Hepatitis C virus infection among teenagers in an endemic township in Taiwan: epidemiological and clinical follow-up studies

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SUMMARY

The aim of the study was to elucidate the epidemiological features of Hepatitis C virus (HCV) infection among teenagers in an endemic area by conducting a mass screening study. We also investigated the clinical outcome of the anti-HCV-positive subjects by conducting subsequent short-term and long-term follow-up studies. All 2837 students of two junior middle schools in Tzukuan, aged 13–16 years, were invited to be screened for anti-HCV, HBsAg, AST and ALT in October 1995. A total of 2726 (96%) students responded. Anti-HCV, HCV RNA and aminotransferase levels were evaluated among anti-HCV-positive students 1 month and 30 months later, respectively. A total of 38 (1·4%; M/F = 22/16) participants were anti-HCV-positive. The anti-HCV-positive students had higher rates of exposures to transfusion, anti-HCV-positive families and surgery. The prevalence (2·8%) of the 7 maritime villages was markedly higher than that (0·7%) of the other 8 villages ($P < 0.001$). Subsequent follow-up studies demonstrated that there might be 5 cases of acute or recent HCV infection, and 6 cases who had recovered from chronic HCV infection.

INTRODUCTION

Hepatitis C virus (HCV) is recognized as the major pathogen of non-A, non-B hepatitis among adults [1, 2]. However, knowledge of HCV infection among the young population is limited. Most data published to date are based on studies of high-risk groups, such as subjects with multiple transfusions for thalassemia, haemophilia, leukaemia, haemodialysis or surgery [3–8]. Furthermore, there have been very few reports so far in the English literature concerning community-acquired HCV infection among teenagers. With the widespread application of anti-HCV tests in the past decade, more and more endemic areas have been discovered (e.g. Arahiro in Japan [9], Egypt [10, 11], and some tropical areas in Africa [12–15]). The previous reports in Taiwan showed that the nation-wide prevalence of anti-HCV among adult voluntary blood donors was < 2·5% [16–19]. In recent years, however, there have been some HCV-endemic areas reported in Taiwan [20–27]. Our previous published studies demonstrated that the anti-HCV prevalence of Tzukuan, one of the endemic areas in Taiwan, reaches 41·6% among adult residents with an annual incidence of 4·5% [22, 23]. Moreover, about 90% of hepato-

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Prevalence of anti-HCV
Maritime villages (A-G)
2.8% (25/887)
Non-maritime villages (a-h)
0.7% (9/1, 251)
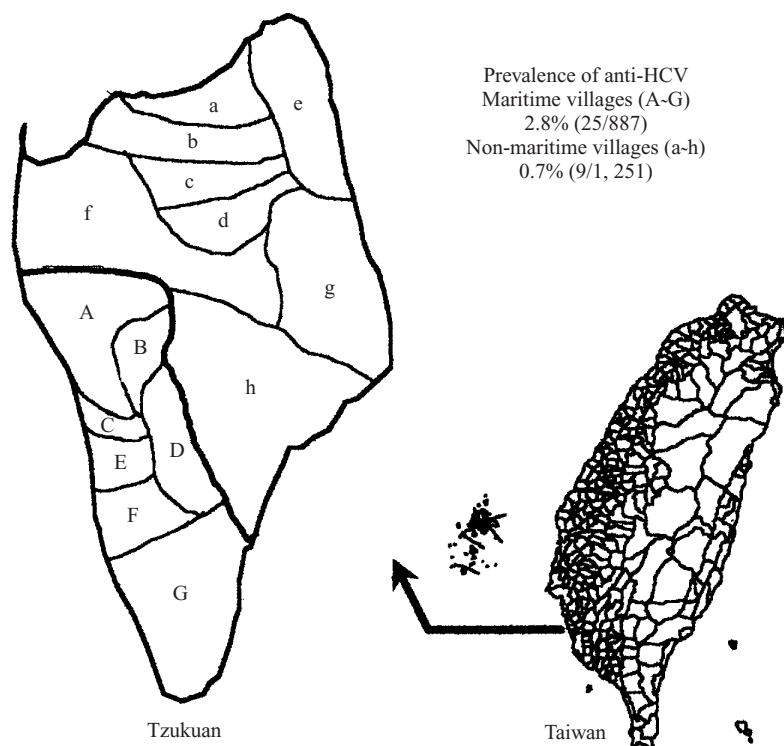
Tzukuan

Taiwan

Fig. 1. Location and villages of Tzukuan Township in southern Taiwan. There are seven maritime villages (A–G) and eight non-maritime villages (a–h).

cellular carcinoma (HCC) patients in this township were HCV-related [21, 28].

