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ORIGINAL ARTICLE

Observational Study

Health care associated infections, antibiotic resistance and clinical outcome: A surveillance study from Sanandaj, Iran

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Author contributions: Soltani J, Poorabbas B, Miri N and Mardaneh J contributions to conception and design, acquisition of data, analysis and interpretation of data; Poorabbas B and Mardaneh J acquisition of data, laboratory performances and interpretation of laboratory data; all authors participated in drafting the article and they critically reviewed the manuscript and approved the final manuscript as submitted.

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Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of Professor Alborzi Clinical Microbiology Research Center, Shiraz, Iran. Once again, the study was reviewed and approved by the Institutional Review Board of Research Committee of the Medical Faculty and Research committee of the Kurdistan University of Medical Sciences, Sanandaj, Iran.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors of the paper declare any conflicting interests (including but not limited to commercial, personal, political, intellectual or religious interests) that are related to the work submitted for consideration of publication.

Data sharing statement: The technical annex, statistical code, and dataset are available from the corresponding author at soltanjaf@gmail.com. The participants gave informed consent for the data sharing.

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Abstract

AIM: To study the antibiotic susceptibility patterns of gram-negative healthcare associated bacterial infections at two tertiary hospitals in the Sanandaj city, Kurdistan Province, Iran.

METHODS: From January 2012 to December 2012, all positive cultures from potentially sterile body fluids were gathered. They sent to professor Alborzi clinical microbiology center in Shiraz for further analysis and susceptibility testing. The antibiotic susceptibility was determined using the Kirby-Bauer method (disk diffusion

technique). The Results were interpreted according to Clinical and Laboratory Standards Institute guidelines against a series of antimicrobials. World Health Organization definitions for Healthcare associated infections were followed.

RESULTS: Seven hundred and thirty-two positive cultures were reported from both hospitals. Seventy-nine isolates/patients fulfilled the study criteria for healthcare associated gram-negative infections. The most frequent bacterial cultures were from the pediatric wards (52%). *Serratia marcescens* (*S. marcescens*) (38%) *Escherichia coli* (*E. coli*) (19%), *Klebsiella pneumoniae* (*K. pneumoniae*) (19%), *Acinetobacter baumannii* (6%), *Enterobacter* species (6%), *Serratia odorifera* (4%) and Pseudomonas species (5%) were the most frequently isolated organisms. The susceptibility pattern of common isolates *i.e.*, *S. marcescens*, *E. coli* and *K. pneumoniae* for commonly used antibiotics were as follows: Ampicillin 3.3%, 6.7%, 20%; gentamicin 73.3%, 73.3%, 46.7%; ceftazidim 80%, 73.3%, 33.3%; cefepim 80%, 86.7%, 46.7%; piperacillin/tazobactam 90%, 66.7%, 86.7%; ciprofloxacin 100%, 73.3%, 86.7%; imipenem 100%, 100%, 100%, respectively.

CONCLUSION: The most effective antibiotics against gram-negative healthcare associated infections are imipenem followed by ciprofloxacin. The resistance rate is high against ampicillin and cephalothin. The high mortality rate (46.1%) associated with *S. marcescens* is alarming.

Key words: *Escherichia coli*; *Klebsiella pneumoniae; Serratia marcescens*; Extended-spectrum beta-lactamase; Nosocomial infections; Antibiotic susceptibility

Core tip: To investigate the antibiotic susceptibility patterns of gram-negative healthcare associated bacterial infections a study was conducted in tertiary hospitals in Sanandaj (a large city in the west of Iran). World Health Organization guidelines for hospital-acquired infections were followed. The results were interesting and provided important information concerning antibiotic resistance, making some antibiotics such as cephalothin almost useless. According to our study, gram-negative health care associated infections are challenging especially in pediatric wards. The most effective antibiotics against gram-negative healthcare associated infections were imipenem followed by ciprofloxacin. The high mortality rate (46.1%) associated with *Serratia marcescens* was alarming.

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INTRODUCTION

Serious bacterial infections that are resistant to commonly available antibiotics have become a major worldwide healthcare problem. They are more severe; require significantly more expensive diagnosis and longer and more sophisticated treatments[1].

Knowledge of the prevalence of antibiotic resistance is a pre-requisite for infection control and essential for public healthcare policy makers to conduct effective responses[2]. Currently, a well-organized nationwide surveillance system is only present in three countries/regions, namely United States, European Union and Thailand[1]. Some studies indicate high bacterial resistance rates in developing countries[3-6]. It is hard to delineate the extent of the problem, since it changes in various healthcare facilities and geographic regions. Most data are retrieved from scattered cross-sectional studies and there is no guideline for rational uses of antibiotics especially at local levels[7]. These factors increase the importance of local surveillance pattern of antibiotic resistance from district hospitals. Based on World Health Organization (WHO) guidelines, antibiotic surveillance should be performed in three levels, *i.e.*, local, intermediate and national[8]. There is a close relationship between antibiotic resistance and health care associated infections. It is estimated that the nosocomial infection rate and associated mortality rate in developing countries are about 15% and 5%, respectively[9,10]. Thirty percent of these rates result from infections caused by gram negative bacteria with a slightly higher rate for mortality[11]. Therefore, they are one of the important causes of mortality in developing countries.

A nationwide surveillance system has not yet been established in Iran. Most of the information about antibiotics resistance is retrieved from cross sectional studies. This study was carried out to assess the antibiotic susceptibility patterns of common gram-negative bacteria isolated from infections of normally sterile body sites. The samples collected from two tertiary hospitals called Tohid and Besat located in the Sanandaj city, Kurdistan Province, Iran. They have 1000 beds including all pediatrics and internal medicine subspecialties, gynecology, general surgery, neurology, neurosurgery, cardiology, cardiac surgery, ophthalmology, and otolaryngology wards.

MATERIALS AND METHODS

Our study was performed from January 2012 through December 2012. Hospital-acquired infections were defined as those occurring 48 h after admission, within 3 d of discharge, or within 30 d of surgery[10]. Samples from potentially sterile body fluids [blood, ascitic fluid,

and cerebrospinal fluid (CSF)] were gathered from various wards from Tohid and Besat hospitals. The specimens were sent to the laboratory in a sterile tube for culture. In clinical microbiology laboratory specimens were cultured on general microbiology media including blood agar, chocolate agar, MacConkey agar and EMB agar (Oxoid Ltd, London, United Kingdom) and incubated at 35 ℃ to 37 ℃ overnight. For isolation fastidious bacteria culture plates were incubated for one week. Then suspicious colonies were recultured and purified. The isolates were identified by gram staining, catalase test, oxidase test, triple sugar iron fermentation, motility, colony color, pigment production, and odor. All bacteria isolated in Sanandaj were sent to professor Alborzi clinical microbiology center in Shiraz for further analysis and susceptibility testing. The transport medium was blood agar slant prepared in screw-cap tubes. In the center, re-cultivation was performed on previously mentioned microbiology media. For final confirmation, biochemical tests were embedded in the API-20E biochemical kit system (Bio-Mérieux, France) and manual biochemical tests were used, according to the manufacturer's instructions. Strains were preserved at -20 ℃ on tryptic soy broth (TSB; Oxoid Ltd, London, United Kingdom) containing 20% (v/v) glycerol.

Susceptibility testing

Disk diffusion method: The antibiotic susceptibility testing was determined using the Kirby-Bauer method (disk diffusion technique). The results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI) against a series of antimicrobials[10].

Escherichia coli (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumonia*) were tested for aminopenicillin resistance by ampicillin and amoxicillin disks, for aminoglycoside by gentamicin, tobramycin and amikacin disks, for fluoroquinolone resistance by ciprofloxacin, ofloxacin and levofloxacin disks, and for third generation cephalosporins by cefotaxime, ceftazidim and ceftriaxone disks. Susceptibility testing to co-trimoxazole, piperacillin + tazobactam and imipenem/meropenem was also investigated. All isolated of *E. coli* and *K. pneumonia* that were resistant to third generation cephalosporin were tested for MIC. In the case of an MIC greater than 1 μg/mL, the bacteria were re-tested for extended-spectrum β-lactamase (ESBL) as explained in the following section[10].

Detection of ESBL in E. coli and K. pneumoniae

Combination disk diffusion method: All *E. coli* and *K. pneumoniae* isolates were screened for ESBL production according to CLSI guidelines using a confirmatory disk diffusion method[4] (30 μg) and cefotaxime+clavulanic acid (30 μg + 10 μg), ceftazidime (30 μg) and ceftazidime+clavulanic acid (30 μg + 10 μg) discs (Mast, United Kingdom) were placed at a distance of 25 mm on a Mueller-Hinton Agar plate incubated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37 ℃. A ≥ 5 mm increase in the diameter of inhibition zone for the combination disc *vs* ceftazidime disc confirmed ESBL production. ESBL producing strain *K. pneumoneae* ATCC 700603 and non-ESBL producing strain *E. coli* ATCC 25922 were used as positive and negative controls.

All *pseudomonas* isolates were tested against a spectrum of antimicrobials including piperacillin, piperacillin + tazobactam, ceftazidime, imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin, tobramycin, amikacin.

RESULTS

During one year study, from January 2012 through December 2012, a total of 30334 and 19557 patients were hospitalized in Besat and Tohid tertiary hospitals respectively. Of these, 4320 and 3180 cultures of potentially sterile body fluids were recorded from two hospitals respectively. Totally, 732 positive cultures were reported from both hospitals. Seventy nine isolates/patients fulfilled the study criteria for health-care associated gram-negative infections. Patients had a mean age of 34.12 ± 30.22 years (range, 0-87 years). Thirty-three percent of patients were female. The specimens were obtained from different body sites that were normally sterile. The sources of specimens were as follows: blood, 72 (92.3%); ascitic fluid, 5 (6.4%); and cerebrospinal fluid, 1 (1.3%). The most frequent bacterial cultures were from the pediatrics (52%) and internal medicine wards (29%) and intensive care units (ICU) including pediatric intensive care unit (PICU) (7.5%) (Tables 1 and 2).

Serratia marcescens (*S. marcescens*) was the most frequently isolated organism (38%) followed by *E. coli* (19%), *K. pneumoniae* (19%), *Acinetobacter baumannii* (*A. baumani*) (6%), *Enterobacter* species (6%), *Serratia odorifera* (4%) and *Pseudomonas* species (5%). The remaining isolates included *Stenotrophomonas maltophilia* (one isolate), and *Klebsiella oxytoca* (one isolate).The isolates were tested for antibiotic susceptibility patterns. The profile of antibiotic resistance is shown in Table 3. Overall the susceptibility pattern varied widely. Among antibiotics with systemic uses, the most effective antibiotic was imipenem followed by ciprofloxacin and levofloxacin. The resistance rate was very high against traditional antibiotics such as ampicillin, amoxicillin, and cotrimoxazole.

Thirteen patients expired during the study. The type and frequency of bacteria among expired patients were as follows: *S. marcescens* 6 patients, *E. coli* 3 patients; *K. pneumoniae* 2 patients, *A. baumani* one patient, and *P. aeruginosa* one patient. The diagnosis of these patients was sepsis in all cases. However, there were other co-morbid underlying diseases in all but one patient. The age prevalence and mortality in each age group were presented in Table 2.

DISCUSSION

Our study detected 79 cases of nosocomial gram-

Table 1 Distribution of bacteria isolated from various hospitals wards

Hospital wards Bacteria	Pediatric /neonatal	PICU	Tertiary internal medicine	Infectious diseases	Surgery	General internal medicine	ICU	Total isolates
Escherichia coli	4	0	6	2	2	0	1	15 (19%)
Klebsiella pneumoniae	9	0	2	0	1	2	1	15 (19%)
Acinetobacter baumannii	0	1	4	0	0	0	0	5 (6.3%)
Pseudomonas aeruginosa	1	0	1	0	0	0	0	2 (2.5%)
Pseudomonas oryzihabitans	1	0	0	0	0	0	0	1 (1.3%)
Serratia marcescens	20	0	6	2	0	2	0	30 (38%)
Enterobacter cloacae	3	0	0	0	0	1	1	5 (6.3%)
Stenotrophomonas maltophilia	0	0	0	0	0	0	1	1 (1.3%)
Klebsiella oxytoca	0	0	0	0	1	0	0	1 (1.3%)
Serratia odorifera	3	0	0	0	0	0	0	3 (3.8%)
Pseudomonas luteola	0	1	0	0	0	0	0	1 (1.3%)
Total isolates in the related wards	41 (52%)	2 (2.50%)	19 (24%)	4 (5%)	4 (5%)	5 (6.5%)	4 (5%)	79 (100%)

PICU: Pediatric intensive care unit; ICU: Intensive care unit.

Table 2 Prevalence of infections and crude mortality by age groups and isolated organisms *n* (%)

Age groups Bacteria	Neonatal (< 28 d)	Infantile (1-11 mo)	Childhood (1-17 yr)	Adults (18-59 yr)	Elderly (> 60 yr)	Total isolates	Crude mortality by bacteria
Escherichia coli	3 (20)[1]	0	5 (33.3)[1]	2 (13.3)[1]	5 (33.3)[1]	15 (19)[2]	3 (23.1)[3]
Klebsiella pneumoniae	10 (66.7)	0	1 (6.7)	4 (26.7)	0	15 (19)[2]	2 (15.4)
Acinetobacter baumannii	3 (60.0)	0	0	1 (20)	1 (20)	5 (5.6)[2]	1 (7.7)
Pseudomonas aeruginosa	0	1 (50)	1 (50)	0	0	2 (2.2)[2]	1 (7.7)
Pseudomonas oryzihabitans	0	0	0	1 (100)	0	1 (1.1)[2]	0
Serratia marcescens	2 (6.7)	6 (20)	11 (36.7)	7 (23.3)	4 (13.3)	30 (33.3)[2]	6 (46.1)
Enterobacter cloacae	0	0	2 (40)	1 (20)	2 (40)	5 (5.6)[2]	0
Stenotrophomonas maltophilia	0	0	0	1 (100)	0	1 (1.1)[2]	0
Klebsiella oxytoca	0	0	0	0	1 (100)	1 (1.1)[2]	0
Serratia odorifera	2 (66.7)	0	1 (33.3)	0	0	3 (3.3)[2]	0
Pseudomonas luteola	0	0	0	0	1 (100)	1 (1.1)[2]	0
Total Isolates in the age groups	20 (25)	7 (8.8)	21 (26.5)	17 (21.5)	14 (17.7)	79 (100)	-
Crude mortality by age	4 (30.8)[3]	0	2 (15.4)	4 (30.7)	3 (23.1)	-	13 (100)

[1]Percent within each isolate; [2]Percent within total isolates of a specific bacteria; [3]Percent within total mortality.

negative infection mostly isolated from blood stream infections (BSIs). The crude mortality rate was calculated as 16.5%. The crude mortality rates that reported in two large series by Marra *et al*[12] and Wisplinghoff *et al*[13] from United States and Brazilian hospitals have been 27% and 40% respectively. The rate of nosocomial BSIs was 16 per 10000 admissions in our hospitals comparing to 60 per 10000 in Wisplinghoff series. Our calculated rates are significantly lower than 2 other series. However, we couldn't deduce strong epidemiological implication because the number of our cases was very low as the duration of our study was shorter comparing to two other studies.

