients was 54.2 years ±8.4 SD; 44 (55%) of them were females **(Table 1)**.

Table (1): Age and gender distribution in all groups

Parameters \ Groups		Cutaneous (N=30)		Musculoskeletal (N=30)		Renal (N=20)		P-value
		Mean±SD		Mean±SD		Mean±SD		
Age		55.07±8.56		51.43±8.[33]		52.15±6.65		0.195
		N	%	N	%	N	%	
Gender	Female	20	66.7%	13	43.3%	11	55.0%	0.192
	Male	10	33.3%	17	56.7%	9	45.0%	

N = Number, SD = Standard deviation.

From the relative frequencies of the presenting clinical manifestations purpuric papules of the lower extremities was the most frequent cutaneous lesion 24/30 (80%) and arthralgia was the most frequent in musculoskeletal manifestations 27/30 (90%) and Diffuse Membranoproliferative glomerulonephritis was the most frequent lesions in patients with renal manifestations 18/20 (90%).Regarding the clinical effects of sofosbuvir and daclatasvir therapy on HCV-MC, the patients with cutaneous manifestations showed significant improvement in their clinical manifestations, 50% of patients with purpuric papules of the lower extremities, 100% of patients with hemorrhagic crusts and/or ulcers, lichen planus and Chronic urticaria. Also,

patients with musculoskeletal manifestations showed significant improvement in their clinical manifestations, 74% and 67% in patients with arthralgia and myalgia respectively.Regarding patients with cutaneous manifestations, leukocytoclastic vasculitis with cryoglobulin deposits was the most frequent pathological manifestation (70%), lymphocytic infiltrate (26.7%) and urticarial vasculitis (3.3%), and all had significant improvement after treatment with sofosbuvir and daclatasvir **(Table 2)**.

Table (2): Clinical presentations, frequency of affected patients and frequency of improved patients in cutaneous and musculoskeletal manifestations

Clinical presentations \ Frequency		Affected patients (% of all group patients)	Improved patients (% of affected patients with the same sign)	P
Cutaneous manifestations	purpuric papules of the lower extremities	24 (80%)	12 (50%)	0.02
	hemorrhagic crusts, and/or ulcers	3 (10%)	3 (100 %)	0.001
	lichen planus	1 (3.33%)	1 (100%)	0.001
	Chronic urticarial	2 (6.66%)	2 (100%)	0.001
	Total	30 (100%)	18 (60%)	0.001
Musculoskeletal manifestations	Arthralgia	27 (90%)	20 (74%)	0.001
	myalgia	3 (10%)	2 (67%)	0.001
	Total	30 (100%)	22 (73%)	0.001

According to pathological presentations in patients with renal manifestations, diffuse membranoproliferative glomerulonephritis was improved on only 16.6% of affected patients with no significant difference before and after treatment **(Table 3)**.

Table (3): Pathological presentations, frequency of affected patients and frequency of improved patients with cutaneous and renal manifestations

Pathological presentations \ Frequency		Affected patients (% of all group patients)	Improved patients (% of affected patients with same pathology)	P
Cutaneous manifestations	Leukocytoclastic vasculitis with cryoglobulin deposits	21 (70%)	12 (57%)	0.001
	Lymphocytic infiltrate	8 (26.7%)	4 (50%)	0.001
	Urticarial vasculitis	1 (3.3%)	1 (100%)	0.001
	Total	30 (100%)	17 (57%)	0.001
Renal manifestations	Diffuse Membranoproliferative glomerulonephritis	18 (90%)	3 (16.6%)	0.191

	Tubulo-interstitial nephritis	1 (5%)	0	> 0.05
	Focal and segmental glomerulosclerosis	1 (5%)	0	> 0.05
	Total	20 (100%)	3 (15%)	0.251

There was significant difference regarding response rates in all patients with mixed cryoglobulinemia following treatment with sofosbuvir and daclatasvir. In cutaneous manifestation group, 18 (60%), 99 (30%), and 3 (10%) patients had complete response, partial response and sustained virological response (SVR) only respectively. In musculoskeletal manifestation group, 22 (73.33%), 6 (20%) and 2 (6.66%) patients had complete response, partial response and only SVR respectively. In group of renal extrahepatic manifestation, the response was complete in 3 (15%) patients, partial in 12 (60%) patients and SVR only in 5 (25%) patients **(Table 4)**.

