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Original Article

A study of Hepatitis A and E virus seropositivity profile amongst young healthy adults in India

Col Atul Kotwal, SM[a,b,*], Maj Harpreet Singh [c], Brig A.K. Verma, (Retd)[d], Brig R.M. Gupta [e], Col Shishir Jain [f], Lt Col S. Sinha [g], Lt Col R.K. Joshi [h], Lt Col Prabhakar Teli [i], Col Vijay Khunga [j], Lt Col Anuj Bhatnagar [k], Lt Col Richa Ranjan [l]

[a] Director, AFMS (Med Research), O/o DGAFMS, Ministry of Defence, New Delhi, India
[b] Professor and Head, Dept of Community Medicine, Army College of Medical Sciences, New Delhi 11001, India
[c] Officer Commanding, SHO Jalandhar Cantt, India
[d] Formerly, Professor & Head, Dept of Community Medicine, Armed Forces Medical College, Pune 411040, India
[e] Dy DGMS (IT), O/o DGMS (Army), L Block IHQ of MoD (A), New Delhi, India
[f] Col Health, HQ 11 Corps, C/o 56 APO, India
[g] ADH, HQ 1 Corps, C/o 56 APO, India
[h] Classified Specialist (Community Medicine & Epidemiology), National Aids Research Institute, Pune, India
[i] DADH, HQ 8 Inf Div, C/o 56 APO, India
[j] Officer Commanding, SHO Agra, UP, India
[k] Assistant Professor, Dept of Community Medicine, Armed Forces Medical College, Pune 411040, India
[l] Classified Specialist (Pathology), Command Hospital (Central Command), Lucknow, India

ARTICLE INFO

Article history:
Received 16 November 2013
Accepted 23 June 2014
Available online 16 July 2014

Keywords:
HAV
HEV
Vaccine
Multi-centric survey
Young adults
Trainees

ABSTRACT

Background: Various Serosurveys and studies provide ample evidence of differing perspectives regarding epidemiology of HAV and HEV in India. This study was conducted to assess the seroprevalence of HAV and HEV and its associated factors with an aim to provide inputs to planners regarding requirement of HAV vaccine.

Methods: A multi-centric cross sectional survey amongst 4175 healthy trainees (young adults) was carried out in training centres, selected by multistage random sampling, giving equal representation to all regions of India. Sample size was calculated by taking prevalence of HAV seropositivity amongst adults as 60% and alpha 0.05.

Results: Seroprevalence for HAV and HEV was 92.68% (95% CI 91.82, 93.47) and 17.05% (15.90, 18.26), respectively. Logistic regression showed that hand washing without soap, regular close contact with domestic animals, consumption of unpasteurized milk and regular consumption of food outside home were risk factors for HAV ($p < 0.05$). For HEV, irregular hand washing, consumption of unpasteurized milk and irregular consumption of freshly prepared food were risk factors ($p < 0.05$).

Conclusion: High level of immunity against HAV among the healthy young adults clearly demonstrates that vaccination against HAV is not required at present in our country. The

* Corresponding author.
E-mail addresses: dratulkotwal@gmail.com, atuljyoti2710@hotmail.com (A. Kotwal).
http://dx.doi.org/10.1016/j.mjafi.2014.06.016

large proportion being susceptible to HEV points towards the requirement of preventive strategies in the form of safe drinking water supply, hygiene, sanitation, increasing awareness and behaviour change with respect to personal hygiene especially hand and food hygiene.

Introduction

Faeco-oral route is one of the leading routes of transmission of infectious diseases and contributes to 4.62 million episodes of diarrhoeal illness worldwide with 1.27 million in SEAR alone.[1] 1562 DALYs per 100,000 are lost in India due to these infections. Amongst the faeco-orally transmitted pathogens, Hepatitis A and Hepatitis E viruses are responsible for acute viral hepatitis and more than 300 million people worldwide suffer from viral hepatitis annually. Nearly 300 deaths per year are attributed to fulminating acute disease and about 15,000 people succumb each year to chronic liver disease. The disease has an enormous impact on health and national economy of many countries including India.[2] Worldwide, Hepatitis A virus (HAV) infection accounts for 1.4 million cases annually with number of cases of HAV in Asian countries ranging from 10 to 30 per 100,000 per year.[3] Exact incidence of Hepatitis E virus (HEV) is unknown with outbreaks of HEV common in parts of world with hot climates and rare in temperate climates.[4]