S. marcescens was the most common organism isolated in our series at about 38% (Table 2). In series reported by Marra *et al*[12] and Wisplinghoff *et al*[13] the organism comprised only 1.7% and 3.5% of all bacteria isolated from BSIs respectively. Meanwhile, the crude mortality rates were reported as 27.4% and 40% of all deaths respectively. This rate calculated as about 46.1% of crude mortality in our series. The *Serratia* species especially the more pathogen serotype (*S. marcescens*) can cause a spectrum of diseases from urinary tract infection to meningitis and overwhelming infections[14]. It has the potential of being multi-resistant by a variety of mechanisms[10]. *S. marcescens* is reported as a nosocomial pathogen that caused outbreaks and endemic healthcare associated infections in many instances[15]. The main mechanism of nosocomial spread is hand to hand transmission. Various other ways of transmissions has been reported as important in *Serratia* transmission including contaminated intravenous solution and caps of bottles containing saline, respirators, arterial pressure monitors, suction traps, contaminated hand washing brushes, colonized disinfectants or soaps, contaminated infant parenteral nutrition fluid, and contaminated whole blood or blood products[15,16]. High isolation rate especially from pediatric wards (46%) and high mortality rate of *Serratia* in our series are alarming signs. They may be reflective of poor infection control in our hospitals.

The frequency of resistant *Serratia* species to penicillins has been reported to be as high as 90%[17]. In our series, the resistance rate for ampicillin and amoxicillin was more than 95%. These high resistance rates are because of the intrinsically resistance chara-

Table 3 Antibiotic susceptibility patterns of gram-negative bacteria isolated from sanandaj hospitals, Iran, 2012 (% susceptible)

	Escherichia coli	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Serratia marcescens*	*Serratia ordorifera*	*Acinetobacter*	*Species enterobacter*	*Pseudomonas aeruginosa*	*Pseudomonas orizihibitans*	*Pseudomonas luteola*	*Stenotropho-monas*	Susceptibility of all Isolates	No. of isolates tested
Ampicillin	1 (6.7)	3 (20)	0 (0)	1 (3.3)	0 (0)	0 (0)	0 (0)	-	-	-	-	5 (6.7)	74
Amoxicillin	2 (13.3)	1 (6.7)	0 (0)	1 (3.3)	0 (0)	0 (0)	1 (20)	-	-	-	-	5 (6.7)	74
Amikacin	14 (93.3)	8 (53.3)	1 (100)	23 (76.7)	2 (66.7)	2 (40)	4 (80)	2 (100)	1 (100)	1 (100)	1 (100)	58 (73.4)	79
Gentamicin	11 (73.3)	7 (46.7)	0 (0)	22 (73.3)	1 (33.3)	0 (0)	4 (80)	2 (100)	1 (100)	1 (100)	0 (0)	48 (60.7)	79
Tobramycin	-	-	-	-	-	-	-	2 (100)	1 (100)	1 (100)	0 (0)	4 (80)	5
Cotrimoxazole	2 (13.3)	6 (40)	0 (0)	21 (70)	2 (66.7)	1 (20)	4 (80)	0 (0)	0 (0)	0 (0)	0 (0)	36 (45.5)	79
Nitrofurantoin	15 (100)	10 (66.7)	0 (0)	6 (20)	2 (66.7)	0 (0)	3 (60)	-	-	-	-	35 (47.2)	74
Cephalothin	6 (40)	5 (33.3)	0 (0)	1 (3.3)	1 (33.3)	0 (0)	1 (20)	-	-	-	-	13 (17.6)	74
Cefixime	10 (66.7)	5 (33.3)	0 (0)	22 (73.3)	1 (33.3)	0 (0)	2 (40)	-	-	-	-	39 (52.7)	74
Cefotaxime	9 (60)	5 (33.3)	0 (0)	22 (73.3)	1 (33.3)	0 (0)	4 (80)	-	-	-	-	40 (54)	74
Ceftazidim	11 (73.3)	5 (33.3)	0 (0)	24 (80)	2 (66.7)	0 (0)	4 (80)	2 (100)	1 (100)	1 (100)	0 (0)	49 (62)	79
Ceftriaxone	8 (53.3)	6 (40)	0 (0)	22 (73.3)	1 (33.3)	0 (0)	3 (60)	-	-	-	-	39 (52.7)	74
Cefepim	13 (86.7)	7 (46.7)	0 (0)	24 (80)	3 (100)	0 (0)	4 (80)	1 (50)	1 (100)	1 (100)	0 (0)	52 (65.8)	79
Ciprofloxacin	11 (73.3)	13 (86.7)	0 (0)	30 (100)	3 (100)	1 (20)	4 (80)	2 (100)	1 (100)	1 (100)	0 (0)	65 (82.3)	79
Levofloxacin	11 (73.3)	13 (86.7)	0 (0)	30 (100)	3 (100)	1 (20)	5 (100)	2 (100)	1 (100)	1 (100)	1 (100)	67 (84.8)	79
Imipenem	15 (100)	15 (100)	1 (100)	30 (100)	3 (100)	5 (100)	5 (100)	2 (100)	1 (100)	1 (100)	1 (100)	79 (100)	79
Meropenem	-	-	-	-	-	-	-	2 (100)	1 (100)	1 (100)	1 (100)	5 (100)	5
Carbenicillin	-	-	-	-	-	-	-	2 (100)	1 (100)	1 (100)	0 (0)	4 (80)	5
Ticarcillin	-	-	-	-	-	-	-	2 (100)	1 (100)	1 (100)	0 (0)	4 (80)	5
Piperacillin	-	-	-	-	-	-	-	2 (100)	1 (100)	1 (100)	0 (0)	4 (80)	5
Piperacillin/ Tazobactam	10 (66.7)	13 (86.7)	1 (100)	27 (90)	3 (100)	0 (0)	4 (80)	2 (100)	1 (100)	1 (100)	-	57 (73)	78
Aztreonam	-	-	-	-	-	-	-	2 (100)	1 (100)	1 (100)	-	3 (75)	4
ESBL(positive)	5 (33.3)[1]	10 (66.7)[1]	-	-	-	-	-	-	-	-	-	16 (51.6)[1]	31[1]
Total susceptible isolates (%)	149 (62.1)	122 (50.8)	3 (18.7)	306 (63.7)	28 (58.3)	10 (12.5)	52 (65)	27 (90)	14 (93.3)	14 (93.3)	4 (30.7)	711 (56.6)[2]	--
Total isolates tested (sum)	240	240	16	480	48	80	80	30	15	15	13	--	1257

[1]Not counted in the sum and susceptibility percents; [2]Total sensitive Isolates (% susceptibility of total tested isolates).

cteristics of the organism as it is the case for penicillin G, amoxicillin-clavulanate, cefuroxime, and narrow-spectrum cephalosporins[10]. In fact, these antibiotics are not only ineffective against the *Serratia* species but they are also strong inducers of AmpC gene causing production of ESBL and treatment failure with third generation cephalosporins. Although the early susceptibility testing against third generation cephalosporins may be promising, this organism may become resistant after 3 to 4 d of treatment[18]. The drug of choice for *Serratia* species is piperacillin. The susceptibility of these organisms in our series to piperacillin-tazobactam was between 90%-100% (mean: 91%). The susceptibility of *Serratia* for ciprofloxacin, levofloxacin, and imipenem were 100% making them advisable beside piperacillin in treating overwhelming infection caused by *Serratia* species. These sensitivity rates are comparable to findings by Wisplinghoff *et al*[13] from United States. They reported the sensitivity rates of *Serratia* to traditional antibiotics ranged from 83.2% to 97.4%. The susceptibility of *Serratia* to cefepim in our study was lower (80%). Overuse of this antibiotic as an empirical choice in our hospitals may be the cause of lower susceptibility[19]. Moderate susceptibility of *Serratia* species to aminoglycosides ranging from 33% to 77% (mean: 73%) make these antibiotics optional for synergistic use with other antibiotics in our district.

E. coli and K. pneumonia were the second most common group of isolated bacteria. Each bacterium comprised 19% of total isolates in our series. It is more than the rate reported by larger studies at about 4.8% to 13.2%[12,13]. The source of infection was blood stream mostly from pediatric and neonatal wards (43%) followed by internal medicine (33%). *E. coli* is the most common causes of neonatal sepsis. Most infections of *K. pneumonia* are acquired in hospital. It is a common cause of neonatal septicemia with high mortality rate. The susceptibility of these organisms to ampicillin and amoxicillin were poor ranging from 6.7% to 20%. These rates may be comparable to the rates reported for *K. pneumonia* (2%-45.5%), however it is far less than the susceptibility rates for *E. coli* (54.2%) in other series[12,13]. The susceptibility rates of *E. coli* and *K. pneumonia* to gentamicin in our series were calculated as 73.3 and 46.7 respectively. These rates are far less than rates from United States reported as 96.1% and 84% respectively[13]. The current WHO recommendation for empirical prophylaxis and treatment of suspected neonatal sepsis is a combination of ampicillin and gentamicin[20]. The quality of evidence for the recommendation of sepsis prophylaxis is categorized as weak and very low quality evidence; and for sepsis treatment is categorized as strong and low quality of evidence. Nevertheless, the efficacy of this antibiotics combination should be re-assessed considering the higher resistance rates to ampicillin and gentamicin in Iran. The susceptibility rate of *E. coli* to ciprofloxacin was 73% compared to previous reports from Iran that measured it at about 46%. The susceptibility rates to third generation cephalosporins were ranged from 53% to 73%. The previous reports from Iran denoted a susceptibility rate to third generation cephalosporins of about 59%[1]. Lower susceptibility to cotrimoxazole and cefixime reflects higher use of the antibiotic in outpatient oral therapy of UTI and gastroenteritis in children. However, low resistance rate of *E. coli* against nitrofurantoin may be due to its infrequent use as a urinary antiseptic and no application in systemic infection.

There are wide therapeutic options for nonresistant cases of *K. pneumonia*. However this is limited to fourth generation cephalosporins and carbapenems for multi-resistant cases[21]. The clinical response to various antibiotics is largely determined by ESBL production by this organism rather than early *in vitro* susceptibility patterns[18]. The rates of ESBL production by *E. coli* and *K. pneumonia* were 33.3% and 66.7%, respectively. Surprisingly, these rates are much lower than the rates reported for European countries (72.7%-100%) and Brasilia (72.3%-91.4%)[12,22]. This difference could be attributed to the different methodologies used in these studies. We had tested all isolates for ESBL production while the other 2 studies had tested only selected bacteria that had been resistant to third generation cephalosporins from selected laboratories. Resistance rates of *E. coli* and *K. pneumonia* to beta-lactam antibiotics were parallel to ESBL production rates. The susceptibility rates of *K. pneumonia* to 3rd generation cephalosporins ranged from 33%-40% while these numbers for *E. coli* species were between 53% and 73.3%. Previous reports from Iran has been calculated the susceptibility of *K. pneumonia* to 3rd generation cephalosporins at 52%[1]. The susceptibility rate for cefepim was similar to 3rd generation cephalosporins. Fortunately, the susceptibility to imipenem for *K. pneumonia* was very high (100%) making this antibiotic the drug of choice for difficult-to-treat infections in our community. This susceptibility rate is much higher compared to previously reported data from Iran (46%)[1]. The resistance rate of *K. pneumonia* to carbapenems in some countries has been reported worrisome[12].

The isolation rate for *Acinetobacter* was 6%, mostly recovered from internal medicine and pediatrics wards. This figure may sound alarming because of its higher rate in comparison with other studies. The reported isolation rates for Acinetobacter baumannii were as 1.3% and 2.7% from two large series from United States[2,13]. However, because of the relatively lower number of total isolated bacteria and short period of our study, the comparison of prevalences may be statistically inaccurate. It should be noted that once the multi-resistant *Acinetobacter* is introduced into a hospital, it could cause recurrent outbreaks and prolonged colonization[23]. It can remain alive under a wide range of environmental conditions[24]. This organism has the potential to be multi-resistant, and the related infections are very difficult to treat. The susceptibility rates reported for *Acinetobacter* from different European countries have been very wide ranged from 4.2% (Romania) to 100% (the Netherlands)[14]. The susceptibility rates for *Acinetobacter* were low in our series. These rates were mostly below 40% (Table 3). However, in contrast to many reports of increasing resistance to imipenem[2,4,5], *Acinetobacter* remained highly susceptible to imipenem (100%) in our hospitals. The resistance rate and prevalence of this organism as a cause of ventilator associated pneumonia is increasing steadily in many countries[11]. However, most of our isolates (80%) were not from ICU wards. The combination of colistin and rifampin, imipenem and rifampin or amikacin may be good choices for multi-resistant *Acinetobacter* in our community[11,24].

The *enterobacter* species accounted for 6% of isolated bacteria. This rate is comparable to rates of isolation from three large series that ranged from 3.9% to 6.1%[2,5,12,13]. Our isolates comprise 5 cases of *Enterobacter cloacae*. In large series, *Enterobacter cloacae* was the most common species isolated from clinical samples[25]. Inducible resistances to 3rd generation cephalosporins by previous use of aminopenicillin and some other antibiotics is a major determinant for selection of antibiotics. Similar to *Serratia*, antibiotic resistance may develop during treatment despite early susceptibility reports. In our series, the organism preserved good susceptibility against imipenem and levofloxacin by

100% susceptibility and against ciprofloxacin, ceftazidim and cefotaxime, cefepim, amikacin, gentamicin, cotrimoxazole and piperacillin-tazobactam by 80% susceptibility.

We found only 4 isolates for *Pseudomonas* species. These comprised two positive cultures for *Pseudomonas aeruginosa*, one for *Pseudomonas orizihibitans* and one for *Pseudomonas luteola*. They were isolated from pediatric ward (2 isolates), internal medicine and ICU (each one isolate). Unexpectedly, they were susceptible to most antibiotics tested. One case was resistant to cefepim. The high susceptibility rates are in contrast to reports from many other studies[2,25,26]. However, the number of isolates was too low to derive a defensible statistical inference. The bacteria may be isolated from new hospitalized patients suffering from community acquired infection and therefore might preserve their primary antibiotics susceptibility. One exception was resistance to cotrimoxazole by all isolates of pseudomonas. High use of cotrimoxazole as a urinary disinfectant and in the treatment of gastroenteritis in Iran may be an explanation for this finding[11,25].

