High prevalence of hepatitis C virus-ribonucleic acid positivity in anti-hepatitis C virus negative renal transplant patients

Brig AK Hooda*, Maj Gen PP Varma, SM, VSM†, Maj Gen GS Chopra, SM, VSM‡, Col Jasmeet Kaur**

ABSTRACT

BACKGROUND

Hepatitis C virus (HCV) infection is common in renal transplant (RT) patients. Some of these patients remain anti-HCV negative despite presence of infection and these are identified by a positive HCV-ribonucleic acid (RNA) test.

METHOD

We studied 404 RT patients for prevalence of HCV-RNA positivity in anti-HCV negative patients. Serum was tested for presence of anti-HCV antibodies using a third generation HCV micro-ELISA (enzyme-linked immunosorbent assay) test, which utilises a combination of HCV structural and non-structural antigens. The RNA was extracted from patient serum for HCV viral quantification using Quiagen QI Amp Viral RNA mini extraction kit. The HCV-RNA viral load was performed on Corbet Rotor Gene 3000 thermocycler using Taqman principle.

RESULTS

About 308 patients were anti-HCV negative and 96 were anti-HCV positive, resulting in prevalence of overt HCV infection of 23.7%. A total of 130 anti-HCV negative patients tested positive for HCV-RNA making a prevalence of occult HCV infection of 42.2%. There was no significant difference in the rate of overt or occult HCV infection between males and females. Patients with HCV infection (whether overt or occult) had received more number of dialysis sessions (62.5 vs 32.2) and blood transfusions (2.78 vs 1.99) when compared to those without HCV infection ($P=0.001$). The mean duration on dialysis was also longer (8.15 months vs 4.53 months) in patients with HCV infection ($P=0.0001$).

CONCLUSION

A direct test for HCV viraemia is important to accurately determine the epidemiology of HCV infection in RT patients who remain anti-HCV negative despite harbouring active HCV infection.

MJAFI 2012;68:123–128

Key Words: hepatitis C; real time PCR; renal replacement therapy

*Consultant (Medicine & Nephrology), Command Hospital (EC), Alipore, Kolkata – 700027, †MG (Med), HQ (Central Command), Lucknow, ‡Deputy Commandant, **Senior Advisor (Pathology & Transplant Immunology), Army Hospital (R&R), Delhi Cantt. – 10, India.

Correspondence: Brig AK Hooda, Consultant (Medicine & Nephrology), Command Hospital (EC), Alipore, Kolkata – 700027.
E-mail: ashokhooda@hotmail.com

Received: 07.07.2011; Accepted: 15.01.2012
doi: 10.1016/S0377-1237(12)60023-9

INTRODUCTION

Hepatitis C virus (HCV) infection is common in patients with end-stage renal disease on renal replacement therapy (dialysis and/or transplantation) despite screening of blood products for anti-HCV and implementation of precaution measures.¹ The prevalence of HCV infection in renal transplant (RT) patients is known to vary widely in different regions of the world.² Even within India, a very wide range of prevalence rates for HCV have been reported in the dialysis population (4.3–45.2%)^{3–5} and among RT recipients (28.9–42%).^{6,7} Adding to the problem of overt HCV infection is the presence of occult HCV infection, which is described as detectable HCV-ribonucleic acid (RNA) in the liver or peripheral blood mononuclear cells (PBMC) in the absence of both serum HCV-RNA and anti-HCV antibodies.⁸ The detection of HCV genome in liver is the most accurate method for the diagnosis of occult HCV infection. However, testing for HCV-RNA in PBMC is an alternative procedure when a liver biopsy is not available. Some studies have also defined occult HCV infection as anti-HCV negative and HCV-RNA positive by polymerase chain reaction (PCR) while evaluating the prevalence of HCV infection in patients on dialysis.^{9–11} But there is a lack of data on the prevalence of occult HCV infection in RT patients. We studied the prevalence of occult HCV infection in RT recipients on our follow-up and analysed the factors that influence this prevalence.

MATERIALS AND METHOD

All RT recipients on follow-up at the Nephrology Centre of Army Hospital (R&R), Delhi Cantt. who had completed at least three months follow-up after transplant were included in the study. A total of 404 patients were studied during a period of two years.

A detailed history of pre-transplant period was obtained in all patients with reference to age, sex, basic disease causing chronic renal failure (CRF), duration on dialysis, and number of dialysis sessions, number of blood transfusions and pre-transplant anti-HCV status. The investigation work-up included anti-HCV antibody and PCR based HCV-RNA quantitative assay.