Against this background, there is a pressing need to elucidate and establish the prevalent and demographic characteristics among young teenagers of this HCV endemic area. Preventive projects of community health based on these data would then be possible. Therefore, we conducted a mass screening study. Besides cross-sectional data, little is known concerning the longitudinal clinical features of community-acquired HCV infection. We also investigated prospectively the short-term and long-term follow-up studies of the anti-HCV-positive teenagers. It represents, to our knowledge, the largest community survey and follow-up studies of HCV infection among a population of this age.

METHODS

Tzukuan Township, located just north of Kaohsiung City in southern Taiwan, was reported to be an HCV-endemic area with the prevalence of 41·6% [22, 23]. The total population is about 40000. There are only two junior high schools in this township attended by 2837 students, aged 13–16 years. Tzukuan comprises

15 villages. Based on the different occupational and geographic background of the residents, 7 villages located in the southwest coast were referred to as 'maritime villages', and the other 8 as 'non-maritime villages' (Fig. 1).

In October 1995, all 2837 students were invited to be screened for HBsAg, anti-HCV, aspartate and alanine aminotransferases (AST and ALT). With the agreement of their parents or guardians, a total of 2726 (96·1%) students participated in this screening. All anti-HCV-positive subjects were tested further for HCV RNA. Meanwhile, a self-filling structured questionnaire was designed to identify possible routes of infection. The risk factors included transfusion, anti-HCV-positive family members, surgery, medical injections or procedures (include dental procedures) and other parenteral routes (including acupuncture, tattoo and body-piercing).

One month after mass screening, all the anti-HCV-positive subjects were invited to a follow-up examination, during which they were tested for AST, ALT, anti-HCV and HBeAg (in HBsAg-positive subjects). Another follow-up to test for AST, ALT, anti-HCV and HCV RNA, was carried out on the anti-HCV-positive subjects in April 1998 (30 months after the initial examination).

Serum AST and ALT levels were measured by an autoanalyser (Hitachi, Tokyo, Japan). The upper normal limits of both AST and ALT were 25 IU/l. We tested HBsAg and anti-HCV by using second-generation enzyme immunoassay (EIA) kits (Abbott Laboratories, North Chicago, IL, USA). The resulting optical density (OD) values of each well measured at 492 nm were recorded. The upper measurable value of OD in anti-HCV EIA was 2·0. All reactive samples were tested in triplicate.

HCV RNA was detected by polymerase chain reaction (PCR) using primers from the 5′-untranslated region (5′-UTR) (COBAS AMPLICOR™ HCV test, Roche Diagnostic Systems Inc., Branchburg, NJ, USA). All the tests were performed in duplicate. Using PCR products of 5′-UTR, genotypes and subtypes of HCV were defined by oligonucleotide hybridization methods (INNO-LiPA™ HCV II test, Innogenetics, Belgium). Percentage with 95% confidence interval (CI) was used to describe the prevalence. χ^2 tests or Fisher exact test were employed to test the difference between prevalence rates of the groups.

RESULTS

Prevalence and geographic difference

A total of 38 out of the 2726 (1·4%) teenagers (aged 13–16 years) were anti-HCV-positive. The prevalence of anti-HCV-positive among 2138 students living in Tzukuan was 1·6% (34/2,138, 95% CI 1·1–2·1%), higher than that among students from other neighbouring townships (4/588, 0·7%, 95% CI 0·02–1·3%) ($P = 0.09$). Among the 34 anti-HCV-positive native residents of Tzukuan, the prevalence (25/887, 2·8%, 95% CI 1·7–3·9%) of the 7 maritime villages was markedly higher than that (9/1,251, 0·7%, 95% CI 0·3–1·2%) of other 8 villages ($P < 0.001$) (Table 1, Fig. 1).

Risk factors of HCV infection

All the 38 anti-HCV-positive students responded to the questionnaire, compared with 2217 of 2688 (82·5%) anti-HCV-negative subjects. As shown in Table 2, history of transfusion ($P < 0.0009$), at least one household family member with HCV infection ($P < 0.0001$) and history of surgical treatments ($P = 0.0253$) were significant risk factors. However, the risk factors of 24 (63·2%) anti-HCV-positive students could not be identified.