Table (4): Response rates in all patients with mixed cryoglobulinemia following treatment with DAAs

Groups / Response	Cutaneous group (N=30)	Musculoskeletal group (N=30)	Renal group (N=20)	Total (N=80)	P
Complete	18	22	3	43	< 0.001
Partial	9	6	12	27	
SVR only	3	2	5	10	

SVR = Sustained virological response.

The effect of sofosbuvir and daclatasvir therapy on serum cryoglobulin levels showed that 90% of mixed cryoglobulinimic patients with cutaneous manifestations, 93.3% of patients with musculoskeletal manifestations and 75% patients with renal manifestations turned to negative after treatment. Our study also, showed negativity of serum cryoglobulin in 87.5% of all included patients with extrahepatic manifestations post-therapy with sofosbuvir and daclatasvir **(Table 5)**.

Table (5): Comparison of serum cryoglobulin before and after treatment with DAAs in patients group with cutaneous, musculoskeletal and renal manifestations

Manifestations / Cryoglobulin2			Negative	Positive	Total	P
Cutaneous	Cryoglobulin1	Positive	27 (90%)	3 (10%)	30 (100%)	0.001
Musculoskeletal	Cryoglobulin1	Positive	28 (93.33%)	2 (6.67%)	30 (100%)	0.001
Renal	Cryoglobulin1	Positive	15 (75%)	5 (25%)	20 (100%)	0.001
Whole sample	Cryoglobulin1	Positive	70 (87.5%)	10 (12.5%)	80 (100%)	0.001

Cryoglobulin1 = serum cryoglobulin level before therapy, Cryoglobulin2 = serum cryoglobulin level post-therapy.

Comparison of laboratory investigations done before treatment with DAAs and after treatment in patients group with cutaneous manifestations and musculoskeletal, there was significant difference as regard RF, C4 and ANA with p- value of 0.001 or less. Laboratory measures among HCV-MC with renal manifestations had significant decrease in RF and significant increase in C4 post-therapy with DAAs with p- value of <0.001, but there was non-significant difference regarding ANA post-treatment **(Table 6)**.

Table (6): Data of investigations before and after treatment with DAAs in different groups

Parameters / Groups	Laboratory investigation	Before treatment	After treatment	Wilcoxon Signed Rank test
		Median (IQR)	Median (IQR)	P
Cutaneous	RF	19.1 (17.4 - 22.3)	5.25 (3 - 6)	<0.001
	C4	18.6 (15.6 - 20.9)	26 (21 - 33)	<0.001
	ANA	0.25 (0.1 - 0.4)	0 (0 - 0.3)	0.002
Musculoskeletal	RF	20.8 (16.5 - 24.5)	4.55 (3.5 - 6.3)	<0.001
	C4	20.6 (19.9 - 21.3)	30.8 (27.8 - 34)	<0.001
	ANA	0.2 (0 - 0.3)	0 (0 - 0.2)	0.002
Renal	RF	23.35 (19.8 - 25.1)	5.5 (4.5 - 6.5)	<0.001
	C4	15.7 (7.7 - 19.2)	20.1 (10.8 - 24.5)	<0.001
	ANA	0.3 (0.1 - 0.5)	0 (0 - 0.5)	0.382

RF = rheumatoid factor, C4 = complement 4, ANA = antinuclear antibody, IQR = Interquartile Range.

There was also significant increase in C3 levels from 69.24±6.93 to 129.27±18.93, from 74.07±5.39 to 126.29±17.12 and from 72.68±9.38 to 113.60±18.30 in patients with cutaneous, musculoskeletal and renal manifestations, respectively and significant increase in eGFR in patients with renal manifestations **(Table 7)**.