Anti-hepatitis C Virus Antibody Assay

Anti-HCV antibodies in serum were tested using a third generation HCV micro-ELISA (enzyme-linked immunosorbent assay), which utilises a combination of antigens with the sequence of both HCV structural and non-structural antigens, i.e. CORE, E1, E2, NS3, NS4, and NS5.

Interpretation is based on a cut-off calculation which is obtained by multiplication of 0.1 with mean absorbance of positive controls and an addition of a factor of 0.10. The absorbance of a patient's sample which is higher than the cut-off value is considered as a positive result and the absorbance less than cut-off value is taken as negative result.

Ribonucleic Acid Extraction

The RNA was extracted from patient serum for HCV viral quantification and genotyping using Quiagen QI Amp Viral RNA mini extraction kit (Cat No 52904) and the protocol followed for extraction was as per the manufacturer's instructions.

Hepatitis C Virus-Ribonucleic Acid Viral Load Quantification by Real Time Polymerase Chain Reaction

The HCV-RNA viral load was performed on Corbet Rotor Gene 3000 thermocycler using Taqman principle. The direct detection of the specific amplicon in fluorescence channels cycling A. FAM and JOE was done along with external standards supplied with the kits. A standard curve was obtained and the fluorescence measured by auto threshold as per the standard curve.

Polymerase Chain Reaction Protocol

The master mix was prepared by adding 14 µL supermix reagent, 5 µL magnesium solution (Mg-sol) and 1 µL IC-1R3 per reaction. To this 20 µL of PCR cocktail and 30 µL of extracted RNA was added. Thermocycler profile for the assay was defined as 50°C for 15 minutes of initial hold, 95°C for 10 minutes of initial denaturation, and cycling of 95°C for 15 seconds, 55°C for 20 seconds, and 72°C for 15 seconds; this cycle was repeated 45 times. The data generated was analysed using Rotor Gene software versions 6.0.33.

Statistical Analysis

Statistical analysis was done using Fisher's exact test and paired *t*-test to ascertain the statistical significance of various parameters.

RESULTS

The study group included 404 RT patients with a mean age of 32.9 years (age group 9–62 years) and male:female ratio of 312:92. Of these, 308 were anti-HCV negative and 96 were

anti-HCV positive, resulting in prevalence of overt HCV infection of 23.7%. A total of 130 patients in the anti-HCV negative group tested positive for HCV-RNA making a prevalence of occult HCV infection of 42.2%. The baseline characteristics and the effect of gender are shown in Table 1. There was no significant difference between males and females either in the rate of overt HCV infection ($P=0.2100$), or occult HCV infection ($P=1.0000$).

Of the 404 patients on follow-up, 320 patients (group A) were transplanted in our centre and 84 (group B) were transplanted in various other centres but were on our follow-up. The effect of centre of transplant on occult HCV infection is shown in Table 2. There was no significant difference in prevalence of occult HCV infection in the two groups ($P=0.4710$).

The effect of three factor, viz. number of dialysis, duration on dialysis, and the number of blood transfusions in the pre-transplant period is shown in Table 3. Patients with both overt and occult HCV infection had received more number of dialysis sessions and blood transfusions and had been on dialysis for a longer period when compared to those without HCV infection, and the difference was statistically significant in all these three parameters.

The study group included patients transplanted between 1990 and 2009. A sub-analysis of the patients transplanted be-

Table 1 Distribution of renal transplant patients by hepatitis C virus positivity and gender (percentage in parentheses).

	Anti-HCV negative (N=308)	Anti-HCV positive (N=96)	P value
Males	233 (75.6)	79 (82.3)	0.2100
Females	75 (24.4)	17 (17.7)	
Mean age (±SD) (yr)	32.6 (±10.25)	33.6 (±8.96)	
HCV-RNA negative	(N=178)	(N=8)	1.0000
Males	133 (57)	7 (8.9)	
Females	45 (60)	1 (5.9)	
HCV-RNA positive	(N=130)	(N=88)	
Males	100 (43)	72 (91.1)	
Females	30 (40)	16 (94.1)	

HCV: hepatitis C virus, RNA: ribonucleic acid, SD: standard deviation.

Table 2 Distribution of renal transplant patients by hepatitis C virus positivity and centre.