Clinical manifestations of anti-HCV-positive students

All the 38 anti-HCV-positive students (male/female = 22/16) were asymptomatic and only one had been diagnosed and treated with interferon one year before the screening. A total of 25 (65·8%) students were HCV-RNA-positive, and 8 (32%) of the 25 HCV-RNA-positive students had elevated ALT levels. The distribution of genotypes in the 25 HCV-RNA-positive subjects was as follows: genotype 1b in 13 (52%) cases, 2a in 3 (12%) cases, 2b in 7 (28%) cases, and unclassified in 2 (8%) cases. None of HCV-RNA-negative students had elevated ALT levels. A total of 21 (55·3%) of the anti-HCV-positive students had optical density (OD) values of anti-HCV tests > 2·0 in repeat tests. Among them, 20 (95·2%) students were HCV-RNA-positive, and 4 (20%) of the HCV-RNA-positive cases had elevated ALT levels (range: 78–111 IU/l). The other 17 (44·7%) had the OD values of anti-HCV tests < 2·0. Only 5 (29·4%) of the 17 cases were HCV-RNA-positive and 3 (60%) had elevated ALT levels (range: 40–72 IU/l). Seven (18·7%) students were HBsAg-positive and only 1 of them was HBeAg-positive.

Short-term follow-up study

All the 38 students were examined again 1 month after the mass screening. They could be divided into four groups according to the sequential changes of anti-HCV tests (Table 3).

Group A (initial OD > 2·0 and follow-up OD > 2·0)

The OD values of anti-HCV tests of the 21 cases were still > 2·0. As mentioned above, 20 of them were HCV-RNA-positive. In this examination, 4 (20%) of them had elevated ALT levels (ranging from 28–281 IU/l). Four HBsAg-positive students were in this group and 1 was HBeAg-positive. His ALT level was 46 IU/l. The other 3 HBeAg-negative students had normal ALT levels. This group most likely represents chronic HCV infection.

Group B (initial OD < 2·0 and follow-up OD > 2·0)

Five students whose initial OD values of anti-HCV tests were < 2·0 had OD values > 2·0 one month later. All 5 students were HCV-RNA-positive. Their initial ALT levels were 13, 14, 35, 73 and 129 IU/l, respectively. In this follow-up study, their ALT levels

Table 1. *Geographical difference of prevalence of anti-HCV among teenagers in Tzukuan*

		Anti-HCV positive	
Geographic groups	No. of students	No. (%)	(95% CI)
Total subjects	2726	38 (1·4)	(1·0–1·8)
Neighbourhood townships	588	4 (0·7)	(0·0–1·3)*
Tzukuan township	2138	34 (1·6)	(1·1–2·1)*
Maritime villages	887	25 (2·8)	(1·7–3·9)†
Non-maritime villages	1251	9 (0·7)	(0·3–1·2)†

* $P = 0.09$; † $P < 0.001$; based on χ^2 tests.

Table 2. *Route of HCV infection among teenagers in Tzukuan*

	Anti-HCV (+) (No. = 38)	Anti-HCV (−) (No. = 2217)	
Route of infection	No. (%)	No. (%)	*P* value
Transfusions	4 (14·3)	23 (1·0)	$P < 0.001$
Anti-HCV (+) families	8 (21·1)	51 (2·3)	$P < 0.001$
Surgery	2 (5·3)	13 (0·6)	$P = 0.025$
Medical injections or procedures	3 (7·9)	149 (6·7)	n.s.
Other parenteral routes*	1 (2·6)	19 (0·9)	n.s.
Not identified	24 (63·2)	2018 (91)	—

* Include acupuncture, tattoo and body-piercing; n.s. non-significant.