We had only one case of *Stenotrophomonas maltophilia*. This organism has the inherent potential to be resistant to many available antibiotics. Cotrimoxazole has been proposed as the drug of choice for the treatment of multi-resistant isolations[27]. However, our isolate was resistant to cotrimoxazole, all Aminoglycosides except amikacin, and many other antibiotics (Table 3).

There are many studies denoting a considerable association between the rate of antibiotic consumption and bacterial resistance pattern both in the hospital and community[4,6,28,29]. It has been proposed that a reduction in antibiotic use would decrease the rate of bacterial resistance[4,30]. Therefore, there is a need to emphasize the judicious use of antimicrobials and pay adequate attention to the subject of "reserve drugs"[25].

Continuous antimicrobial surveillance is necessary to determine the changing status of antibiotic resistance in local, provincial and national referral hospitals. Effective strategies/guidelines should be established to minimize the misuse of existing antimicrobials.

Our study was performed using data collection from two large hospitals in Sanandaj for one year; however the number of isolate were not enough to conclude strong epidemiological deduction. A study with a longer duration based on a detailed surveillance system is needed to monitor antibiotic resistance continuously.

We found a high bacterial resistance rate isolated from healthcare associated infections in our hospitals. The most effective antibiotics against gram-negative healthcare associated infections were imipenem followed by ciprofloxacin. The resistance rate was high against ampicillin and cephalothin. The high mortality rate (46.1%) associated with *S. marcescens* was alarming. A national surveillance program is essential to monitor the extent of resistance continuously, emphasize rational use of antimicrobials, and conduct effective measures to improve patient management outcome.

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COMMENTS

Background

Knowledge of the prevalence of antibiotic resistance is a pre-requisite for infection control and essential for public healthcare policy makers to conduct effective responses. A nationwide surveillance system has not yet been established in Iran. Most data are retrieved from scattered cross-sectional studies and there is no guideline for rational uses of antibiotics especially at local levels. It is hard to delineate the extent of the problem, since it changes in various healthcare facilities and geographical regions.

Research frontiers

In recent years, the status of antibiotic resistance has changed very rapidly. Iran had one of the highest antibiotic consumption rates. Fears regarding that the irrational use of antibiotics have resulted in a high level of antibiotic resistance. Few studies from Iran indicate high resistance rate. No antibiotic stewardship has yet been established in this country.

Innovations and breakthroughs

The present study is the only evidence based data from this county. It was carried out to assess the antibiotic susceptibility patterns of common gram-negative bacteria isolated from infections of normally sterile body sites. The patients were from two tertiary hospitals called Tohid and Besat located in the Sanandaj city, Kurdistan Province, Iran. The study was performed from January 2012 through December 2012.

Applications

The data in this study recommended that antibiotic resistance is alarming in this county. Moreover, this study also provided readers with important information regarding the clinical implication of current status of antibiotic resistance.

Terminology

Sanandaj is the center of Kurdistan province in the west of Iran with a population of about 480000. The samples were collected from two tertiary hospitals called Tohid and Besat located in the Sanandaj. They have 1000 beds including all pediatrics and internal medicine subspecialties, gynecology, general surgery, neurology, neurosurgery, cardiology, cardiac surgery, ophthalmology, and otolaryngology wards.

Peer-review

Available papers concerning antimicrobial resistance in Sanandaj are scarce. The authors in this study analyzed the characteristics and outcomes of infections of sterile body sites especially blood stream infection. This study showed that the most effective antibiotics against gram-negative healthcare associated infections were imipenem followed by ciprofloxacin. The results were interesting and provided important information concerning antibiotic resistance, making some antibiotics such as cephalothin almost useless. The high mortality rate (46.1%) associated with *Serratia marcescens* was alarming.

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CASE REPORT

Hepatitis B surface antigen escape mutations: Indications for initiation of antiviral therapy revisited

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Abstract

Approximately 240 million people are chronically infected with hepatitis B. The implementation of rigorous vaccination programs has led to an overall decrease in the prevalence of this disease worldwide but this may also have led to emergence of viral mutations that can escape the protection of hepatitis B surface antibody. As this phenomenon is increasingly recognized, concern for transmission to vaccinated individuals has also been raised. Herein, we describe two cases where the suspected presence of a hepatitis B surface antigen escape mutation impacted the decision to initiate early antiviral therapy, as well as provide a brief review of these mutations. Our findings described here suggest that a lower threshold for initiating therapy in these individuals should be considered in order to reduce the risk of transmission, as vaccination does not provide protection.

Key words: Hepatitis B virus; Hepatitis B surface antigen; Escape mutant; Hepatitis B immunoglobulin; Vaccination

Core tip: Hepatitis B surface antigen escape mutations are being increasingly recognized, along with concern for the risk of transmission to vaccinated individuals.

The management of these patients and the natural history of the disease remain controversial due to insufficient data. However, transmission of this mutated virus to vaccinated individuals has been reported in the literature. Herein, we discuss two different clinical scenarios that led to the initiation of early antiviral therapy in order to decrease the risk of transmission to others due to the suspected presence of this mutation.

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INTRODUCTION

The World Health Organization estimates that over 2 billion people worldwide have been infected with hepatitis B virus (HBV). Approximately 240 million are chronically infected and therefore are at risk for life-threatening complications as a consequence of this disease[1]. The introduction of universal vaccination at birth, as well as other HBV immunization campaigns, has led to a significant reduction in the transmission of HBV in endemic countries[2,3]. However, with the success of these immunization strategies, there have been increasing reports of mutant viruses that develop despite vaccination, leading to concern about the threat they may pose to the public. These mutant viruses have been found to occur as a result of selection pressure from vaccination, hepatitis B immunoglobulin (HBIG) and have even been reported to occur spontaneously[4-6]. Despite increasing awareness of the emergence of hepatitis B surface antigen (HBsAg) escape mutant virus, there is no consensus on the treatment and management of individuals infected with these mutants. At present, there is also no easily available assay to diagnose these individuals when they are suspected of harboring HBsAg escape mutants. Here we highlight two clinical cases in which recognition of the presence of HBsAg escape mutations influenced the decision to initiate early antiviral therapy, as well as provide a brief review of HBsAg escape mutants.

CASE REPORT

Case 1

A 35-year-old G1P0 Korean woman presented at 13 wk gestation for evaluation of HBV discovered on routine prenatal screening. She was HBV treatment naïve and had never received a liver biopsy. She otherwise had no significant past medical or surgical history. She moved to the United States from South Korea at the age of 17. Her family history is significant for chronic HBV in both her mother and sister but no history of hepatocellular carcinoma (HCC). She reported a history of vaccination for HBV as a child.

Screening for virologic markers was positive for HBsAg, hepatitis B surface antibody (anti-HBs), hepatitis B core IgG antibody (anti-HBc IgG) and hepatitis B e antigen (HBeAg). She had negative markers to hepatitis B core IgM antibody (anti-HBc IgM) and hepatitis B e antibody (anti-HBe). Serum HBV DNA was detected with a viral level of 1707 copies/mL, mildly elevated alanine aminotransferase (ALT) level of 31 U/L (normal < 19 U/mL) for women)[7] and aspartate aminotransferase (AST) level of 31 U/L. Her alpha-fetoprotein (AFP) level was mildly elevated at 29.7 ng/mL, but this was felt to be consistent with her pregnancy, as AFP is known to be produced by human fetus and an abdominal ultrasound was negative for any suspicious liver lesion.

Her HBV DNA and ALT levels were monitored closely during her pregnancy without any evidence of hepatitis flare. She was sexually active with her husband who had previously been vaccinated for HBV but had not developed anti-HBs.

The patient was counseled on standard prevention of perinatal transmission with active and passive immunization of the infant. However, due to the concerns of transmission with HBV escape mutant, despite maintaining a low viral load throughout her pregnancy, she was started on treatment with tenofovir 300 mg orally once a day at 32 wk gestation, as HBV vaccination and administration of HBIG would be ineffective against HBsAg escape mutant HBV. The infant received hepatitis B vaccination as well as HBIG at birth and follow-up so far has shown the child to free of HBV infection.

Case 2

A 49-year-old Laotian man with chronic hepatitis B was referred for evaluation of a liver lesion discovered on screening ultrasound. He had previously been diagnosed with HBV in 2008, had no prior anti-HBV therapy, no prior liver biopsy, nor jaundice or other signs of hepatic decompensation. His past medical history was significant for latent tuberculosis, not treated given concern for isoniazid hepatotoxicity, and chronic sinusitis. He was born in Laos to Chinese immigrant parents and moved to the United States at the age of 16. He is married to a registered nurse, and they have a 17-year-old son. His family history is significant for a sister with chronic HBV, his father with HCC, and his mother with gastric cancer.

Screening for virologic markers was positive for HBsAg, anti-HBs, anti-HBc IgG, and anti-HBe. He had negative markers to anti-HBc IgM and HBeAg. Serum HBV DNA was detected with a viral level of 6100 copies/mL and mildly elevated ALT of 52 U/L (normal < 30 U/L for men)[7] and normal AST of 25 U/L. His AFP was normal at 2 ng/mL. Liver ultrasound showed a stable 9 mm × 10 mm × 8 mm lesion consistent with a hemangioma and a new 3 mm × 3 mm × 3 mm lesion in the right lobe of the liver. Follow-up magnetic resonance imaging did not demonstrate any evidence of HCC or cirrhosis. FIBRO*spect* Ⅱ analysis was consistent

with F0-F1 (no fibrosis-portal tract fibrosis).

His wife was previously vaccinated against HBV and had protective anti-HBs as part of her employment screening as a nurse but reported that three years ago she was prevented from donating blood due to positive HBsAg. Previously, she was a regular blood donor. Although the patient was asymptomatic with a low HBV DNA level, normal ALT, and a reassuring FIBRO*spect*, given the possibility that he likely infected his previously vaccinated wife with HBV escape mutant, the decision was made to start him on antiviral therapy to prevent further transmission among household contacts such as his son.

DISCUSSION

HBV with coexisting HBsAg and anti-HBs have long been reported in up to 10%-25% of patients with chronic hepatitis B, but were initially felt to be variants of no clinical importance[8]. However, several studies have since shown that the coexistence of HBsAg and anti-HBs is associated with mutations in the a determinant region of the HBsAg[9-12]. The significance and impact of these mutations was first recognized in 1988 when Zanetti *et al*[13] reported infants born to HBsAg carrier mothers who developed breakthrough infections despite receiving HBIG and HBV vaccine at birth. A single point mutation was identified in the surface antigen region from guanosine to adenosine at nucleotide position 587, resulting in amino acid (aa) substitution from glycine to arginine at position 145 in the a determinant of the HBsAg. This *G145R* mutation alters the conformation of the a determinant so that the neutralizing antibodies induced by vaccination are no longer able to recognize the virus, thereby resulting in breakthrough infection[13]. Since this discovery, other surface gene (S-gene) mutations with the same ability to evade immunization and infect vaccinated individuals have been reported, leading to increasing concern that these mutations may overcome the wild type and infect those who have been vaccinated. These mutations were also later recognized to occur after administration of HBIG in liver transplant recipients[14]. In addition, S-gene mutations have also been found to occur spontaneously, hypothesized to be due to the pressure of the host immune system, although the mechanism by which this occurs remains unclear[10,15].

At present, the clinical significance of HBsAg escape mutations remains controversial. A mathematical model proposed in 1998 by Wilson *et al*[16] predicted the disappearance of wild-type HBV in 200 years and the emergence of the G125R mutant as the common HBV in 60-100 years, based on the assumption that the current vaccination does not protect against this mutation. Several surveys in Taiwan have shown that the proportion of mutant viruses in HBV-infected children had increased significantly since the implementation of the universal vaccination program: 7.8% in 1984 just before the program implementation to 28.1% in 1994 and 23.1% in 1999[3,17]. A more recent epidemiologic survey published by Hsu *et al*[18] on the other hand, showed that with the reduction in the total number of children infected with HBV as a result of universal vaccination program, the prevalence of HBV mutants has actually decreased over time. This was also followed by a study published by Lai *et al*[19] which confirmed the decreased prevalence of HBV mutants in Taiwan. By measuring HBsAg, anti-HBs and anti-HBc from various age groups in 2007, the authors found that the HBsAg carrier rate, anti-HBc seropositive rate and infection rate was significantly lower in those who were born after the initiation of the vaccination program in Taiwan as compared to those who were born before the program. However, when compared across age groups, there was a significant increase in the HBV DNA positive rate for those who were 18-21 years of up to 3% as compared to those of younger age. In addition, the prevalence of HBsAg mutants was 2.63% in those > 18 years of age, but only 0.10% in those younger than 18. Thus, the authors concluded that although the prevalence of HBV infection has decreased with universal vaccination, continued monitoring for the presence of HBV infection is important due to the risk of mutant strains developing, particularly as this population continues to age[19].

As discussed above, the long-term impact that these HBsAg escape mutations may have on the natural history of chronic HBV remains unknown. On a public health level, some studies have suggested that these viruses lack stability and tend to result in lower levels of viremia, thus perhaps explaining why the viruses have not become as large of a threat to immunization programs as originally predicted[20]. On an individual level, however, there has been data to suggest that these patients may be at increased risk for active chronic hepatitis with higher HBV DNA levels and more advanced fibrosis[9]. There is also concern that the accumulation of mutations may lead to failure of recognition of HBsAg by currently available diagnostic assays, thereby leading to a missed diagnosis of chronic HBV infection[21,22]. In addition, there is a real concern regarding the risk of transmission to others, as vaccination does not provide protection from these mutated viruses. The risk of horizontal transmission is not currently well defined, but there are prior case reports in the literature[23,24]. One Taiwanese study identified that at least 26% of the HBV mutant-infected children who experienced immunization failure were born to non-carrier mothers, implying the possibility of horizontal infection or a spontaneous emergence of the mutant during the course of chronic infection[18].

In summary, while the threat that HBsAg escape mutants poses to the world at this time does not seem to be on as large a scale as initially feared, its significance may be considerable on individual patient levels. The possibility of transmitting the virus to other household and sexual contacts is real and needs to be addressed. In situations where the risk of sexual, vertical, or horizontal transmission is present, patients and their

family members should be counseled carefully, and consideration should be given towards having a lower threshold to initiating potent antiviral therapy in these individuals to reduce the risk of transmission. Studies have clearly shown that the coexistence of HBsAg and anti-HBs is not as rare as once thought. Therefore, screening tests for HBV should routinely include HBsAg, anti-HBs and anti-HBc, especially in those who have close contact with individuals with chronic HBV infection.

COMMENTS

Case characteristics

Herein are two cases of individuals with chronic hepatitis B presenting with coexisting hepatitis B surface antigen (HBsAg) and anti-HBs without symptoms.

Clinical diagnosis

Case 1 is a 35-year-old pregnant, but otherwise asymptomatic woman presenting with newly diagnosed hepatitis B virus (HBV) infection and Case 2 is a 49-year-old man with chronic HBV infection who likely transmitted HBV to his wife despite immunity from prior vaccination.

