

RESEARCH NOTE

COMPARISON OF Vi SEROLOGY AND NESTED PCR IN DIAGNOSIS OF CHRONIC TYPHOID CARRIERS IN TWO DIFFERENT STUDY POPULATIONS IN TYPHOID ENDEMIC AREA OF INDIA

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Abstract. For detection of chronic typhoid carriers, nested PCR targeting flagellin the gene of *Salmonella enterica* subspecies *enterica* serotype Typhi was carried out on DNA extracted from hepatobiliary specimens from 424 autopsies which were apparently free from gallbladder pathology on postmortem examination. The second study population was 508 healthy volunteers, who did not suffer from typhoid fever during the preceding year and whose sera were subjected to detection of carriage by estimation of Vi antibody levels using an indirect hemagglutination assay. Males of both study populations had comparable rates of detection by the two methods, 6.3% by PCR and 4.1% by Vi serology. Similarly, females in both study groups had comparable frequency of detection of chronic typhoid carriage using the two methods, *ie* 13.1% by PCR and 15.1% by Vi serology. *S. Typhi* specific immunosuppression could be speculated in females of 51-60 years as only 40% were positive by Vi serology against 100% by nested PCR. Vi serology may be recommended for community based detection of chronic typhoid carriers.

Key words: chronic typhoid, nested PCR, Vi serology, carriers, India

INTRODUCTION

About 2-3% of individuals become chronic typhoid carriers following symptomatic or asymptomatic typhoid infection

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(Cvjetanovic *et al*, 1971). Since *Salmonella Typhi* is a human restricted pathogen, these carriers are mostly responsible for endemicity and outbreaks of infection in the community. Persistence of *S. Typhi* in carriers has also been linked with development of carcinoma of the gallbladder (Caygill *et al*, 1971; Welton *et al*, 1979; Mellemgard and Gaarsler, 1988; El Zayadi *et al*, 1991; Nath *et al*, 1997, 2008; Shukla *et al*, 2000).

Current standard practice to detect chronic typhoid carriage is by isolating the Typhi serotype from consecutive stool specimens (≥ 3) and if possible better from hepatobiliary specimens. However, culture isolation is tedious and almost always associated with low sensitivity (Gupta *et al*, 2006; Mohan *et al*, 2006). Positive fecal culture for *S. Typhi* has been reported after 196 negative culture results in a chronic carrier (Christae, 1987). Whereas none of the 17 bile specimens from healthy individuals yields serotype Typhi, 4 isolations could be made from 72 patients with gallbladder ailments (Nath *et al*, 1997).

Serological testing, although cheap and easy to perform, has its inherent limitations and drawback. Vi antibody (ViAb) detection either by indirect hemagglutination assay (IHA) or ELISA provides some clues regarding chronic carrier state but is associated with some ambiguity. Reports based on ViAb detection have shown the prevalence of chronic carriage to be 1.8% in Vietnam (Gupta *et al*, 2006) and 13% among healthy Indians (Mohan *et al*, 2006). Interestingly, fecal isolation rate hardly exceeds 1% (Gupta *et al*, 2006; Mohan *et al*, 2006). In suspected cases of acute typhoid fever PCR-based detection method could detect *S. Typhi* in 92.8% of cases against 29.8% by culture (Prakash *et al*, 2005). Therefore, it becomes prudent to look into the role of ViAb detection in the diagnosis of chronic typhoid carriers in comparison with PCR-based-detection of *S. Typhi* as gold standard. As ethically it is extremely difficult to get hepatobiliary specimens from live healthy subjects, we decided to collect these specimens from dead bodies, almost always victims of un-natural deaths, and serum samples from an apparently healthy population residing in the same geographical area. In his study, we have compared ViAb detection by IHA in

healthy individuals with that of nested PCR of the flagellin gene (*fliC*) in hepatobiliary specimens from corpses for the diagnosis of chronic typhoid carriage in the Gangetic plain of North India, an endemic zone for typhoid fever.

MATERIALS AND METHODS

Sample collection

A total 424 corpses (mostly victims of road traffic accidents, devoid of visible gallbladder pathology) and 508 healthy adults (not having history of typhoid fever for at least a year) were included in the present study. This study was conducted from July 2005 through June 2007 in a tertiary care medical center, University Hospital of Banaras Hindu University, Varanasi, located in the eastern part of North India. The study design was approved by the Ethics Committee of the University and written consent was obtained from family members of the deceased. Autopsied liver tissue, bile and gallbladder tissue specimens were brought to the Post Graduate Department of Forensic Medicine of the University Hospital within 18 hours of death. From live subjects, 5 ml of blood were collected in a sterile tube by venipuncture and serum was separated within 2 hours of collection and stored at -20°C till used.

Isolation of *S. Typhi*

Bile was inoculated directly onto blood agar, McConkey agar (MA) (Hi Media, Mumbai, India), xylose lysine deoxycholate agar (XLD agar) (Hi Media, Mumbai, India) and Selenite F broth (Hi Media, Mumbai, India) for enrichment culture. Inoculated media were incubated overnight at 37°C aerobically. Subcultures were made from Selenite F broth onto XLD and MA. Gallbladder, bone marrow and liver tissue specimens were

Table 1
Age and sex distribution of chronic typhoid carriers in a healthy population of north India detected by IHA and in autopsies by nested PCR.