Table (7): Data of investigations before and after treatment with DAAs in different groups

Parameters / Groups	Laboratory investigation	Before treatment	After treatment	Paired t test
		Mean±SD	Mean±SD	P
Cutaneous	C3	69.24±6.93	129.27±18.93	0.001
Musculoskeletal	C3	74.07±5.39	126.29±17.12	0.001
Renal	C3	72.68±9.38	113.60±18.30	0.001
	eGFR	49.70±8.12	65.80±12.45	0.001

C3 = complement 3, eGFR = estimated glomerular filtration rate, SD = Standard deviation.

DISCUSSION

HCV is well known for having high immunogenic capacity, converting HCV infection in a systemic disease. Following the identification of HCV as the largely prevalent etiologic agent of MC, researchers in several studies have evaluated the therapeutic efficacy of antiviral drugs such as IFN-α, with or without ribavirin, in this condition. These

studies consistently reported lower rates of SVR in HCV-positive patients with MC than in those without MC. In addition, IFN-based regimens were characterized by severe adverse events, often leading to therapy discontinuation *(Dammacco and Sansonno, 2013)*. Our study found that HCV patients proven to have MC, showed female predominance in patients with cutaneous and renal manifestations but male predominance in patients with musculoskeletal predominance, and this was in line with *Mohammed and his Colleagues (2010)*, whom reported that cryoglobulinemia was significantly more common in the female population with HCV. Also, there was no significant difference regarding age in all groups.

Serum cryoglobulins were found in approximately 30-40% of HCV patients, with 5 to 30% of these patients manifesting with MCS. The pathophysiology is unclear, with both direct and indirect theories proposed. The direct theory, supported by evidence that HCV is lymphotropic and that the cryoprecipitate contains high concentrations of viral genome, beside that HCV induces B cells to produce immunoglobulins. Alternatively, the indirect theory proposes that the virus provokes MC via chronic immune stimulation *(Cacoub et al., 2016)*. In our study, only 12.7% of HCV patients had positive serum cryoglobulin, the difference may be due to sampling and the race of patients.

In the present study, purpuric papules (80%) were the most frequent sign in MC patients with cutaneous manifestations in agreement with *Dammacco and his Colleagues (2013)* whom reported that Cryoglobulinemic vasculitis was most evident on the skin as palpable purpura, seen in virtually all patients with cryoglobulinemia. Arthralgias were a common complaint of HCV-infected patients in the current study and were reported in 90% of MC patients with musculoskeletal symptoms, these results came in agreement with *Palazzi and his Colleagues (2016)*. Proteinuria was the frequent sign (75%) in MC patients with renal manifestations in our study, and also, some patients manifested with macroscopic hematuria (5%), nephrotic syndrome (10%) and nephritic syndrome (10%). These results were in accordance with the findings of *Cacoub and his Colleagues (2016)* whom reported that renal manifestations cover a variety of disorders ranging from slightly proteinuria and hematuria, to nephrotic and nephritic syndromes, as well as renal insufficiency in a lesser extent in HCV-related MC patients suffering from kidney disease.

Diffuse membranoproliferative glomerulonephritis (MPGN) was the most common pathological finding in MC patients with renal affection 18/20 (90%), in agreement with *Dammacco and his Colleagues*, whom reported that the most common renal manifestations of HCV infection were essential mixed cryoglobulinemia (MC) leading to membranoproliferative glomerulonephritis (MPGN), MPGN without cryoglobulinemia, and membranous glomerulonephritis. In our patients with MC cutaneous manifestations, leukocytoclastic vasculitis (LCV) with cryoglobulin deposits was found in about 70% of patients, lymphocytic infiltrate and urticarial vasculitis in 26.7%, and 3.3% respectively. At SVR12, there was

improvement in 57%, 50% and 100% of patients respectively. These results are in agreement with *Rutledge and his Colleagues (2018),* whom reported that LCV was detected in 50% of skin biopsy specimens in HCV-MC patients.