Differential diagnosis

Due to the history of prior vaccination and the presence of concomitant HBsAg and anti-HBs, it was felt likely that both patients were infected with HBsAg escape mutants.

Laboratory diagnosis

Both patients had serologies positive for HBsAg, anti-HBs, slightly abnormal liver enzymes and quantifiable HBV DNA tests.

Treatment

Initiation of antiviral therapy with tenofovir in both cases.

Related reports

Reports of HBsAg mutant strains of HBV have been increasingly recognized with the introduction of universal vaccination. There have been published case reports of this mutation transmitted to others despite the presence of protective antibodies from prior HBV vaccination.

Term explanation

HBsAg escape mutants are due to mutations in the a determinant region of the HBsAg. These mutations allow the virus to escape neutralizing antibodies from the administration of hepatitis B immunoglobulin and HBV vaccine.

Experiences and lessons

Physicians should maintain a level of suspicion in patients with coexisting HBsAg and anti-HBs in the appropriate clinical situation, and should counsel their patients on the risks and benefits of early treatment.

Peer-review

A limitation of this paper is the inability to perform molecular confirmation of HBsAg escape mutations. However, this is not available in real-world clinical practice. This manuscript describes two interesting case studies that generate clinically relevant questions for an infrequent but important issue in the management of patients with HBV infection.

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CASE REPORT

Fulminant isolated cardiac sarcoidosis with pericardial effusion and acute heart failure: Challenging aspects of diagnosis and treatment

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Informed consent statement: The patient involved in this study gave his verbal informed consent authorizing use and disclosure of his protected health information.

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Abstract

This case report illustrates challenging aspects of diagnosis and treatment of isolated sarcoid heart disease (SHD) and the role of cardiovascular magnetic resonance (CMR) imaging. Here, we present a previously healthy 45-year-old man, who was admitted with pericardial effusion and symptoms of acute heart failure. CMR followed by targeted left ventricular endomyocardial biopsy (EMB) revealed the diagnosis of isolated SHD. The combined use of CMR and EMB was crucial in diagnosing SHD. Furthermore, this case report demonstrates the value of CMR for monitoring response to therapy and lesion healing.

Key words: Heart failure; Cardiac sarcoidosis; Cardiovascular magnetic resonance imaging; Endomyocardial biopsy; Internal cardiac defibrillator

Core tip: This case report illustrates the challenging aspects of diagnosis and treatment of isolated sarcoid heart disease (SHD) and the role of cardiac magnetic resonance imaging (CMR) in diagnosis. Due to the use of CMR followed by targeted left ventricular endomyocardial biopsy the diagnosis of isolated SHD could be achieved. Most importantly, this case supports the use of CMR as an extremely useful non-invasive technique for monitoring response to therapy and lesion

healing in the course of heart failure.

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INTRODUCTION

Sarcoidosis is a granulomatous multisystem disorder of unknown etiology which has a wide range of manifestations affecting a variety of organs[1]. The prevalance of sarcoidosis varies with ethnicity (4.7-64/100000)[2]. Approximately 2%-7% of patients with sarcoidosis suffer from clinical cardiac manifestations[3,4]. However, several studies reveal a much higher prevalance of 20%-50% of patients with asymptomatic sarcoid heart disease (SHD) or even up to 70%-85% in autopsy studies[4,5]. Interestingly, the prevalence of isolated cardiac sarcoidosis is much higher in Japanese patients[6].

CASE REPORT

A 45-year-old man was referred to the emergency unit with syncope and temporary hemiparesis. His medical history was unremarkable besides splenectomy many years ago due to trauma. Physical examination at admission was notable for bilateral pleural effusions. Extensive neurological examinations including cranial computed tomography, brain magnetic resonance imaging (MRI), electroencephalography and lumbar puncture did not reveal any pathology. Transthoracic echocardiography revealed pericardial effusion with beginning hemodynamic relevance, possibly leading to syncope. Thus, pericardial paracentesis was performed and drained 1.8 L of hemorrhagic, sterile effusion. Subsequent laboratory findings showed increased cardiac markers (Troponin 2320 pg/mL, Creatinkinase 224 U/L, NT-proBNP 6731 ng/L) and electrocardiogram revealed abnormalities with ST-segment depression. Thus, coronary angiography was performed, which excluded coronary artery disease. Follow-up echocardiography during the next days revealed a high grade mitral regurgitation due to annular enlargement secondary to left ventricular (LV) dilatation (left ventricle end-diastolic diameter: 74 mm) and papillary muscle dysfunction, severely reduced ejection fraction (EF 30%), diastolic dysfunction and regional wall motion abnormalities of the lateral wall. Nevertheless, the etiology of the pericardial effusion and myocardial injury still remained unclear. Therefore, cardiovascular magnetic resonance (CMR) imaging was performed revealing the following findings: Severely impaired global systolic function (EF 36%), extensive edema on T2-weighted short-tau inversion recovery images as well as necrosis on late gadolinium enhancement (LGE) with a non-ischemic pattern of the lateral wall, but also the left ventricular septum (Figure 1). These findings were suspicious but not specific for sarcoidosis. Thus, we performed targeted endomyocardial biopsy (EMB) in the lateral LV wall and immunohistology revealed SHD. Interestingly, additional laboratory results were unremarkable with normal angiotensin-converting enzyme blood levels as well as normal different antibodies [anti-neutrophil cytoplasmatic antibodies (ANCAs), antinuclear antibodies, rheumatoid factors, antibodies to double-stranded DNA (anti-dsDNA), complement factors, interleukin-2 receptor]. Apart from that, computed tomography and chest X-ray excluded typically findings of pulmonary sarcoidosis such as bihiliar lymphadenopathy.

We initiated medical heart failure therapy as recommended in current guidelines[7]. After diagnosing SHD, high-dose glucocorticoid therapy with prednisone was initiated and gradually reduced over months. The follow-up-visits revealed an improved NYHA class (NYHA I-II) and clinical symptoms. Most importantly, follow-up CMRs after 1, 3 and 6 mo after initiation of the glucocorticoid therapy demonstrated resorption of edema consolidation of scar and improved systolic LV function (EF 41%) and reduced LV volumes enddiastolic volume from 290 to 276 mL, endsystolic volume from 192 to 163 mL (Figure 1). Considering the improved left ventricular function, consolidation of scar by CMR and the absence of ventricular arrhythmias on repeated Holter-ECG, we decided not to implant an internal cardiac defibrillator (ICD) for primary prevention of sudden cardiac death in this individual patient. No arrhythmic events occurred over more than one year of clinical follow-up so far.

DISCUSSION

First, this case report highlights that the diagnosis of isolated SHD is challenging. SHD has a wide range of clinical cardiac manifestations, *e.g.,* conduction abnormalities, ventricular arrhythmias, sudden cardiac death, congestive heart failure, valve involvement, and rarely as in this patient with pericardial effusion[1]. Conventional echocardiography appears to be not sufficient to diagnose or to exclude SHD. However, speckle tracking echocardiography has been recently discussed as a novel tool to diagnose SHD. Tsuji *et al*[8] proposed three dimensional speckle tracking radial strain as potential method to distinguish between dilated cardiomyopathy and SHD. Others groups have reported early detection of global longitudinal strain in patients with new onset SHD[9,10]. But so far, further studies are required to evaluate strain analysis as a non-invasive method in diagnosing cardiac involvement of sarcoidosis. Furthermore, data about lesion healing and therapy monitoring with strain analysis is missing compared to CMR. Nevertheless, further studies are required to better understand the potential incremental value of these techniques.

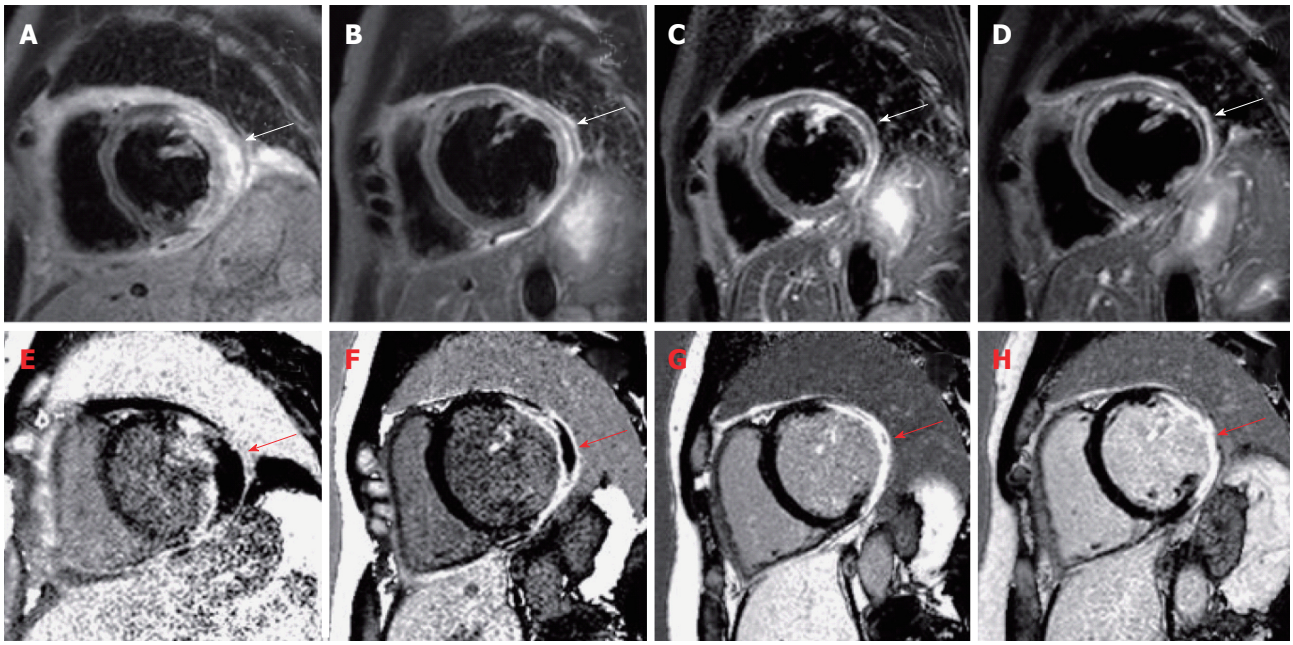

Figure 1 Note the hypointense core of the lesion on late gadolinium enhancement images, indicating massive myocardial injury with potentially myocardial hemorraghe, similar to the pattern of microvascular obstruction that can be found in patients with acute myocardial infarction. T2-weighted short-tau inversion recovery images show hyperintense areas of myocardial edema (A) and necrosis/fibrosis on Late-Gadolinium-Enhancement images (E) of the left ventricular and septal wall before therapy (A + E). Cardiovascular magnetic resonance was repeated after 1 mo (B + F), 3 mo (C + G) and 6 mo (D + H) of glucocorticoid treatment demonstrating impressive resorption of edema and consolidation of scar.

Contrarily, CMR and targeted EMB are established tools in diagnostic evaluation of suspected SHD[2,11,12]. However, the sensitivity of EMB in SHD is only 20%-30% due to the focal appearance of non-caseating granulomas and thus false negative results, but CMR targeted EMB seems to improve the sensitivity of EMB[2,11,13]. Based on different recommendations and consensus documents, EMB should be performed in recent onset heart failure (HF) or > 3 mo duration of HF, in particular if associated with new ventricular tachyarrhythmias or second/third degree atrioventricular block or rapidly deteriorating HF[2,12].

CMR is a valuable non-invasive tool to detect SHD and to monitor therapy response as shown in this case report[2,14,15]. Conduction abnormalities and/or life threatening ventricular tachyarrhythmias are common in SHD and are related to myocardial inflammation, necrosis and/or fibrosis and scar. Consequently, implantation of ICD should be carefully evaluated in all patients suffering from SHD[16-18]. However, recent reports indicate significant rates of inappropriate shocks and device complications in patients with SHD[19,20]. As shown in this case report, CMR could be used to tailor therapy in patients with SHD. On one hand, presence and extent of scar in LGE as a measure of substrate for ventricular arrhythmia could predict risk for sudden cardiac death[21]. On the other hand, edema resorption and scar consolidation on CMR, as demonstrated in this case report, could be used to identify patients with controlled disease responding to immunosuppressive therapy. Thus, CMR seems to be helpful in risk stratification and a may be used as an adjunctive tool to guide therapy in patients with SHD. However, it is important to note that estimating risk for arrhythmia and sudden cardiac death requires careful and individual decision-making as well as informed patients.

COMMENTS

Case characteristics

A 45-year-old man with no significant medical history was referred to the emergency unit with syncope followed by symptoms of acute heart failure.

Clinical diagnosis

An unclear cardiomyopathy was found clinically accompanied by pericardial effusion with hemodynamic relevance and severe mitral regurgitation.

Differential diagnosis

Myocardial infarction, dilated cardiomyopathy, giant cell myocarditis, viral myocarditis, cardiac sarcoidosis.

Laboratory diagnosis

Laboratory findings showed increased cardiac necrosis markers (Troponin, Creatinkinase, NT-proBNP), but normal angiotensin-converting enzyme blood levels as well as normal antibodies (pANCA, cANCA, antinuclear antibodies, rheumatoid factor, anti-ds-DNA, complement factors, interleukin-2 receptor).

Imaging diagnosis

Cardiovascular magnetic resonance (CMR) revealed severely impaired global systolic function with extensive edema on short-tau inversion recovery images and necrosis on late gadolinium enhancement images with a non-ischemic pattern of the lateral wall, but also the left ventricular septum.

Pathological diagnosis

Cardiac sarcoidosis.

Treatment
The authors initiated standard heart failure therapy and high-dose glucocorticoid therapy with prednisone, which was gradually reduced over months.

Related reports
Only 2%-7% of patients with sarcoidosis suffer from clinical cardiac manifestations.

Term explanation
Sarcoidosis is a granulomatous multisystem disorder of unknown etiology which has a wide range of manifestations affecting a variety of organs.

Experiences and lessons
This case highlights the challenging diagnosis and treatment of sarcoid heart disease (SHD) and the value of combining CMR with targeted endomyocardial biopsy. Moreover, this case report demonstrates the value of CMR for monitoring response to therapy in SHD.

Peer-review
This case report was well written and worth to be published in the journal.

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CASE REPORT

Hepatitis C virus positive patient diagnosed after detection of atypical cryoglobulin

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Author contributions: Ongen B, Aksungar FB, Cicek B, Serteser M and Akyar I conceived and gathered data for the case report; Cicek B obtained written informed consent from the patient; Coskun A and Unsal I were involved in literature search and data analysis; Ongen B and Aksungar FB wrote the manuscript; all authors reviewed and edited the manuscript and approved the final version of the manuscript.

Institutional review board statement: Acıbadem University School Of Medicine, Acıbadem Hospitals Review Board had approved the case for publication.