	Group A (N=320) Patients transplanted in Army Hospital (R&R), Delhi		Group B (N=84) Patients transplanted in other centres		P value
	HCV-RNA negative	HCV-RNA positive	HCV-RNA negative	HCV-RNA positive	
Anti-HCV negative	140	107	38	23	
Anti-HCV positive	5	68	3	20	
Overt HCV infection (%)	73/320 (22.81)		23/84 (27.38)		0.3896
Occult HCV infection (%)	107/247 (43.31)		23/61 (37.7)		0.4710

HCV: hepatitis C virus, RNA: ribonucleic acid.

Table 3 Effect of duration on dialysis and number of dialysis sessions and blood transfusions.

	No. of dialysis sessions			Duration on dialysis (mo)			No. of blood transfusions		
	Mean	Range	±SD	Mean	Range	±SD	Mean	Range	±SD
Anti-HCV negative (N=308)	32.25	0–236	35.94	4.53	0–44	5.14	1.99	0–25	3.24
Anti-HCV positive (N=96)	62.55	1–310	64.03	8.15	0–48	8.02	2.78	0–38	4.75
P value		0.0001			0.0001			0.0019	
<i>Anti-HCV negative group</i>									
HCV-RNA negative (N=178)	29.99	0–200	32.48	4.30	0–44	4.98	1.96	0–25	3.26
HCV-RNA positive (N=130)	35.34	2–236	40.11	4.83	0–27	5.35	2.03	0–25	3.23
P value		0.0001			0.001			0.0331	
<i>Anti-HCV positive group</i>									
HCV-RNA negative (N=8)	55.5	7–120	36.32	7.50	2–14	4.34	4.75	0–15	6.01
HCV-RNA positive (N=88)	63.19	1–310	66.08	8.21	0–48	8.29	2.60	0–38	4.62
P value		0.8565			0.3964			0.6807	

HCV: hepatitis C virus, RNA: ribonucleic acid, SD: standard deviation.

fore and after 2000 was done. The prevalence of anti-HCV positivity in patients transplanted before 2000 was 35% and in those transplanted after 2000 was 21.1% ($P=0.0455$).

There was no statistically significant difference in the levels of HCV-RNA in the overt and occult infection groups, the mean HCV-RNA in the occult HCV infection group being 8932648489.61 cpm and 54519556.14 cpm in the anti-HCV positive group ($P=0.3100$). HCV genotyping was studied in 59 patients and the genotypes 1, 3, and 4 were present in 52, two, and five patients, respectively. Figure 1 shows a representative graph of the readout of HCV on real time PCR showing positive control and patient runs. Figure 2 shows a representative electropherogram showing sequencing-based assay of the HCV genotype 1a.

DISCUSSION

The original report on occult HCV infection described patients with abnormal liver function tests who were anti-HCV and serum HCV-RNA negative but had an occult HCV infection in liver and in PBMC.¹² This criteria has been modified by some workers to define occult HCV infection as anti-HCV negative and HCV-RNA positive by PCR while evaluating the prevalence of HCV infection in patients on dialysis.^{9–11} We used the same criteria and performed quantitative HCV-RNA assay by real time PCR to assess the prevalence of occult HCV infection in RT patients on our follow-up.

The reported incidence of HCV infection in RT patients in various centres in India varies from 28.9% to 42%.^{6,7} The prevalence of anti-HCV positivity or overt HCV infection in our study was 23.7%. This included patients transplanted between 1990 and 2009 and on further analysis, the prevalence has progressively decreased over the years in our centre also. The prevalence of anti-HCV positivity in patients transplanted before 2000 was 43.8% and in those transplanted after 2000 was 21.9% ($P=0.0455$). One of the reasons for this decrease in prevalence could be the mandatory screening by anti-HCV

testing of blood products before transfusion which came in to practice in India in the 1990s. However, there is still a residual risk of HCV transmission due to donations in the anti-HCV window period of about 1 in 100,000 transfusions of cellular products.¹³ Forcic et al tested 2,718 anti-HCV negative plasma pools and found that 2.1% were HCV-RNA positive.¹⁴ Their study confirmed the necessity of testing HCV-RNA in blood products to increase the safety of transfusions.