Because the activity of MC usually correlates with viremia, therapy should be directed toward the potential causal agent. The efficacy and safety of all oral DAAs therapyin HCV-MC are largely unknown, *Gragnani et al. (2015)* and few studies investigated the efficacy of sofosbuvir-based DAA regimens for HCV patients generally and for HCV-MC patients specifically. The results of the current study pertaining to the therapeutic efficacy of the combined use of sofosbuvir and daclatasvir as oral DAAs, revealed that 100% of patients had SVR12 and clearance of cryoglobulins in 87.5% of patients in all groups, with significant improvements in eGFR (in renal manifestations group), CBC, AST, ALT, total bilirubin, direct bilirubin, serum albumin, ESR and AFP in all groups, with 54% of the included patients achieved a complete response, 34% a partial response and 12% had only SVR response. This indicated the higher therapeutic efficacy and safety of sofosbuvir and daclatasvir in managing HCV-MC. In agreement with these findings, *Sise et al. (2016)* reported that 83% of HCV-MC patients showed SVR12 rates for sofosbuvir based DAA regimens. Also, our findings were in line with *Bonacci et al. (2017),* and *Saadoun. et al. (2017)* whom reported an abrupt decay of HCV-RNA with DAAs with a rapid improvement of the clinical manifestations of MC that may allow the reduction or even cessation of the traditional immunosuppressive therapy. *Lauletta and her Colleagues (2017)* reported that at SVR12, cryoglobulins disappeared or decreased by at least 50% in 77.3% of their patients. Another Egyptian study found that the combined use of sofosbuvir and daclatasvir as oral DAAs revealed 100% SVR12 and clearance of cryoglobulins, with significant improvements in Child-Pugh score, liver fibroscan status, hemoglobin levels, platelet counts, liver functions, and eGFR and creatinine levels, with 87% of the included patients showing a complete response and 13% a partial response without any side effects *(Hassan et al., 2018).* Our findings revealed significantly higher RF with significantly lower C3 and C4 serum levels among the included HCV-MC in all groups, with significant improvements in their level following DAAs. In line with our data, *Gragnani et al. (2016)* and *Hassan et al. (2018)* had reported similar findings. On another study, RF and C4 levels are less influenced by DAAs within the short period of 12 weeks after stopping therapy and may represent independent markers not only of clinical activity but also of persistently activated B-cell clones *(Lauletta G. et al., 2017).*

Lauletta and herColleagues (2017) reported that at SVR12, cryoglobulins disappeared or decreased by at least 50% in 77.3% of patients in patients treated with DAAs, serum cryoglobulins disappeared in 90%, 93% and 75% of patients with extrahepatic cutaneous, musculoskeletal and renal manifestations after treatment with DDAs despite SVR12 was 100%. The difference between us and mentioned study may be due to different sampling of patients, genotype, and DAAs regimen used in the treatment.

CONCLUSION

DAAs, in the form of Sofosbuvir/Daclatasvir ± Ribavirin regimen, were a treatment of choice for HCV infection and should be considered in cases of HCV-MC as they were associated with clinical improvement in most patients.

DISCLOSURE

The authors report no conflicts of interest in this work.

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تأثير العلاج بعقاقير مضادات الفيروسات المباشرة على الجلوبيولين البارد المختلط فى المرضى المصريين المصابين بفيروس الإلتهاب الكبدي سي

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خلفيـــة البحـــث: يعـــانى مـــا يصـــل إلـــى 74٪ مـــن المرضـــى المصـــابين بفيـــروس التهـــاب الكبـــد (ســـي) مـــن مظـــاهر خـــارج الكبـــد ذات صـــلة بفيـــروس إلتهـــاب الكبـــد بمـــا فـــي ذلـــك أمـــراض المناعـــة الذاتيـــة، والجلوبيـــولين البـــارد المخـــتلط والأورام اللمفاويـــة غيـــر الهودجكينية.