Informed consent statement: Written informed consent from the patient is obtained in the clinic.

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Abstract

A 60-year-old male patient presented with jaundice and dark urine for three days, icteric sclerae and skin rash on his legs for six months. Laboratory investigations revealed an atypical cryoglobulinemia with high hepatitis C virus (HCV)-RNA levels. Imaging studies showed cholestasis was accompanying HCV. Capillary zone electrophoresis using immunosubtraction method revealed a polyclonal immunoglobulin G and immunoglobulin A (IgA) monoclonal cryoglobulin and that IgA lambda was absent in immunofixation electrophoresis. After a liver biopsy, chronic hepatitis C, HCV related mixed cryoglobulinemia and cryoglobulinemic vasculitis were diagnosed and antiviral therapy was initiated. Our HCV patient presented with cryoglobulinemic symptoms with an atypical cryoglobulinemia that was detected by an alternative method: Immunosubtraction by capillary electrophoresis. Different types of cryoglobulins may therefore have a correlation with clinical symptoms and prognosis. Therefore, the accurate immunotyping of cryoglobulins with alternative methods may provide more information about cryoglobulin-generated pathology.

Key words: Cryoglobulinemia; Hepatitis C; Immunosu-

btraction; Immunotyping; Electrophoresis

Core tip: We describe atypical IgA monoclonal cryoglobulinemia as the presenting symptom of chronic hepatitis C. Immunotyping of the cryoglobulin was performed with capillary zone electrophoresis with immunosubtraction method which is an alternative method to classical immunofixation electrophoresis. Accurate immunotyping of cryoglobulins with alternative method provide more information about cryoglobulin-generated pathology in atypical patients.

Ongen B, Aksungar FB, Cicek B, Akyar I, Coskun A, Serteser M, Unsal I. Hepatitis C virus positive patient diagnosed after detection of atypical cryoglobulin. *World J Clin Cases* 2016; 4(3): 81-87 Available from: URL: http://www.wjgnet.com/2307-8960/full/v4/i3/81.htm DOI: http://dx.doi.org/10.12998/wjcc.v4.i3.81

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded RNA virus that causes chronic liver disease since, in most affected patients, the immune system cannot completely clear the virus. Patients may develop a variety of extra-hepatic manifestations including arthritis, arthralgia, fibromyalgia, lympadenopathy and skin lesions. These various symptoms may sometimes lead to misdiagnosis and inappropriate therapies. The interaction of immune system cells and the surface proteins of HCV can cause immunological symptoms similar to those observed in autoimmune disorders. In addition, chronic immune response to HCV can produce cryoglobulins resulting in vasculitis-related skin ulcers and immune complex related nephropathy[1].

Cryoproteins are immunoglobulins in a form that precipitates in serum and plasma[2] at low temperatures. Wintrobe and Buel first described Cryoglobulinemia in 1933, and it was clinically associated with palpable purpura, arthralgia and weakness, also known as the Meltzer's triad. Cryoproteins precipitate at temperatures below 37 ℃ and redissolve upon warming. They have clinical importance as they form intravascular precipitates, leading to clinical consequences such as obstruction in peripheral vessels resulting in Raynoud phenomenon, and immune-complex related vasculitis in skin, peripheral nerves and kidneys[3]. Three types of cryoglobulins have been defined depending on their immunoglobulin composition; Type I cryoglobulins are monoclonal immunoglobulins most frequently made of immunoglobulin M (IgM), followed by immunoglobulin G (IgG), and IgA. They are associated with immunoproliferative disorders like multiple myeloma and Waldenström macroglobulinemia[4]. Type II and type III cryoglobulins are polyclonal immunoglobulins, occasionally associated with monoclonal ones, that are considered to be mixed cryoglobulins[5]. Mixed cryoglobulins are associated with infectious and chronic inflammatory diseases, and constitute 90% of all types of cryoglobulins[6]. Among patients who have mixed cryoglobulinemia, 92% have HCV, 1.8% have hepatitis B virus (HBV) infection[5,6]; whereas only 5% of the patients with HCV infection show clinical signs of cryoglobulinemia[3]. Here we present a HCV positive patient with atypical cryoglobulinemia that was suspected by the discordant complete blood count (CBC) results. Immuno-typing of the cryoglobulin was carried out with capillary zone electrophoresis-immunosubtraction method (CZE/IS).

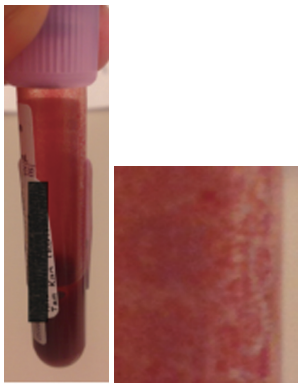

Figure 1 Agglutinations on the complete blood count tube. It was anticoagulated with K2-EDTA, analyzed at room temperature and red blood cell, Hemoglobin and Hematocrit results were discordant with each other.

CASE REPORT

A sixty-year-old male patient presented with jaundice and dark urine that had started 3 d previously. He had had a rash on his legs for 6 mo and all examinations at that time were stated to be normal. Despite steroid therapy, there was no improvement in his rash. Physical examination revealed a BP of 120/70 mmHg, icteric sclerae and skin and there were diffuse rashes on arms and legs. An ultrasonographic scan of liver revealed cholestasis with minimal parenchymal hepatosteatosis. Laboratory investigations showed discordance in RBC, haemoglobin and haematocrit values in CBC and when blood sample tube was inspected agglutinations on the walls of the tube were remarkable (Figure 1). Upon suspicion of cryoglobulinemia, a second sample was obtained from the patient. The new sample was collected in a tube which was incubated at 37 ℃ and CBC was repeated after keeping it in the incubator for 20 min at 37 ℃. This time results were concordant (Table 1) hence a sample was obtained at appropriate conditions in order to investigate cryoglobulins and a positive cryocrit was detected (Figure 2). Laboratory investigations yielded the following results: Serum protein electrophoresis was normal, cryoglobulins were positive, rheumatiod factor (RF) was positive,

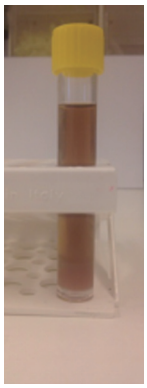

Figure 2 Cryoglobulin sediment at 4 ℃.

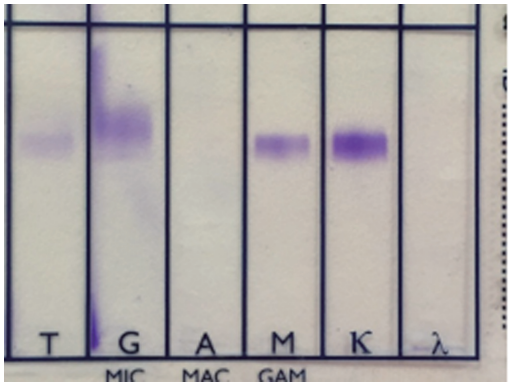

Figure 3 Cryoglobulin immunofixation electrophoresis with SAS-1 agarose gel (Helena, United Kingdom). A polyclonal IgG and monoclonal IgM kappa are detected. T lane shows total protein electrophoresis of cryoglobulin and an absent albumin band shows washing and isolating of the cryoprecipitate is successfully performed.

Complement 3 and 4 (C3, C4) levels were normal, anti-HCV antibody was positive, HCV RNA levels 6770728 IU/mL and HCV was type1bgenotype. Clinical Chemistry results were as follows (reference ranges are in parenthesis): Serum Urea concentration 66 mg/dL (17-49 mg/dL); Creatinine 0.97 mg/dL, (0.8-1.3 mg/dL); Total bilirubin, 3.8 mg/dL, (0.2-1.2 mg/dL); Conjugated bilirubin, 3.39 mg/dL (< 0.2 mg/dL), Alanine aminotransferase, 548 U/L (16-63U/L), Aspartate aminotransferase, 251 U/L (15-37 U/L); Gama-glutamyl transferase, 3126 U/L (15-85U/L); Total protein, 6.32 g/dL (6.4-8.3 g/dL); Albumin, 2.76 g/dL (3.5-5.0 g/dL); Prothrombin time (PT), 10.6 (10-14 s); International normalized ratio for PT, 0.96 (0.8-1.25). Pathological examination of a liver biopsy revealed a moderate cholestatic injury accompanying chronic hepatitis, and degenerative and dysplastic changes in bile ducts. The patient was diagnosed with chronic hepatitis C, HCV related mixed cryoglobulinemia and cryoglobulinemic vasculitis. Antiviral therapy, (ledipasvir and sofosbuvir-Harvoni) was initiated.

Detection and characterization of cryoglobulin

In the first sample that was obtained at room temperature, the cryoglobulins were agglutinated and may have acted as cold agglutinins, leading to the agglutination of erythrocytes, providing falsely low measurements of RBC and HCT, whereas the hemoglobin measurement was not affected since erythrocytes were hemolyzed prior to analysis.

Table 1 Complete blood count results at room temperature *vs* 37 ℃[1]

	At room temperature	At 37 ℃	Reference ranges
White blood cells	13.93	15.27	3.8-10.0 × 10^3/μL
RBC	2.0	3.1	4.0-6.2 × 10^6/μL
Hemoglobin	12.1	12.6	13.0-17.5 g/dL
Hematocrit	22	32.5	40%-52%
Mean corpuscular volume	111.7	103.5	80-95 fL
Mean corpuscular hemoglobin	61.4	40.1	25-34 pg
Mean corpuscular hemoglobin concentration	55	38.8	31-37 g/dL
RBC distribution width	Cannot be calculated	17.1	11.2%-15%
Platelets	124	142	150-400 × 10^3/μL

[1]Measurements are performed from different samples. RBC: Red blood cells.

For detection of cryoglobulins, prior to sample withdrawal, sample tube was warmed up to 37 ℃, and transported to the laboratory at 37 ℃. It was incubated at 37 ℃ until serum was separated. Separated serum was transferred to secondary tubes, and evaluation was carried out by incubating the tubes at 4 ℃ for seven days[4]. Tubes were inspected every day for any precipitate presence. At day 6 and 7 a precipitate was obvious and the cryocrit was measured to be 15% (Figure 2). Samples were incubated at 37 ℃ for 30 min and the precipitate dissolved. In order to separate cryoglobulins from other proteins in serum such as albumin, cryoprecipitate was washed with saline at 4 ℃, and then it was centrifuged at 1500 rpm for five minutes at 4 ℃. Supernatant was removed and saline, with the same volume of supernatant, was added. Washing was repeated for 3 times. Finally with the added saline sample it was dissolved at 37 ℃[7]. Total protein and immunoglobulin concentrations in cryocrit were analyzed; immuno-typing of cryoglobulins were made using immunofixation by agarose gel electrophoresis, and CZE/IS. Absence of an albumin band in agarose gel electrophoresis indicated washing was complete. A polyclonal band at IgG heavy chain and monoclonal bands at IgM heavy chain and kappa light chain were remarkable in agarose gel electrophoresis (Figure 3). In capillary electrophoresis, albumin band was also absent, and besides polyclonal IgG and IgA gamma-globulins there was monoclonal subtraction at IgM heavy chain and kappa light chain (Figure 4). IgA lambda was absent in IFE (Figure 3). Total protein, Immunoglobulin and light chain concentrations in the cryocrit were as follows: Total protein 200 mg/dL, IgA 2.2 mg/dL, IgG

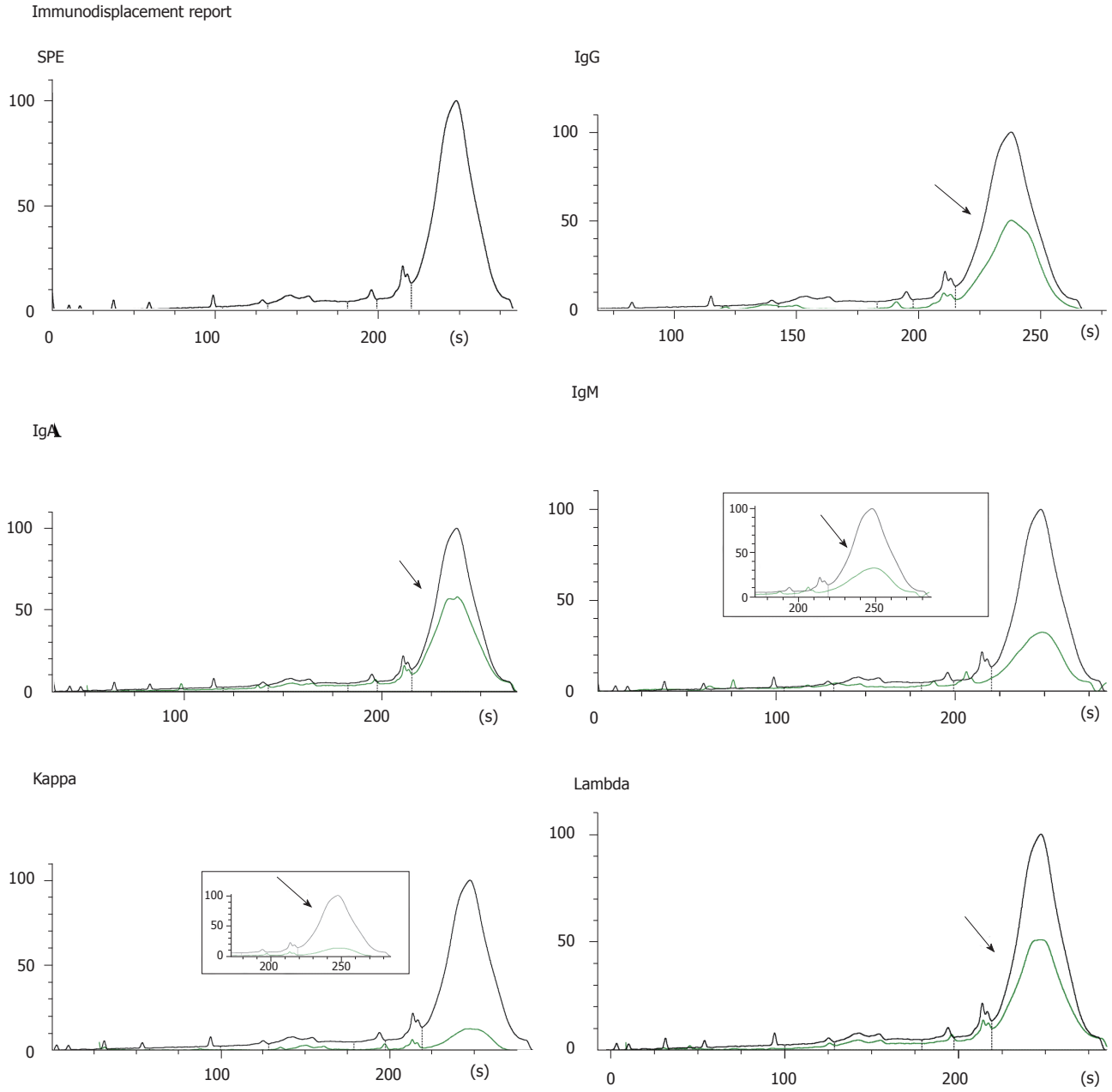

Figure 4 Cryoglobulin immunosubtraction was performed with V8 automated clinical capillary electrophoresis (Helena, United Kingdom). Arrows indicate specifically subtracted parts of immunoglobulins which mean cryoglobulin is composed of these. In this report, a mixed cryoglobulin is present: Monoclonal IgM kappa and polyclonal IgG and IgA heavy chains together with lambda light chain are detected (report shows heavy and light chains separately). Small frames indicate zoomed traces for monoclonal IgM kappa. Serum protein electrophoresis (SPE), shows total protein electrophoresis of cryoglobulin and an absent albumin band indicates washing and isolating of the cryoprecipitate is successfully performed.