HAV infection largely depends on sanitary and hygiene conditions of populations. HAV infection in children is generally self-limiting but it causes significant morbidity in adults. Seroprevalence of HAV differs significantly in different socioeconomic groups at 85.3% and 64.5% in upper and lower socioeconomic groups, respectively in an Indian study[5] and 68.8% and 79.7% in Bangladesh.[6] A study conducted in Delhi has shown seroprevalence of HAV as 57% in individuals less than 35 yrs and 92% in more than 35 yrs with no socioeconomic disparity.[7] Lowest seropositivity rates have been reported in Kerala (10.3%) amongst children below 5 yrs of age.[8] The available data show heterogeneity among population regarding endemicity of HAV. Epidemics of HAV, mainly affecting young adults have occurred in different parts of the country, e.g. in Delhi,[9] Kerala,[10,11] Shimla.[12]

HEV is also self limiting and clinically indistinguishable from other hepatitis infections. Disease affects mainly young adults between 15 and 45 yrs with increased mortality among pregnant women.[13] Ratio of clinical to subclinical infection has not yet been determined. Seroprevalence of HEV has been reported to be lower as compared to HAV. Hospital based study among jaundiced patients in a tertiary care hospital in Manipur reported 18% cases due to HEV,[14] while a similar study in Pakistan reported the proportion as 5.3%.[15] Various epidemics have occurred due to HEV e.g. in Kashmir valley,[16] Kanpur[17] and Satara district of Maharashtra.[18]

Armed forces have been no exception to viral hepatitis as it accounted for 4.11% and 3.78% of total hospital admissions during 2005 and 2006 respectively. Average duration of hospital stay was 34.04 and 40.25 days respectively in 2005 and 2006. HAV contributed 0.24 per 1000 hospital admissions while other unspecified viral hepatitis including HEV to 3.37 per 1000 hospital admissions in the year 2006.[19] In an Armed Forces study, conducted in a tertiary care hospital with study individuals mainly being army trainees, HAV and HEV have been found to be responsible for 33% and 45.4% cases respectively amongst hepatitis patients.[20]

Outbreaks as well as sporadic cases of HAV and HEV not only lead to loss of man days but also have a direct effect on fighting efficiency of the troops. Outbreaks of HEV have been reported sporadically from various parts of the country.[21,22] Vaccine against HAV is available worldwide to immunize susceptibles and a vaccine against HEV has shown promising results in phase II and III trials.[23]

Indian Armed forces comprise of men from all regions and socioeconomic status and data on seroprevalence of HAV from the whole country suggests heterogeneity regarding age, socioeconomic status and region while no data are available on HEV in our country so far. Since, no exhaustive study has been carried out either in the armed forces or in the country, this study was conducted to assess the current seroprevalence of Hepatitis A and E infection among young healthy adults joining the Armed Forces, with a view to guide the policy decision on vaccination.

Material and methods

A multi-centric cross sectional survey was conducted in years 2010 and 2011 amongst healthy young adults of eleven (11) training centres, selected by multistage random sampling, giving equal representation to all regions of India.

The purpose and methodology of the study was explained to all study participants and informed consent was obtained. Pretested questionnaire was used to collect data on demographic profile and various risk factors associated with Hepatitis A and E infection. . The participants were asked to answer regarding their practices before joining the training centre. The filled questionnaire and the blood sample of each study participant was coded to relate the results of ELISA later for data entry and analysis.

Briefly, 5 ml of blood was collected under aseptic precautions in red top vacutainer from the cubital vein. The serum was separated and stored in sterile cryovials which was transported to the testing lab in storage boxes, each with capacity of 64 vials. Cold chain was maintained. ELISA for detection of anti-HAV IgG and anti-HEV IgG antibodies was carried out on each of the samples following protocols as per

Table 1 – Distribution of Study participants as per age, education, residence and family income.

	N	%
Education (N = 4168)		
≤10th	191	4.58
>10 ≤ 12th	3940	94.53
Graduate and above	37	0.89
Area of residence (N = 4166)		
Rural	3271	78.51
Urban	895	21.49
Family income (N = 4175)		
≤5000	3019	72.31
5001–10000	772	18.49
10001–15000	95	2.27
15001–20000	104	2.49
>20000	185	4.44

kits manufactured by standard reputed companies. Tests were carried out at the hospitals with ELISA readers nearest to the centres selected.