الهـــدف مـــن البحـــث: دراســـة تـــأثير العـــلاج بعقـــاقير مضـــادات الفيروســـات المباشـــرة علـــى الجلوبيـــولين البـــارد المخـــتلط فـــى المرضـــى المصـــريين المصـــابين بفيـــروس الإلتهاب الكبدي (سي).

المرضـــى وطـــرق البحـــث: اشـــتملت الدراســـة علـــى 80 مريضـــاً مصـــابين بالتهـــاب الكبـــد الوبـــائي (ســـي) ولـــديهم أعـــراض خـــارج الكبـــد، بمـــا فـــي ذلـــك المظـــاهر الجلديـــة (30 مريضـــاً)، وأعـــراض العضـــلات الهيكليـــة (30 مريضـــاً) والمظـــاهر الكلويـــة (20 مريضـــاً). وكـــان جميـــع المرضـــى يعـــالجون لأول مـــرة باســـتخدام عقـــاقير مضـــادات الفيروسات المباشرةنظام سوفوسبوفير/دكلاتاسفير± ريبافيرين.

نتـــائج البحـــث: ظهـــرمن التـــرددات النســـبية للمظـــاهر الســـريرية حطاطـــات فرفريـــة مـــن الأطرافالســـفلية هـــو المـــرض الأكثـــر شـــيوعًا (80 ٪) بـــين المرضـــى المصـــابين بالمظـــاهر الجلديـــة، وكـــان ألـــم المفاصـــل الأكثـــر شـــيوعًا فـــي المرضـــى الـــذين لـــديهم أعـــراض العضـــلات الهيكليـــة (90 ٪)، وكـــان إلتهـــاب كبيبـــات الكلـــى الغشـــائي المنتشـــر هـــو المـــرض الأكثـــر شـــيوعا لـــدى المرضـــى الـــذين يعـــانون مـــن المظـــاهر الكلويـــة (90٪)، وحـــدث زيـــادة ملحوظـــة فـــي مســـتويات الجلوبـــولين البـــارد وعامـــل الروماتويـــد فـــى الـــدم مـــع انخفـــاض كبيـــر فـــي مســـتويات ســـي 3 وســـي 4 فـــي الـــدم فـــي جميـــع المجموعـــات، وأظهـــر المرضـــى المشـــمولين فـــى الدراســـة إنخفاضًـــا كبيـــرًا فـــي متوســـط مســـتويات الجلوبيـــولين البـــارد لـــديهم مـــن إيجـــابي إلـــى ســـلبي، مـــع إنخفـــاض كبيـــر فـــي

تركيزات عامل الروماتويد وأرتفاع في مستويات سي 3 وسي 4 في الدم يقترب من القيم الطبيعية، وكان هناك تحسناً في المظاهر السريرية للمرضى المصابين بأعراض خارج الكبد نتيجة الجلوبيولين البارد المختلط بدرجات متفاوتة تتراوح من 71.42٪ في المرضى الذين يعانون من التهاب كبيبات الكلى الغشائي المنتشر إلى 98.4٪ في المرضى الذين يعانون من فرفرية، كما أظهر 87٪ من المرضى المشمولين إستجابة كاملة (التعافي السريري والفيروسي والمناعي)، كم أن 13٪ أظهروا إستجابة جزئية (التعافي الفيروسي والمناعي دون التحسين السريري للمظاهر المرتبطة بالجليوبولين البارد فى الدم).

الإستنتاج: العلاج بعقاقير مضادات الفيروسات المباشرة، في شكل نظام (سوفوسبوفير/دكلاتاسفير± ريبافيرين)، مفضل لمرضى التهاب الكبد الوبائي (سي)، ويجب أن تؤخذ في الاعتبار في الذين لديهم مظاهر خارج الكبد نتيجة الجلوبيولين البارد المختلط لأنها مرتبطة بالتحسن السريري في معظم المرضى.