28 mg/dL, IgM 108.5 mg/dL, total kappa 31.5 mg/dL, total lamda 11.8 mg/dL.

DISCUSSION

HCV has been defined as a both heterotropic and lymphotropic virus and it may exert chronic stimulus to the immune system through different viral proteins. Chronic stimulation of the B-cells by HCV epitopes may trigger increase in some B-cell subpopulations causing the production of oligoclonal and monoclonal antibodies. Those antibodies may end up as cryoglobulins and/or cold agglutinins[8]. Only 5% of HCV patients with cryoglobulinemia have clinical symptoms. Most patients, infected with the HCV have no obvious clinical symptoms, and generally patients do not know they are infected with the virus. This was the case with our patient, too. He had no clinical symptoms other than cryoglobulinemic symptoms until development of jaundice three days previously occurring probably with the increase in cholestasis.

Healthy individuals may have cryoglobulins at low concentrations (< 0.06 g/L), which do not cause any clinical symptoms[9]; however, cryoglobulins must be

Table 2 Classification of cryoglobulins and laboratory findings[4,18]

Cryoglobulin type	Content	Related diseases	Laboratory findings
Type I	Monoclonal immunoglobulins (IgG, IgM or IgA) or Bence Jones protein/ monoclonal free light chains	Multiple Myeloma Waldenstrom Macroglobulinemia Lymphoproliferative disease related monoclonal gammopathy Light chain disease	Precipitation within 24 h Hyperviscosity
Type II (mixed)	Monoclonal immunoglobulins (IgG, IgM or IgA) and polyclonal immunoglobulins (usually IgG)	HCV Essential cryoglobulinemia Sjogren's syndrome Rheumatoid arthritis Chronic lymphocytic leukemia	Precipitation within 7 d HCV positivity Decreased C3 Decreased C4 Decreased CH50
Type III (mixed)	Polyclonal immunoglobulins	Essential cryoglobulinemia Sjogren's syndrome Systemic lupus erythematosus Viral infections (HBV, CMV, EBV, HIV) Endocarditis Biliary cirrhosis	Increased autoantibodies such as ANA, ENA, AMA

Type I cryoglobulin concentrations are > 5 g/L, so that they tend to precipitate within 1 d; whereas in mixed cryoglobulinemia, development of precipitation can take a couple of days[2]. After the precipitation is observed samples should be incubated at 37 ℃ again and dissolution of the precipitates at this temperature should be confirmed. If the precipitates do not dissolve at 37 ℃, test should be reported to be negative. HBV: Hepatitis B virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; ANA: Antinuclear antibodies; ENA: Extractable nuclear antigen antibodies; AMA: Antimitochondrial antibodies; HIV: Human immunodeficiency virus.

investigated in presence of Raynaud phenomenon, peripheral cyanosis or ischemia, skin purpura, membranoproliferative glomerulonephritis, chronic HCV and HBV[10]. Circulating mixed cryoglobulins are much more common and their prevalence is stated to be 40%-50% in chronic HCV patients[11]. HCV related cryoglobulinemia is thought to be a result chronic antigenic stimulation of the humoral immune system however other clinical viral infections including HBV are not associated with the same high prevelance[11]. Biochemical grounds for why cryoglobulins precipitate at cold temperatures is not clearly understood. Protein sizes, concentration, hydrophobic content and strength of ionic bonds are thought to contribute; precipitating proteins are observed to have relatively greater ratio of hydrophobic amino acids and lower number of tyrosine and sialic acid residues[12]. IgM-RF-IgG complexes are thought to be an important factor for cryoprecipitation in mixed cryoglobulinemia[13]. Development of cryoaggregates can trigger vasculitis, whereas changes in chloride and calcium concentrations have been suggested as an influencing factor in kidneys and nerves, where cold exposure is not the case[14,15]. Our patient had recurrent lower extremity rash which is one of the vasculitic symptoms seen in especially type Ⅱ and type Ⅲ cryoglobulinemia. Although there are studies indicating the presence of bile duct abnormalities in HCV patients, a direct correlation between these abnormalities and cryoglobulinemia was not shown[16,17]. Table 2 illustrates types of cryoglobulins and laboratory findings.

Sample withdrawal and transport are the most important and the critical steps for detection of cryoglobulins. Several groups have been described different analytic approaches for detection of cryoglobulins[4,10,18-20]. The main reason for false negative cryoglobulin results is incorrect withdrawal and transport procedures. United Kingdom National External Quality Assurance Scheme (UKNEQAS) organization conducted a research about detection and reporting of cryoglobulins in 137 laboratories; only in 36% of the laboratories the analysis was done without letting the temperature drop below 37 ℃ during serum separation, sample transport and centrifugation[7].

After washing and isolating the cryoprecipitates, cryoglobulin quantification and characterization is essential for follow up and prognosis of the patients[21]. Total protein and immunoglobulin content can be measured by nephelometry while immunotyping can be performed by immunofixation electrophoresis (IFE). CZE/IS is an alternative method to IFE for immunotyping of monoclonal M-spike in immunoproliferative malignancies.We have used both IFE and CZE-IS methods for immunotyping of the cryoprecipitate in our patient and found out their results were slightly different. In the IFE study a polyclonal IgG and monoclonal IgM kappa bands were detected however CZE-IS study revealed a monoclonal IgM kappa and a polyclonal IgA lambda besides a polyclonal IgG content. With these findings we think we are facing an atypical mixed cryoglobulinemia, between Type Ⅱ and Ⅲ which may have caused cryoglobulinemic symptoms in our patient.

During IFE, specific antibodies are overlaid after electrophoresis and the corresponding immunoglobulin heavy and light chains are bound and stained. IFE is a highly sensitive and specific method to classify monoclonal immunoglobulin[22]. With the development of CZE, Immunosubtraction method is began to be used as an alternative method combined with CZE for identifying monoclonal immunoglobulins. Immunosubtraction, separates serum proteins after incubating serum with antisera for heavy and light chains, thus removing them, and detection is based on their absence when compared to serum protein electrophoresis. In CZE, the sample runs through the narrow capillary tubes and direct protein detection is performed by a measurement at 200 nm, eliminating the need for staining. We have detected especially IgA lambda by CZE/IS, which was not detected with IFE in this particular patient.

As it is previously stated only 5% of HCV patients with cryoglobulinemia have clinical symptoms[3], however, our patient had cryoglobulinemic symptoms with an atypical cryoglobulinemia. Hence, different

types of cryoglobulins may have a correlation with the presentation of clinical symptoms. Although our theory must be confirmed with additional case reports we conclude that accurate immunotyping of cryoglobulins with alternative methods like CZE/IS may provide opportunities for proper management of these special patients.

COMMENTS

Case characteristics

A sixty-year old male patient presented with jaundice and dark urine for 3 d and rash on his legs for six months.

Clinical diagnosis

Jaundice and dark urine with a rash on legs for six months.

Differential diagnosis

All vasculitic syndromes, viral hepatitis, autoimmune diseases.

Laboratory diagnosis

There was a discordance in red blood cells, haemoglobin and haematocrit values in complete blood count and when blood sample tube was inspected agglutinations on the walls of the tube were remarkable. A cryocrit was positive with an atypical presentation.

Imaging diagnosis

An ultrasonographic scan of liver revealed cholestasis with minimal parenchymal hepatosteatosis.

Pathological diagnosis

Moderate cholestatic injury accompanying with chronic hepatitis, and degenerative and dysplastic changes in bile ducts revealed in liver biopsy.

Treatment

Ledipasvir and sofosbuvir were initiated for hepatitis C virus (HCV) theraphy.

Related reports

Chronic immune response to HCV can produce cryoglobulins resulting in vasculitis-related skin ulcers and immune complex related nephropathy. These various symptoms may sometimes lead to misdiagnosis and inappropriate therapies.

Term explanation

Cryoglobulinemia, cryoproteins are immunoglobulins in a form that precipitates in serum and plasma at low temperatures resulting in various vasculitic symptoms.

Experiences and lessons

Different types of cryoglobulins may have a correlation with clinical symptoms and prognosis. Accurate immunotyping of cryoglobulins with alternative methods may provide more information about cryoglobulin-generated pathology.

Peer-review

A case report written by Ongen *et al* describes a unique case with HCV infection that was diagnosed by the presence of mixed cryoglobulinemia. They analyzed the characteristics of the methods for the detection of various cryoglobulins that are essential for the diagnosis of cryoglobulinemia. The case is interesting and their analysis regarding the methods for the detection of cryoglobulin such as capillary zone electrophoresis with immunosubtraction or agarose gel electrophoresis provides important information to the readers.

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CASE REPORT

Combination therapy with daclatasvir and asunaprevir for dialysis patients infected with hepatitis C virus

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Abstract

The standard antiviral therapy for dialysis patients infected with hepatitis C virus (HCV) is (pegylated) interferon monotherapy, but its efficacy is insufficient. Oral direct-acting antiviral agents (DAAs) have recently been developed for chronic hepatitis C patients. However, some DAAs have contraindications for chronic renal failure (CRF). Daclatasvir and asunaprevir are metabolized largely in the liver and are not contraindicated in CRF. Combination therapy with daclatasvir and asunaprevir was used for 4 dialysis patients infected with genotype 1b HCV. One patient had viral breakthrough, and the 3 others had sustained virological response 12. One patient was admitted for heart failure and percutaneous coronary intervention due to concomitant ischemic disease. Heart failure was unlikely to be caused by the combination therapy, as it was probably due to water overload. The patient continued to receive the combination therapy after the remission of the heart failure. The combination therapy was well tolerated in the other patients.

Key words: Hepatitis C; Oral drug; Daclatasvir; Asunaprevir; Dialysis

Core tip: Oral combination therapy with the direct-acting antiviral agents daclatasvir and asunaprevir, which are

both metabolized largely in the liver, is a very useful strategy for dialysis patients infected with genotype 1b hepatitis C virus. Although there were only 4 dialysis patients, the combination therapy was effective and showed a relatively favorable safety profile. One patient was admitted for heart failure with or without pneumonitis and percutaneous coronary intervention, although the causal relationship between these adverse events and the combination therapy was interpreted as negative. Our case reports warrant further studies, although careful observation during the treatment is needed.

Sato K, Yamazaki Y, Ohyama T, Kobayashi T, Horiguchi N, Kakizaki S, Kusano M, Yamada M. Combination therapy with daclatasvir and asunaprevir for dialysis patients infected with hepatitis C virus. *World J Clin Cases* 2016; 4(3): 88-93 Available from: URL: http://www.wjgnet.com/2307-8960/full/v4/i3/88.htm DOI: http://dx.doi.org/10.12998/wjcc.v4.i3.88

INTRODUCTION

Combination therapy with daclatasvir and asunaprevir has been reported to have a sustained virological response (SVR) rate of over 80% after 24 wk of therapy in chronic hepatitis C patients with genotype 1b[1,2] and is available in Japan. These 2 direct-acting antiviral agents (DAAs) are recommended for patients with chronic hepatitis C with genotype 1b based on Japan Society of Hepatology guidelines for the management of hepatitis C virus (HCV) infection[3] in Japan.

Currently, the Kidney Disease Improving Global Outcomes and the Japanese Society for Dialysis Therapy recommend antiviral therapy for dialysis patients infected with HCV[4,5]. The standard antiviral therapy for dialysis patients infected with HCV has been (pegylated) interferon (IFN) monotherapy because of the contraindication of ribavirin due to potential renal toxicity. However, the efficacy of (pegylated) IFN monotherapy has been insufficient for patients infected with HCV.

Although some oral DAAs are contraindicated for chronic renal impairment, daclatasvir and asunaprevir are both metabolized largely in the liver and are not contraindicated in chronic renal failure. Thus, we treated dialysis patients infected with genotype 1b HCV. The resistance-associated variants (RAVs) were analyzed by the PCR-invader method[6] or direct sequencing[7].

We received written informed consent from all 4 patients. The submitted case reports comply with the Declaration of Helsinki. Here, we report 4 dialysis patients infected with genotype 1b HCV that were treated with the combination therapy of daclatasvir and asunaprevir as case reports. To our knowledge, our report is the first to show the effectiveness of oral DAAs for dialysis patients in Japan.

CASE REPORT

In all patients, the HCV genotype was 1b and the severity of liver disease was judged as chronic hepatitis based on the laboratory data and imaging. After we started this combination therapy, we principally checked the laboratory data and adverse events on a weekly basis. The laboratory findings, treatments and outcomes of all cases are shown in Table 1.