All the reported studies from India on HCV infection in RT patients are based on the detection of HCV infection using anti-HCV, which is relatively insensitive in RT patients, rather than the more sensitive HCV-RNA. Since, the antibody test does not define viraemia in all cases, the detection of HCV-RNA is necessary for diagnosis of active HCV infection in these patients. As many as 42.2% of our anti-HCV negative patients had occult HCV infection. Negative serology in spite of HCV viraemia has also been documented in HD patients.¹⁵ Seth et al reported HCV-RNA positivity despite anti-HCV negativity in two of six renal allograft recipients who died of liver failure.¹⁶ Contrary to this, in some individuals serology is positive without detectable viraemia and this occurs both in the immunosuppressed and immunocompetent. Such a situation has been explained on the basis of three factors, viz. non-establishment of a carrier state, low-level undetectable viraemia, and intermittent viraemia.^{15,17} The former is more likely in RT patients and the latter two are more likely in a dialysis setting. In our study HCV-RNA was undetectable in 8.3% of the patients with a positive serology.

There were two sets of patients in our study, namely those transplanted in our centre and a smaller set of patients who were transplanted in various other centres from across the country but were subsequently under our follow-up care. On sub-analysis of these two sets, we found no difference in the prevalence of either overt or occult HCV infection, an indicator that HCV infection is an issue of concern in all centres providing renal replacement therapy in India.

Since, HCV infection in most patients of chronic kidney disease is acquired during the period on dialysis, we analysed

Figure 1 Readout of hepatitis C virus on real time polymerase chain reaction showing positive control and patient runs.

Figure 2 Electropherogram showing sequencing-based assay of hepatitis C virus genotype 1a.

the effect of number of dialysis sessions, duration on dialysis and number of blood transfusions on the prevalence of overt and occult HCV infection. Patients with both occult as well as overt infection had been on dialysis for a longer duration when compared with patients without HCV infection and the difference was statistically significant ($P=0.0001$). The positive relation of HCV infection with longer duration on dialysis has been reported in other studies also.^{3,18,19} The major reason for this is the nosocomial route of transmission including use of

contaminated equipment and patient-to-patient exposure.^{19,20} The recommended remedial measures to prevent this nosocomial acquisition of HCV infection are strict adherence to universal precautions and standard infection control measures and segregation of infected patients from uninfected patients with use of dedicated dialysis machines for HCV positive patients.^{21,22}

Similarly, patients with both occult as well as overt infection had received significantly higher number of transfusions when

compared with patients without HCV infection ($P=0.001$). The role of multiple blood transfusions as the major risk factor in developing HCV infection has been emphasised in earlier studies from India.¹⁸

Literature is divided on the clinical implications of the HCV infection on patient and graft survival in RT patients. A multivariate analysis showed similar seven-year patient and graft survival rates in anti-HCV positive and anti-HCV negative RT recipients.²³ In another study, anti-HCV positive RT patients had a better five-year survival than anti-HCV negative patients.²⁴ In an earlier study we had found HCV infection to be a bad prognostic indicator for patient and graft survival duration in RT recipients and infections were the commonest cause of death in our patients.²⁵ To assess the progression of liver disease and its impact on graft and patient survival, further long-term studies are required using HCV-RNA as the diagnostic test for HCV infection.

The present study shows that HCV infection is common in our population of RT patients and the factors responsible for the high prevalence are longer duration on dialysis and more number of blood transfusions in the pre-transplant period. A significant number of these patients have occult infection with HCV-RNA positivity and negative anti-HCV serology. A direct test for HCV viraemia is thus important to accurately determine the epidemiology of HCV infection and to assess the impact of chronic HCV infection on morbidity and mortality in RT patients. The HCV-RNA should be done in all patients on dialysis whether anti-HCV positive or anti-HCV negative before they undergo RT. Those with HCV infection, so detected, should be treated before transplant. In addition it should also be done in all patients with persistently deranged transaminases but negative anti-HCV.

Intellectual Contributions of Authors

Study concept: Brig AK Hooda, Maj Gen PP Varma, SM, VSM, Maj Gen GS Chopra, SM, VSM

Drafting and manuscript revision: Brig AK Hooda, Col Jasmeet Kaur

Statistical analysis: Brig AK Hooda

Study supervision: Maj Gen GS Chopra, SM, VSM, Maj Gen PP Varma, SM, VSM

CONFLICTS OF INTEREST

This study has been funded by research grants from the O/o DGAFMS.