Age group (years)	Male			Female		
	Vi No. positive/ total cases(%)	PCR No. positive/ total cases(%)	Value (Fisher's exact test)	Vi No. positive/ total cases(%)	PCR No. positive/ total cases(%)	Value (Fisher's exact test)
1-30	0/157(0)	3/103(3)	-	15/147(10.2)	2/51(4)	0.14
31-60	11/107(10)	16/158(10.1)	0.56	21/87(24)	13/62(21)	0.40
61-80	0/5(0)	1/41(2)	-	0/5(0)	0/9(0)	-
Total	11/269(4.1)	20/302(6.6)		36/239(15.1)	15/122(12)	0.29

Vi male vs female $p = 0.00002$; PCR male vs female $p = 0.045$

first homogenized by a sterile plastic microfuge adopted pestle, and then processed in the same way as for bile.

Serological studies

Antibodies against Vi antigen (ViAb) were measured following the method of Barrett (1985). The titer $\geq 1:160$ was considered as cut-off for diagnosing typhoid carriage.

Detection of *fl*C** specific nucleotide sequence of *S. Typhi*

DNA extraction from different types of samples was conducted after homogenization using the phenol-chloroform method (Ho *et al*, 1995). Reference strain of *S. Typhi* (MTCC 3216) was spiked into each type of the sample before isolation of DNA to standardize the isolation as well as amplification protocol. Nested PCR was performed as described by Song *et al* (1997) modified according to Frankel (1994). Primers for first round PCR to amplify a 495 bp fragment were ST1 (5'-TAT GCC GCT ACA TAT GAT GAG-3') and ST2 (5'-TTA ACG CAG TAA AGA GAG-3'), and for nested PCR to amplify a 364 bp fragment ST3 (5'-ACT CGT AAA ACC ACT ACT-3') and ST4 (5'-TGG AGA CTT CGG TCG CGT AG-3').

Statistical method

Fischer's exact test was used to analyze the association between study variables.

RESULTS

Detection rate of chronic typhoid carriers by IHA in healthy population was observed to be 9.3% (47/508), while the rate in cadavers by nested PCR was 8.3% (35/424). The difference in the above detection rates is statistically not significant ($p > 0.05$). Healthy male population was found to have typhoid carriage of 4.1% by Vi serology, not statistically different ($p > 0.05$) from males of deceased group of 6.3% (Table 1). Similarly in females of healthy volunteers, the rate of detection of chronic typhoid carriage by IHA was (15.1%) not statistically different ($p > 0.05$) from that in corpses by nested PCR (13.1%).

Age group of 31-60 years was found to have highest number of carriers, with Vi- and PCR-based methods detecting comparable rates in males and in females. When analysis was performed with the age group blocks of 10 years, males in their fourth decade of life were observed with

the highest rate of chronic typhoid carriers by both methods [19% (5/26) by IHA and 20% (8/40) by PCR in cadavers]. Highest detection rate (40%, 6/15) in female volunteers was observed in fifth decade by Vi serology. This decade of life of female corpses was also observed with the highest (100%, 5/5) detection rate by PCR also. In females of age group of 41-50 years detection rates by both methods 26.9%, 7/26 by IHA and 21.2%, 7/33 by PCR are not statistically different ($p>0.05$) from those in males of the same age group.

None of the hepatobiliary specimens yielded positive results for *S. Typhi*.

DISCUSSION

Vi antibody detection rate by IHA was very similar to that by nested PCR (based on amplification of pathogen specific sequence), indicating that ViAb detection might be a reliable method to detect chronic typhoid carriage. Our observation is in contrast to the suggestions made by Gupta *et al* (2006) that the elevated titers of ViAb in an individual may be due to the presence of other Vi carrying bacteria *viz* *S. Paratyphi C*, *S. Dublin* and *Citrobacter freundii*. The utility of ViAb in detecting chronic typhoid carriers is further augmented by the observation of comparable rates of detection in males and females of respective age groups by both the methods. However, females in age group 51-60 years were the exception. It is difficult to explain this discrepancy, but Typhi specific immunosuppression in females of this age group (post menopausal) may be one reason. Apart from the comparable prevalence of chronic typhoid carriers in corresponding age group of both sexes, it is of interest to note the prevalence rate for carriers in the ratio of 4:1 in female versus male by serological method and 2:1 by

PCR method. In the present study, however, there was the possibility of false negative cases by PCR, as the volume of specimens and sites from which the specimens were collected might be limiting factors. Surprisingly, a recent PCR-based study has reported significantly higher association of chronic typhoid carriage with malignant as well as benign gallbladder diseases as compared to healthy controls (Nath *et al*, 2008). Also female to male ratio for both of the diseases was observed to be 3:1 (Nath *et al*, 2008). These observations indicate that ViAb based detection method is definitely relevant for detection of chronic carriage in the community because of its ease of performance and economy.

In summary, the present study focused on the comparison of two completely different technologies using two different populations and different specimens. In the light of extremely poor isolation of *S. Typhi* from chronic typhoid carriers, (Christae, 1987), Vi based serological method appeared to be relevant. This is the first study of its kind where utility of ViAb based detection system was evaluated with the extremely sensitive and specific nested PCR-based method (Prakash *et al*, 2005). Although, ViAb detection by IHA was promising, it may be necessary to carry out repeated cultures with improved isolation techniques and to perform nested PCR-based detection of *S. Typhi* in fecal samples of subjects who are found positive by serological method.

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