Case 1

A 62-year-old female is receiving dialysis due to chronic renal failure caused by chronic glomerulonephritis at 26 years of age. Chronic hepatitis C was diagnosed at 39 years of age probably due to post-transfusion hepatitis after transfusion at 28 years of age. She received a liver biopsy approximately 10 years ago, and the histology showed fibrous portal expansion without bridging fibrosis. At that time, the platelet count was already several tens of thousands. Thus, she has had thrombocytopenia for unknown reasons since that time. The severity of liver disease was judged as chronic hepatitis based on other laboratory data and imaging. She received peginterferon α-2a monotherapy, but the virological response was partial. Regarding the RAVs, the L31 amino acid mutation was negative, but the D168E and Q80L amino acid mutations were positive and the Y93H mutation was slightly positive. We fully informed her using general information including the SVR rate of the combination therapy of daclatasvir and asunaprevir and provided her with some information about the effect and safety of this combination therapy for dialysis patients. However, she had a strong desire to receive this combination therapy. Thus, we began this combination therapy. The serum viral load disappeared at week 2 of therapy, but reappeared at week 4 of therapy. After we confirmed the positivity of the serum HCV RNA in weeks 7 and 8, we diagnosed a viral breakthrough, and the combination therapy was discontinued. The reason is that the most recent guidelines from the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labor and Welfare recommends consideration for the discontinuation of antiviral therapy in the case of viral breakthrough referring to the reappearance of HCV RNA while still on therapy in patients who have a > 1 $\log_{10}$ IU/mL increase in HCV RNA above the nadir. The maintenance of combination therapy for the patient who had viral breakthrough may promote multiple drug resistance. The patient's adherence to both drugs was 100% until then. During the therapy, there were no adverse events, and the therapy was well tolerated. After the viral breakthrough, we checked the RAVs approximately 2 and 9 mo after the discontinuation of the combination therapy. The mutation profile of the RAVs was almost the same as that of the baseline, but the virus newly achieved a V170I amino acid mutation. She took 600 mg ursodeoxycholic acid before starting the combination therapy and continued to take it during the combination therapy and after the discontinuation of the combination therapy. We increased the dose up to 900 mg because

Table 1 Laboratory findings at baseline, treatments and outcomes of dialysis cases

Parameters	Case 1	Case 2	Case 3	Case 4
Age (yr)	62	61	72	70
Sex	Female	Male	Male	Male
BMI (kg/m^2)	17.9	20.9	19.4	22.3
HCV genotype	1b	1b	1b	1b
Cause of dialysis	CGN	CGN	CGN	DM
IFN-based therapy: Outcome	PEG-IFN-α2a: partial response	Naive: NA	Naive: NA	Naive: NA
At the start of therapy				
HCV RNA (log_{10} IU/mL)	4.7	6	6.3	4.9
ALT (IU/L)	54	18	36	19
AST (IU/L)	36	20	31	13
WBC (cells/μL)	1660	3780	4330	5200
Hemoglobin (g/dL)	10.5	11.3	12.4	11.6
Platelets (cells/μL)	72000	204000	146000	218000
RAVs at baseline	D168E, Y93Y/H, Q80L	None	None	None
Severity of liver disease	Chronic hepatitis	Chronic hepatitis	Chronic hepatitis	Chronic hepatitis
Treatment and outcome				
Daclatasvir dosage (mg)	60	60	60	60
Asnaprevir dosage (mg)	200	200	200	200
Week of serum HCV RNA disappearance	2	3	3	2
Adherence to daclatasvir	100%[1]	100%	100%	100%
Adherence to asnaprevir	100%[1]	100%	100%	100%
Weeks of therapy	9	24	24	24
Response	Breakthrough	SVR12	SVR12	SVR12
Adverse events	None	None	None	Heart failure, Pneumonitis, Myocardial ischemia

[1]The patient's adherence to both drugs was 100% until the date of the discontinuation of the combination therapy. BMI: Body mass index; CGN: Chronic glomerulonephritis; DM: Diabetes mellitus; NA: Not applicable; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; WBC: White blood cells; RAVs: Resistance-associated variants; SVR: Sustained virological response.

of the flare-up of serum alanine aminotransferase (ALT) after the discontinuation of the combination therapy, and the serum ALT then improved. The time course after the start of the combination therapy is shown in Figure 1.

Case 2

A 61-year-old male is receiving dialysis due to chronic renal failure probably caused by chronic glomerulonephritis at 28 years of age. The transmission source of hepatitis C may have been a blood transfusion when he received a cadaveric renal transplant at 30 years of age. He did not receive interferon-based therapy. He had no RAVs that were associated with the combination therapy. We started this combination therapy, and the patient's adherence to both drugs was 100%. Serum HCV RNA became undetectable in week 3 of therapy, and then he achieved rapid virological response (RVR) and SVR12. During the therapy, there were no adverse events, and the therapy was well tolerated.

Case 3

A 72-year-old male is receiving dialysis due to chronic renal failure caused by chronic glomerulonephritis since 1977. The transmission source of hepatitis C may have been a blood transfusion. His medical history included prostate cancer at 64 years of age, radiation proctitis due to radiation therapy for the prostate cancer, and a cerebral lacunar infarction at 65 years of age. Moreover, he had arrhythmia, bronchial asthma, goiter, and secondary hyperparathyroidism. He did not take any concomitant-use forbidden or combined-use caution medications and did not receive interferon-based therapy. He had no RAVs that were associated with the combination therapy. We started this combination therapy and the patient's adherence to both drugs was 100%. The serum HCV RNA became undetectable on week 3 of therapy, and then he achieved RVR and SVR12. During the therapy, there were no adverse events, and the therapy was well tolerated.

Case 4

A 70-year-old male is receiving dialysis due to chronic renal failure arising from diabetic nephropathy in 2014. The transmission source of hepatitis C could not be identified. He had not received interferon-based therapy. He had no RAVs that were associated with the combination therapy. As he had taken nifedipine as a combined-use caution, nifedipine was discontinued after consultation with an attending cardiovascular specialist. Thus, we started this combination therapy. On day 60 of the therapy, he experienced difficulty breathing and admitted himself to the hospital where he was receiving dialysis. He was diagnosed with a heart failure that was unlikely to bear a causal relationship to the combination therapy, but rather was probably due to water overload, as postulated by an attending nephrologist in the hospital. The duration of hospitalization was 6 d. On day 1 after discharge, he experienced difficulty breathing and was hospitalized again. He was diagnosed as suffering from heart failure and pneumonitis and received antibiotics and volume control through dialysis. Then, he was discharged without symptoms. He could continue to receive the combination therapy with daclatasvir and asunaprevir after the remission of heart failure. As the attending nephrologist suspected that the deterioration of cardiac function might be a cause of heart failure in this episode, he asked the attending cardiovascular specialist to check the condition of the coronary arteries. Then, he admitted himself to our hospital to receive percutaneous coronary intervention

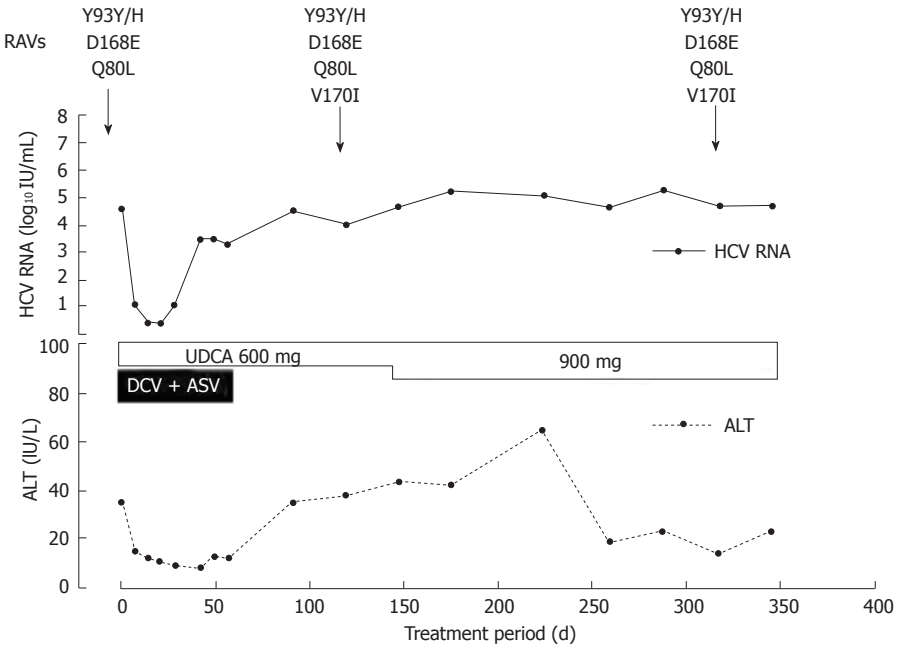

Figure 1 Clinical course of Case 1 of viral breakthrough during the combination treatment with daclatasvir and asunaprevir. RAVs: Resistance-associated variants; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; UDCA: Ursodeoxycholic acid; DCV: Daclatasvir; ASV: Asunaprevir.

on day 101 of therapy, and ischemia was diagnosed and treated by a drug-eluting stent (XIENCE Alpine®, Abbott Vascular Japan Co., Ltd., Minato-ku, Tokyo, Japan). After discharge from the hospital, he could continue to receive the combination therapy with daclatasvir and asunaprevir safely until the target completion date. Twenty-six days after the completion of the combination therapy, he was admitted to our hospital to recheck the condition of the coronary arteries, and ischemia was not diagnosed. The serum HCV RNA became undetectable in week 2 of therapy, and then he achieved RVR and SVR12. The patient's adherence to both drugs was 100%.

DISCUSSION

The major findings from these case reports are that the combination therapy with daclatasvir and asunaprevir was effective for dialysis patients and generally well tolerated. Sofosbuvir and ledipasvir have been recently approved and are available in Japan. The treatment period of this drug combination therapy is 12 wk, which is half the length of the 24-wk combination therapy with daclatasvir and asunaprevir. Moreover, there is little possibility of developing serious adverse events such as elevation of transaminase levels in the combination therapy of sofosbuvir and ledipasvir. However, this combination therapy has a flaw in that it is contraindicated in patients with chronic renal failure (eGFR < 30 mL/min per 1.73 m^2) and dialysis. Thus, the combination therapy with daclatasvir and asunaprevir is quite useful for dialysis patients and superior to the combination therapy of sofosbuvir and ledipasvir in this regard. To our knowledge, this is the first report that shows the effectiveness of the oral DAAs for dialysis patients in Japan.

The standard therapy for IFN therapy in dialysis patients includes pegylated IFN α-2a, natural IFN α, recombinant IFN α-2b, and natural IFN β. Only approximately one-third of hemodialysis patients with chronic hepatitis C achieve SVR with standard IFN monotherapy[4]. The SVR rate is 14%-75% by pegylated IFN α-2a monotherapy[5]. As these trials include patients with various genotypes, we have difficulty accurately evaluating the SVR rate in dialysis patients by (pegylated) IFN monotherapy. In our case reports, however, 3 dialysis patients without RAVs achieved SVR12. Regarding Case 1, perhaps we should not have treated her in light of RAVs at baseline, even though she had a strong desire to receive this combination therapy. By selecting the subjects to treat by examination of RAVs, a high SVR rate in dialysis patients with genotype 1b HCV would be expected.

The heart failure observed in Case 4 is unlikely to bear a causal relationship to the combination therapy, as mentioned previously. As dialysis patients are likely to develop heart failure based on the disease itself or iatrogenically, we should pay close attention to the development of heart failure during the combination therapy.

To treat dialysis patients, a better understanding of the metabolism of daclatasvir and asunaprevir is quite important. Daclatasvir is the substrate of CYP3A and metabolized mainly by CYP3A4, which is a member of the cytochrome P450 superfamily of enzymes and mainly found in the liver and the intestine. In the single oral administration of daclatasvir, it is eliminated 88% in fecal matter and 6.6% in urine. Asunaprevir is the substrate of OATP1B1 and OATP2B1 and metabolized mainly by CYP3A. In the single oral administration of

asunaprevir, it is eliminated 84% in fecal matter and less than 1% in urine. Thus, both drugs are mainly eliminated through the fecal route.

In end-stage renal disease (ESRD) foreign patients, the area under the curve (AUC) values for total and unbound daclatasvir were 26.9% and 20.1% higher than those with normal renal function by the single oral administration of 60 mg daclatasvir, respectively. The AUC and Cmax values for asunaprevir were 10.1% lower and 28.6% higher than those with normal renal function in a twice a day for 7-d repeated dose study in ESRD foreign patients, respectively.

Generally, dialysis patients are likely to take various medicines due to their complications. Daclatasvir and especially asunaprevir have concomitant drugs that should be cautiously used. We should cease or change concomitant drugs that should be avoided or carefully used before the combination therapy. Calcium-channel blockers are one such concomitant drug class to be carefully used. In Case 4, we discontinued nifedipine for ischemic heart disease in advance and started the administration of the combination therapy after we verified that there was neither an increase in blood pressure nor an angina attack. Thus, the concomitant drugs and complications were completely checked in advance, and the application of this combination therapy was cautiously considered, as is also the case for patients not receiving dialysis. The administration of the combination therapy may be performed after the discontinuation or change of a combined-use caution situationally.

The combination therapy of daclatasvir and asunaprevir sometimes causes elevated transaminase levels, and this adverse event can develop at any time during the therapy. Thus, we should frequently check for liver dysfunction, regardless of dialysis. The 4 patients in our case reports did not experience an elevation in transaminase levels. As the dialysis patients are likely to have concomitant drugs and complications, we need to have more careful follow-up for them.

Our case reports have limitations. The sample size of our case reports is very small, and the serum concentrations of daclatasvir and asunaprevir during the therapy were not measured.

In summary, the combination therapy of daclatasvir and asunaprevir was a very useful strategy for dialysis patients infected with genotype 1b HCV. Large prospective studies of the combination therapy of daclatasvir and asunaprevir for dialysis patients are needed in the near future to confirm the results of our case reports.

COMMENTS

Case characteristics

Almost free of symptoms in all 4 cases.

Clinical diagnosis

Dialysis patients infected with hepatitis C virus (HCV).

Laboratory diagnosis

The blood draw showed hepatitis C viremia and chronic renal failure.

Imaging diagnosis

Abdominal computed tomography or echography revealed that all cases were considered chronic hepatitis according to the severity of liver disease.

Treatment

All cases were treated with daclatasvir and asunaprevir.

Term explanation

Direct-acting antiviral agents (DAAs): DAAs are molecules that target specific nonstructural proteins of the virus and disrupt viral replication and infection. Resistance-associated variants (RAVs): RAVs have been detected in the treatment-naive HCV as well as after drug exposure and are thought to result from genetic variation inherent in the virus itself as well as selective pressure from drugs.

Experiences and lessons

The combination therapy of daclatasvir and asunaprevir is a very useful strategy for dialysis patients infected with genotype 1b HCV, but careful selection of subjects for treatment should be performed by examination of the RAVs.

Peer-review

The authors reported combination therapy with daclatasvir and asunaprevir for four dilaysis patients infected with HCV genotype 1b. Except for one patient which was discontinued after viral breakthrough, this combination therapy was quite effective, however long term observation and monitoring of patients should be done. Overall, this combination therapy was well tolerated in patients. This case report will be useful as a base for further investigate the effectiveness of the combination therapy of daclatasvir and asunaprevir in dialysis patients.

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CASE REPORT

Hypertension in the liver clinic - polyarteritis nodosa in a patient with hepatitis B

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Author contributions: Laroia ST worked on concept, design, clinical and imaging work up, data acquisition, analysis and manuscript writing; Lata S did the clinical data interpretation and helped in editing of the manuscript; Laroia ST designed the study and wrote the manuscript.

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Informed consent statement: All study participants provided informed written consent prior to study enrolment.

Conflict-of-interest statement: The authors declare that they have no conflict of interest whatsoever including but not limited to commercial, personal, political, intellectual, or religious interests.