REFERENCES

1. Fabrizi F, Lunghi G, Ganeshan SV, Martin P, Messa P. Hepatitis C virus infection and the dialysis patient. *Semin Dial* 2007;20:416–422.
2. Einollahi B, Alavian SM. Have all the questions regarding the HCV infection in renal transplant recipients been answered? *Int J Nephrol Urol* 2009;1:1–3.
3. Chawla NS, Sajiv CT, Pawar G, Pawar B. Hepatitis B and C virus infections associated with renal replacement therapy in patients with endstage renal disease in a tertiary care hospital in India – prevalence, risk factors and outcome. *Indian J Nephrol* 2005;15:205–213.
4. Agarwal SK, Dash SC, Irshad M. Hepatitis C virus infection during haemodialysis in India. *J Assoc Physicians India* 1999;47:1139–1143.
5. Chattopadhyay S, Rao S, Das BC, Singh NP, Kar P. Prevalence of transfusion transmitted virus infection in patients on maintenance haemodialysis from New Delhi, India. *Haemodial Int* 2005;9:362–366.
6. Radhakrishnan S, Abraham P, Raghuraman S, et al. Role of molecular techniques in the detection of HBV-DNA and HCV-RNA among renal transplant recipients in India. *Ind J Med Res* 2000;111:204–211.
7. Agarwal SK, Dash SC, Irshad M, et al. Impact of hepatitis C virus infection on renal transplant outcome in India – a Single Centre Study. *J Assoc Physicians India* 2000;48:1155–1159.
8. Barril G, Castillo I, Arenas MD, et al. Occult hepatitis C virus infection among hemodialysis patients. *J Am Soc Nephrol* 2008;19:2288–2292.
9. Yakaryilmaz F, Gurbuz OA, Guliter S, et al. Prevalence of occult hepatitis B and hepatitis C virus infections in Turkish hemodialysis patients. *Ren Fail* 2006;28:729–735.
10. Rai RR, Mathur A, Mathur D, et al. Prevalence of occult hepatitis B & C in HIV patients infected through sexual transmission. *Trop Gastroenterol* 2007;28:19–23.
11. Jain P, Nijhawan S. Occult hepatitis C virus infection is more common than hepatitis B infection in maintenance haemodialysis patients. *World J Gastroenterol* 2008;14:2288–2289.
12. Castillo I, Pardo M, Bartolome J, et al. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis* 2004;189:7–14.
13. Van der Poel CL. Hepatitis C virus and blood transfusion: past and present risks. *J Hepatol* 1999;31(Suppl 1):101–106.
14. Forcic D, Zgorelec R, Branovic K, Kosutic-Gulija T, Santak M, Mazuran R. Incidence of hepatitis C virus RNA in anti-HCV negative plasma pools in Croatia. *Transfus Apher Sci* 2001;24:269–278.
15. Chopra GS, Gupta RM, Gedela SR, Varma PP, Rai R, Nema SK. Hepatitis C virus infection in haemodialysis patients: “Wolf in Sheep’s Clothing”. *MJAFI* 2005;61:241–244.
16. Seth AK, Anand AC, Gedela SR, Varma PP, Baliga KV. Rapid progression of hepatitis C-induced liver failure in renal allograft recipients. *Indian J Gastroenterol* 2006;25:155–156.
17. Chan TM, Lok ASF, Cheng IK, Chan RT. Prevalence of HCV infection in dialysis patients: a longitudinal study comparing the results of RNA and antibody tests. *Hepatology* 1993;17:5–8.
18. Murthy KK, John GT, Abraham P, Talaulikar GS, Thomas PP, Jacob CK. Profile of hepatitis B and hepatitis C virus infections in dialysis and renal transplant patients 1997–2001; CMCH Vellore. *Indian J Nephrol* 2003;13:24–28.
19. Aroldi A, Lampertico P, Montagnino G, et al. Natural history of hepatitis C virus infection in adult renal graft recipients. *Transplant Proc* 2005;37:940–941.
20. Zacks SL, Fried MW. Hepatitis B and C and renal failure. *Infect Dis Clin North Am* 2001;15:877–899.
21. Pereira BJG, Levey AS. Hepatitis C virus infection in dialysis and renal transplantation. *Kidney Int* 1997;51:981–999.

22. Jadoul M. Epidemiology and mechanisms of transmission of the hepatitis C virus in haemodialysis. *Nephrol Dial Transplant* 2000; 15(Suppl 8):39–41.
23. Einollahi B, Hajarizadeh B, Bakhtiari S, et al. Pretransplant hepatitis C virus infection and its effect on the posttransplant course of living related allograft recipients. *J Gastroenterol Hepatol* 2003;18:836–840.
24. Siren S, Nurhan OF, Munire T, Sedat B, Zubeyde A, Mehemt H. Renal transplant offers better survival in HCV-infected end-stage renal disease patients. *Tissue Antigens* 2002;60:559.
25. Narula AS, Hooda A, Anand AC, Patrikar S. Impact of hepatitis C virus infection in renal transplant recipients. *Indian J Gastroenterol* 2005;24:151–154.