Sample size was calculated for each centre taking seroprevalence for Hepatitis A as 60%, with alpha 5% and size worked out to be 369 (370) with a total sample size for 11 centres as 4070. Adding 5% for refusals, a total of 4275 participants were included. However, 06 (0.14%) participants in one of the centres gave a probable history of Hep A vaccination and 94 (4.13) were excluded either due to refusal to be part of the study (21) or filling up questionnaire but not providing blood sample (73). A total of 4175 participants were included in the study. Study participants within each centre were chosen by simple random sampling. A data base was created in MS Excel and was analysed using SPSS ver 14.0. 95% confidence intervals were calculated. Chi square test for trend and multivariate analysis were used to analyse the data.

Results

The mean age and sd of study participants were 19.88 years ± 1.38 years. Table 1 depicts the education level, area of residence prior to joining and family income of the study participants. Seroprevalence for IgG antibodies for Hepatitis A virus among the study participants was found to be 92.68% (95% CI: 91.82–93.47) and IgG antibodies for Hepatitis E virus was found to be 17.05% (95% CI: 15.90–18.26). The distribution based on seropositivity for hepatitis A and E has been described in Table 2.

The bivariate analysis between various correlates and immunity against HAV and HEV among the study participants was carried out. All the variables showing statistically significant association with immunity to HAV and HEV were included in respective model of multivariate analysis by logistic regression. Results of these models have been depicted in Tables 3 and 4. Logistic regression shows that hand washing without soap, regular close contact with domestic animals, consumption of unpasteurized milk and regular consumption of food outside home were risk factors for HAV ($p < 0.05$). For HEV, irregular hand washing, consumption of unpasteurized milk and irregular consumption of freshly prepared food were risk factors ($p < 0.05$).

Discussion

The proportion of study participants from rural areas (78.51%) was more in our study as compared to the census figure of 68.85%.[24] The seropositivity for HAV shows that approximately 93% of the study participants were immune and only 7.32% were susceptible to hepatitis A virus infection, this is in consonance with the high endemicity zone as per the WHO.[25] Our results are also similar to a cross sectional population based study (85.9%) in the age group of 16–25 years.[26] Seropositivity rates of around 90% were found in a study conducted on voluntary blood donors aged 18–25 years from city and suburbs of Pune[27] and in a population based cross sectional study in Mumbai in age group of 11–30 years.[28] However, seroprevalence findings of our study are in contrast to the findings of 54.1% in age group of 15–24 years among healthy outpatients of a hospital in New Delhi[8] and 62.6% among medical students of New Delhi in age group of 18–22 years.[29] This difference might be attributable to the regional differences, urban – rural differences amongst the

Table 2 – Distribution of study participants based on Seropositivity for Hepatitis A and E virus.

Training centre	Station	Presence of IgG antibody [N (%) 95% CI]	
		Against Hepatitis A ($n = 3974$)[a]	Against Hepatitis E ($n = 3992$)[b]
I	Ahmadnagar	372 (97.89) 95.89, 99.09	67 (17.63) 13.93, 21.84
II	Bengaluru	370 (100.00) 99.00, 100.00	3 (0.81) 0.17, 02.35
III	Bareilly	332 (94.05) 91.05, 96.28	2 (22.16) 18.03, 26.74
IV	Pune	373 (92.10) 89.03, 94.53	42 (10.34) 07.56, 13.73
V	Jabalpur	323 (86.83) 82.96, 90.09	31 (8.36) 05.75, 11.65
VI	Jabalpur	315 (86.36) 81.34, 88.81	45 (12.20) 09.84, 15.98
VII	Lucknow	337 (95.74) 93.07, 97.60	32 (8.96) 06.21, 12.42
VIII	Namkum	366 (95.31) 92.69, 97.20	21 (5.47) 03.42, 08.24
IX	Pune	321 (84.25) 80.20, 87.76	50 (13.08) 09.87, 16.88
X	Roorkee	332 (94.05) 91.05, 96.28	82 (22.16) 18.03, 26.74
XI	Shillong	242 (94.90) 91.44, 97.26	7 (3.00) 01.22, 06.09
TOTAL		3683 (92.68) 91.82, 93.47	681(17.05) 15.90, 18.26

[a] 201 samples for Hep A.
[b] 183 for Hep E were either inadequate/haemolysed during processing of blood samples.

Table 3 – Multivariate analysis – HAV.