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Abstract

Chronic hepatitis caused by hepatitis B virus (HBV) is an endemic disease in India. It is associated with extra-hepatic manifestations like polyarteritis nodosa (PAN) which is a vasculitis like disorder, presenting in subacute or chronic phase; involving visceral and systemic vessels. It should always be considered as a possible etiology of hypertension in an underlying setting of hepatitis B. We describe a 56-year-male patient with a history of chronic HBV who presented to the outpatient clinic with history of recent onset hypertension and suspected liver disease. Further work up for the cause of recent hypertension included a contrast computerized tomography of abdomen, which revealed concomitant pathologies of chronic liver disease and multiple aneurysms in bilateral kidneys. This case illustrates the unusual presentation of extrahepatic manifestation of viral hepatitis in the form of PAN of kidneys. PAN as an independent entity may be missed in specialized clinics evaluating liver pathologies, due to its insidious onset, atypical clinical symptoms and multi-systemic manifestations. The knowledge of extrahepatic, renal and vascular manifestations of hepatitis B unrelated to liver disease should be considered by physicians at the time of diagnosis and management of patients with HBV.

Key words: Hepatitis B; Polyarteritis nodosa; Hypertension; Liver; Extra-hepatic; Vascular

Core tip: Extra hepatic manifestation of viral hepatitis B infection may be its first presenting symptom. This unusual presentation may be in the form of hypertension in a middle aged patient which is usually a part of vasculitis like disease process such as polyarteritis nodosa (PAN). PAN as an independent entity may be missed in specialized clinics evaluating liver pathologies, due to its insidious onset, atypical clinical symptoms and multi-systemic involvement. It is prudent for diagnosticians and physicians to be aware of this entity and its imaging features, which may help as pointers to its diagnosis.

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INTRODUCTION

India has a vast carrier pool of hepatitis B and C viruses, which are the leading causes of chronic hepatitis in the country[1]. Most of these patients, reporting to specialized liver hospitals and institutions are usually investigated and managed, primarily for liver and related issues. A small percentage (usually 6%-7%) may have subclinical or overt extrahepatic manifestations of hepatitis[2,3]. These include glomerulonephritis, polyarteritis nodosa (PNA), aplastic anemia, cryoglobulinemia and other immune related disorders. It has been hypothesized that immune related mechanisms are responsible for such systemic extrahepatic manifestations in patients with hepatitis B[4].

CASE REPORT

A 56-year-old gentleman who had long standing history of hepatitis B antigen positive, presented to our liver hospital out-patient clinic with recent onset hypertension. No obvious associated symptoms were present. No history of fever, rash, arthralgia, visual disturbances, headaches, fatigue or change in urinary output could be elicited. Findings of physical examination were unremarkable. Examination of peripheral pulses revealed no asymmetry, bruit or radio-femoral delay. Renal bruit was absent. Laboratory tests revealed microcytic hypochromic anemia with a hemoglobin level of 10.5 g/dL (Reference range 13-17 g/dL). The differential leukocyte counts were normal. Liver function tests showed mildly elevated liver enzymes with Aspartate Aminotransferase of 76 IU/L (Reference range 5-40 IU/L) and Alanine Aminotransferase of 104 (Reference range 10-40 IU/L). Serum proteins, Albumin: Globulin ratio and the bilirubin levels were normal. The renal function tests showed normal blood urea, serum creatinine, serum electrolytes and uric acid levels. Markers for hepatitis viruses showed the following results: Hepatitis B surface antigen (HBsAg) was positive with negative hepatitis C antibodies. HBsAg and HBe antigen were reactive. Hepatitis B virus (HBV) DNA was tested by real time polymerase chain reaction. Viral load at the time of presentation in the patient was 5.8×10^7 IU/mL.

Multiphase dynamic computed tomography (CT) of the abdomen was performed to evaluate the status and extent of parenchymal chronic liver disease. It showed imaging features of irregular contours of the liver, prominent caudate lobe and widened periportal space consistent with chronic liver disease (Figure 1). Incidentally, CT angiography revealed multiple aneurysms of main right and left segmental renal arteries, seen predominantly on the left side (Figure 2).

Immunology profile including antinuclear antibody and antimyeloperoxidase antibodies (anti MPO) were negative. These findings were in accordance with a diagnosis of polyarteritis nodosa. Differential diagnoses of segmental arterial mediolysis (which is a non-inflammatory vasculopathy), Microscopic polyangiitis and granulomatosis with polyangiitis (which are ANCA-associated systemic vasculitides) were considered. These disorders have some features similar to those of classic PAN, with the additional involvement of renal glomeruli and pulmonary capillaries. These could however, be excluded with the help of imaging and laboratory findings, viral markers and subsequent therapeutic response. As per institute policy and standard guidelines, the patient was prescribed corticosteroids for initial two weeks to control the inflammatory damage to organs. Antiviral therapy with Lamivudine was started after the course of short term steroids to bring down the viral load and seroconversion of the patient. The follow up serology revealed conversion of hepatitis B e antigen to anti HBe antibody after six months. The patient made a good recovery without any significant side effects and tolerated the therapy well.

An abdominal CT performed after one year demonstrated no increase in the size and extent of the aneurysms and appeared as stable disease. The extent of liver disease also remained stable with no change in the liver size and no signs of decompensation or portal hypertension.

DISCUSSION

Pathophysiology and incidence

About one percent of HBV infected patients and less than one percent patients of HCV infection are known to develop PAN as an extrahepatic manifestation of liver disease[5,6]. These patients can develop features of PAN as early as, six months post infection[7]. Thirty percent patients of PAN have HBV infection as their etiological factor whereas the rest are idiopathic[5]. Microaneurysms

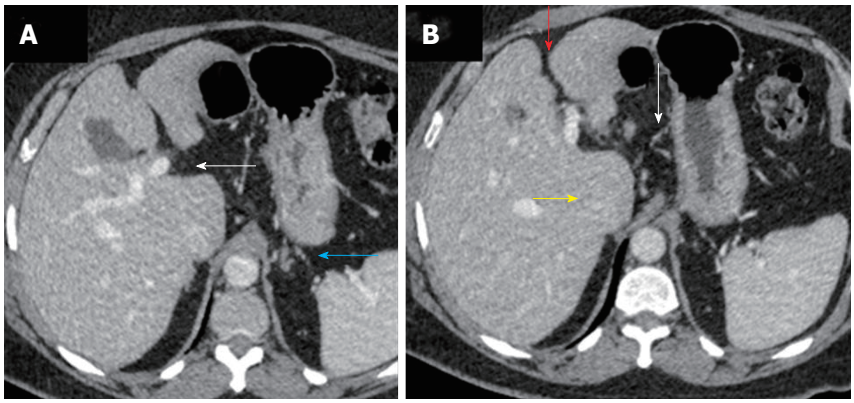

Figure 1 Triple phase dynamic contrast enhanced computed tomography performed for the upper abdomen showed features of chronic liver disease, predominantly on the portal venous phase. A: Subtle sign of periportal space widening (white arrow) and few small collaterals in the peri-gastric location (blue arrow) suggesting features of chronic liver disease on computed tomography scan; B: Irregular liver margins seen better in the inter-lobar fissure (red arrow) with mild caudate enlargement (yellow arrow) and perigastric collaterals (white arrow).

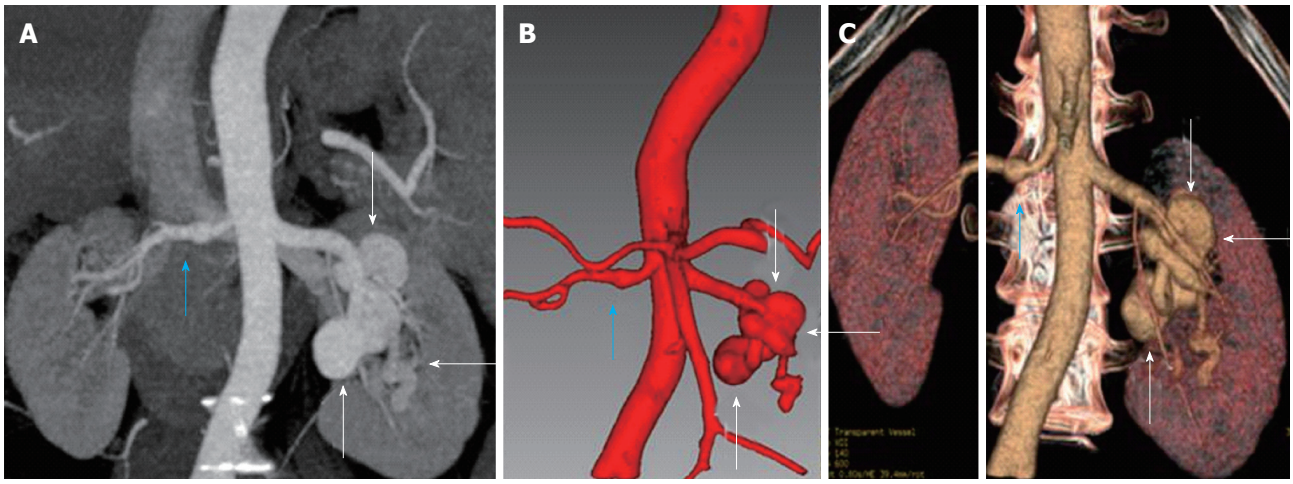

Figure 2 Reformatted images of the bilateral renal artery aneurysms, in right main renal artery and the segmental left renal artery divisions. A: Coronal minimum intensity projection reformat of arterial phase of the triple phase computed tomography (CT) scan showing right main renal artery aneurysm (blue arrow) and multiple left segmental division branch aneurysms (white arrows); B: Coronal reformats of arterial phase of the CT angiogram performed using Myrian Intrasense software for liver showing right main renal artery aneurysm (blue arrow) and multiple left segmental division branch aneurysms (white arrows); C: Coronal reformats of arterial phase of the CT angiogram performed using Myrian Intrasense software for liver showing right main renal artery aneurysm (blue arrow) and multiple left segmental division branch aneurysms (white arrows).

are the most common presentation of PAN and are predominantly seen to involve the vasculature supply of kidneys, mesentery and liver[8]. Circulating immune complexes containing viral proteins, deposit in vessel walls of visceral arteries and induce focal inflammation resulting in stenosis and microaneurysms[9].

Diagnosis and clinical features

The diagnosis of HBV is based on serological demonstration of viral markers namely HBsAg, anti-HBsAg, immunoglobulin M, anti hepatitis B core (HBc) antibody and HBV DNA[1]. Clinical presentation of PAN includes involvement of cutaneous and peripheral nerves, vessels as well as multisystemic vasculitis. Diagnosis may be made with the help of muscle, skin or nerve biopsy and in cases of visceral involvement by angiography to demonstrate multiple aneurysms[10]. Isolated multiple bilateral renal aneurysms are very rare and the most common cause would be systemic vasculitis such as seen in PAN, as was present in our patient. Hypertension is an indirect presentation of renal artery aneurysms which cause segmental dilatation and stenosis of the renal vessels due to aneurysm formation.

Management

In a case where hepatitis B is concomitantly present with an extrahepatic manifestation such as PAN, management would consist of combined immunosuppressant and antiviral therapy[4]. Recent role of plasmapheresis has been emphasized in clearing up circulating immune complexes in this group of patients. Remission of PAN is associated with the loss of HBV DNA replication. This was also observed in the patient described in our

study above. The 5-year survival has been predicted as 75%[11].

Complications

Renal or peri-renal haematomas may result from the rupture of microaneurysms. The indications for treatment of a renal artery aneurysm are the presence of intra-aneurysmal clots, hypertension and potential for rupture.

Hypertension in the liver clinic may be the only manifestation of PAN; hence this association should be ruled out using imaging techniques such as CT angiography, for better management of this subgroup. All physicians need to be aware of concomitant presentation of PAN as hypertension and systemic vascular aneurysms in a patient of chronic liver disease.

COMMENTS

Case characteristics

A middle aged gentleman with history of positive hepatitis B antigen, presented to the out-patient clinic of the liver care hospital with recent onset hypertension.

Clinical diagnosis

The physical examination including examination of renal bruit, peripheral pulses and clinical history pertaining to the symptoms were unremarkable, hence essential hypertension with chronic liver disease was the working clinical diagnosis.

Differential diagnosis

Included: Essential hypertension with underlying liver disease, renal artery stenosis with chronic liver disease, chronic renal and liver disease leading to hypertension.

Laboratory diagnosis

The patient's laboratory tests revealed microcytic hypochromic anemia with a hemoglobin level of 10.5 gm/dL. The differential leukocyte counts were normal. Liver function tests showed mildly elevated liver enzymes with aspartate aminotransferase of 76 IU/L (Reference range 5-40 IU/L) and alanine aminotransferase of 104 (Reference range 10-40 IU/L). Serum proteins, Albumin: Globulin ratio and the bilirubin levels were normal. The renal function tests showed normal blood urea, serum creatinine, serum electrolytes and uric acid levels. Hepatitis B surface antigen was positive. Hepatitis B surface antigen and HBe antigen were reactive. Hepatitis B virus DNA was tested by real time polymerase chain reaction. Viral load at the time of presentation in the patient was 5.8×10^7 IU/mL.

Imaging diagnosis

Multiphase dynamic computed tomography (CT) of the abdomen showed irregular contours of the liver, prominent caudate lobe and widened periportal space consistent with chronic liver disease with incidental CT angiography revealing multiple aneurysms of the main right and segmental left renal arteries.

Pathological diagnosis

Immunology profile including antinuclear antibody and antimyeloperoxidase antibodies (anti MPO) were negative and no further biopsy was carried out to rule out cause of the vasculitis like process due to the well-known association of polyarteritis nodosa (PAN) with hepatitis B infection and classical imaging findings.

Treatment

As per institute policy and standard guidelines, the patient was prescribed corticosteroids for two weeks to control the inflammatory damage to organs. Antiviral therapy with Lamivudine was started after the course of short term steroids to bring down the viral load and seroconversion of the patient. The follow up serology revealed conversion of hepatitis B e antigen to anti HBe antibody after six months.

Related reports

Very few cases of isolated renal, multiple bilateral aneurysms, which are usually otherwise seen in systemic vasculitis have been reported in literature. In our case study, the unusual finding of PAN indirectly presenting as hypertension due to renal artery aneurysms with resultant segmental dilatation and stenosis in a case of underlying chronic liver disease has been highlighted.

Term explanation

PAN is an immune system related disorder where circulating immune complexes containing viral proteins, deposit in vessel walls of visceral arteries and induce focal inflammation resulting in stenosis and microaneurysms commonly involving the vasculature supply of kidneys, mesentery and liver.

Experiences and lessons

Hypertension in the liver clinic may be the only manifestation of PAN and should be ruled out using imaging techniques such as CT angiography for better management of this subgroup. All physicians need to be aware of concomitant presentation of PAN as hypertension and systemic vascular aneurysms in a patient of chronic liver disease.

Peer-review

This study reports the case of PAN in a 56-year-male patient with a history of hepatitis B infection. This case illustrates the unusual presentation, hypertension, of extrahepatic manifestation of viral hepatitis in the form of PAN of kidneys. This case has the reference value for clinical settings. The manuscript's presentation is well and readable.

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