List of CME 2012

S. no.	Comd	Venue	Subject	Topic	Approved date	Type of events
1	Navy	INHS Asvini	Medicine	68th Annual conference of Bombay Medical Congress	11–12 Feb 2012	Inter Comd
2	Western Comd	MH Jalandhar	PSM	Life Style Disease	18 Feb 2012	Intra Comd
3	Western Comd	AH (R&R)	Orthopaedics	Advance Anthroplasty Update	3–4 Mar 2012	Inter Comd
4	South West Comd	MH Jaipur	Endocrinology	Current Trends in Diabetes Mellitus	10–11 Mar 2012	Intra Comd
5	Western Comd	AH (R&R)	Gastroenterology	Controversies in Gastroenterology	17–18 Mar 2012	Inter Comd
6	Western Comd	CH (WC)	Surgery	Head Injury	1–2 Apr 2012	Intra Comd
7	Central Comd	AFMSD Lucknow	Med Store	Pharmaco Vigilance	7–8 Apr 2012	Inter Comd
8	Air Force	IAM	Av Med	Night Vision Goggles-third Eye	14–15 Apr 2012	Inter Comd
9	Air Force	CH (AF) Bengaluru	Surgery	Rattling Out Complications in Surgery	26–27 May 2012	Inter Comd
10	Central Comd	MH Namkum	Medicine	Management of Common Emergencies	9–10 Jun 2012	Intra Comd
11	AFMC	AFMC	Ophthalmology	Current Concepts & Future Trends in Ophthalmology	16–17 Jun 2012	Inter Comd
12	AFMC	AFMC	Psychiatry	Frontiers in Military Psychiatry	21–22 July 2012	Inter Comd
13	Northern Comd	CH (NC)	Surgery	Surgery in War	6–7 Aug 2012	Inter Comd
14	Central Comd	AMC C&C	Medicine	Physician Exec: Challenges & Perspective for Armed Forces Medical Services	11–12 Aug 2012	Inter Comd
15	Air Force	CH (AF) Bengaluru	Radiology	Update of Imaging & Interventional Radiology	25–26 Aug 2012	Inter Comd
16	Navy	INHS Jeevanti	Av Med	Aero Medical Challenges in Newer Aircraft Induction	5–6 Sep 2012	Intra Comd
17	AFMC	AFMC	Medicine	CME on 'Milestones in Medicine'	8–9 Sep 2012	Inter Comd
18	Central Comd	CH (CC)	Medicine	Recent Advances in Medicine	15–16 Sep 2012	Inter Comd
19	Southern Comd	CH (SC)	Endocrinology	Recent Trends in Endocrinology	22–23 Sep 2012	Inter Comd
20	AFMC	AFMC	Pathology	Expanding Vistas in Pathology and Haematology—An Update	7–8 Oct 2012	Inter Comd
21	Western Comd	AH (R&R)	Neurology	Recent Advances in Neurosciences	13–14 Oct 2012	Inter Comd
22	Navy	INHS Asvini	Marine Med	XVIII Annual Conference of Marine & Allied Sciences along with CME on Current Concept & Human Performance in High Pressure Environment	20–21 Oct 2012	Inter Comd
23	Western Comd	CH (WC)	Medicine	Recent Advances in Clinical Practice	27–28 Oct 2012	Inter Comd
24	AFMC	AFMC	Dental	10th National Dental Update	3 Nov 2012	Inter Comd
25	Eastern Comd	CH (EC)	Medicine	Controversies in Medical Management	4 Nov 2012	Inter Comd
26	Western Comd	AH (R&R)	Urology	Advanced Laparoscopy in Urology	17–18 Nov 2012	Inter Comd
27	Southern Comd	MH Jodhpur	Anaesthesia	Airways Management Workshop for MOs/RMOs	24–25 Nov 2012	Intra Comd
28	Northern Comd	CH (NC)	Anaesthesia	Recent Advances in Interventional Pain Mgt in Trauma victims & Chronic Pain States	1–2 Dec 2012	Inter Comd