S No	Variable	Adjusted Odds Ratio	95% CI
1	**Hand washing**		
	With soap	1	1.005, 2.136*
	Without soap	1.465	
2	**Domestic animals**		
	No	1	1.059, 1.938*
	Yes	1.433	
3	**Type of milk consumed**		
	Packaged & pasteurised	1	1.380, 2.965*
	Open and unpasteurised	2.023	
4	**Consumption of food outside home**		
	Infrequently	1	1.856, 10.519*
	Frequently	4.064	
5	**Treatment of water**		
	Boiling	1	0.897, 1.746
	Other	1.252	
6	**Consumption of freshly prepared food**		
	No	1	0.062, 1.064
	Yes	0.257	

*$p < 0.05$.

study population and also because of different settings of our study and the study conducted in Delhi.

No declining trend of HAV seropositivity was observed when this study findings were compared with earlier studies like a multi-centric study[30] in 2001 (80.8% vs 88.30%) and a study in Pune.[27] This level of immunity among large proportion of healthy young adults suggests an asymptomatic exposure to HAV. Based on the findings of this study, no epidemiological transition in epidemiology of HAV seems to be taking place.

High level of sero-negativity for anti-HEV shows that large proportion of young adults is susceptible to infection by hepatitis E virus. As per WHO,[31] the seroprevalence in our country varies from 10 to 40% and our study has shown a similar pattern with overall being 17.05% (range 15.90–18.26%). A South Indian study[32] estimated seroprevalence of 8% among healthy blood donors, antenatal women and patients for elective surgeries aged 16–40 years while a study in Mumbai estimated it as 25.8% in age group of 11–20 years in a residential colony.[28]

Table 4 – Multivariate analysis – HEV.

S No	Variable	Adjusted Odds Ratio	95% CI
1	**Hand washing**		
	Regular	1	1.555, 2.796*
	Irregular	2.085	
2	**Type of milk consumed**		
	Packaged & pasteurised	1	1.385, 3.338*
	Open and unpasteurised	2.166	
3	**Consumption of freshly prepared food**		
	Regularly	1	1.050, 5.731*
	Irregularly	2.454	
4	**Hand washing**		
	With soap	1	0.727, 1.498
	Without soap	1.043	
5	**Domestic animals**		
	No	1	0.818, 1.390
	Yes	1.067	
6	**Treatment of water**		
	Boiling	1	0.817, 1.404
	Other	1.071	
7	**Water supply**		
	Tap	1	0.800, 1.421
	Other sources	1.066	
8	**Place of residence**		
	Urban	1	0.729, 1.395
	Rural	1.009	
9	**Education status**		
	>10th	1	0.689, 2.483
	≤10th	1.400	

*$p < 0.05$.

Conclusion

The large proportion of healthy young adults with pre-existing immunity to HAV does not pose risk of personal morbidity and operational ability. The consideration of HAV vaccination as recommended by WHO in this subset of population (healthy young adults) across the country will not only have a tremendous unnecessary economic burden on the meager resources and constrained economy of our country but also an immunological assault on the individuals with pre-existing immunity. Public-health agencies in our country will have to carefully reassess the need for mass or universal vaccination depending on other factors such as the disease burden, available resources and priorities. In view of this high immunity, it would be more pertinent to improve our water supply, sanitation and eating practices rather than recommending vaccine for all. Only after improvements in hygiene, sanitation and water, which may take few years, we can reassess the value of this vaccine in children.

The low seropositivity among healthy young adults shows an increased susceptibility to HEV infection. The risk is increased manifold when conglomeration of individuals takes place in slums, refugee camps, training camps, etc. The dominance of HEV in the whole spectrum of viral hepatitis both sporadically and in outbreaks makes it a suitable candidate for vaccination whenever the same is available. The coordinated efforts towards the research on effectiveness of the candidate vaccine are required but at this juncture preventive strategies aiming at providing clean drinking water, proper sewage disposal and health education in form of hand hygiene practices are of utmost importance. These preventive strategies coupled with efforts to improve housing and living conditions, management of crowds during fairs, festivals, etc., are immediately required for prevention of HEV infection.

Conflicts of interest

All authors have none to declare.

Acknowledgement

This paper is based on Armed Forces Medical Research Committee Project No 3946/2009 granted by the office of the

Directorate General Armed Forces Medical Services and Defence Research Development Organisation, Government of India.

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