Table 3. *Characteristics of follow-up studies of the anti-HCV-positive teenagers based on anti-HCV tests*

	OD of anti-HCV		Short-term (*n* = 38)			Long-term (*n* = 31)		
			Subjects	HCV RNA (+)	ALT ↑	Subjects	HCV RNA (+)	ALT ↑
Groups	Screening	1 month later	No. (M/F)	No. (%)	No. (%)	No. (M/F)	No. (%)	No. (%)
A	> 2·0	> 2·0	21 (13:8)	20 (95·2)	4 (20·0)*	17 (10:7)	15(88·2)†	6 (35·3)*
B	< 2·0	> 2·0	5 (3:2)	5 (100)	4 (80·0)*	4 (2:2)	3(75·0)†	1 (25·0)*
C	< 2·0	< 2·0	6 (4:2)	0	0	5 (3:2)‡	0	0
D	< 2·0	(—)	6 (2:4)	0	0	5 (2:3)§	0	0

* All cases with elevated ALT were positive for HCV RNA.
† HCV RNA became negative in 2 cases of group A and 1 case of group B.
‡ Anti-HCV became non-reactive in 3 cases of group C.
§ Anti-HCV was weakly positive (OD = 0·77) in one case.

increased to 25, 30, 85, 343 and 364 IU/l, respectively. One of them was HBsAg-positive and HBeAg-negative. This group most likely represents acute or recent HCV infection.

Group C (initial OD < 2·0 and follow-up OD < 2·0)

Six students were still anti-HCV-positive, although the OD values of both initial and follow-up tests were repeatedly < 2·0. All 6 students had normal ALT levels and all of them were HCV-RNA-negative. One HBsAg-positive student was included.

Group D (initial OD < 2·0 and follow-up anti-HCV-negative)

Another 6 students were anti-HCV-negative 1 month after the mass screening. None of them had abnormal

ALT levels. HCV RNA assays showed negative in all of them. One of them was HBsAg-positive.

Long-term follow-up study

A total of 31 students (male = 17, female = 14) were followed 30 months after initial screening. They were 17 students of group A, 4 of group B, 5 of group C and 5 of group D (Table 3). All 17 students of group A remained anti-HCV-positive with the OD values > 2·0. HCV RNA was negative in 2 (11·8%) cases. Six HCV-RNA-positive cases had abnormal ALT values (range: 43–163 IU/l). Among cases of group B, the OD values of all 4 follow-up cases remained > 2·0. One (20%) of them became HCV-RNA-negative. Only 1 HCV-RNA-positive case was with mild ALT elevation (47 IU/l). Among 5 students of group C, none was HCV-RNA-positive or had ALT elevation. Three (60%) cases became anti-HCV-negative. All 5 students of group D remained HCV-RNA-negative with normal ALT levels. However, 1 of them had a weak reaction in anti-HCV tests with an OD value (= 0·770) slightly higher than the cut-off value.

DISCUSSION

This large-scale community study confirms the prevalence of anti-HCV-positive among young teenagers in this endemic area is higher than those of previous reports in our country [16–19]. In this study, the prevalence (1·6%) of anti-HCV-positive in this township was similar to that (1·9%) of our previous report among young teenagers in another HCV-endemic township (Paisha) of Taiwan [20]. Since HCV infection is an acquired infectious disease and the prevalence of HCV infection increases with age, the prevalence is related to cumulating risks of environment [16]. The cumulating risks for HCV infection of young teenagers in HCV-endemic areas are almost equal to those of adults in other areas of Taiwan. The prevalence of anti-HCV among young teenagers was much lower than that among adults in Tzukuan. If these students still live in such an environment of high incidence, their prevalence might increase with age and reach the same level as that of present adults several years later. Based on the epidemiological data of our study, there is a pressing need to implement control measures on this population.

Iatrogenic factors (e.g. medical injections, acupuncture and tattooing) were the major vehicles for HCV infection among several endemic areas reported in Taiwan [24–27]. Sexual or vertical transmission routes were thought to be negligible in this country [26]. However, the routes of HCV transmission among the teenagers have not been fully clarified [29–32]. In our study, the anti-HCV-positive students had higher rates of exposures to transfusion, anti-HCV-positive family members and surgery. Among these, risk factor of anti-HCV-positive families was the one which was easy to underestimate because anti-HCV testing had not been conducted in other family members at the time of our study. Our results show that the possible routes of HCV infection in Tzukuan might partly be different from those of other HCV endemic areas in our country. However, possible risk factors could not be clearly identified among 63·2% of anti-HCV-positive students. It might indicate that environmental factors also play important role in the HCV transmission.

Considering the geographic difference, our results demonstrated that difference in prevalence exists between students from Tzukuan and other townships. Meanwhile, the prevalence of the maritime areas was significantly higher than that of the non-maritime areas (2·8% vs. 0·7%; P < 0·001). Most of the residents in the 7 maritime villages are fishermen of low socioeconomic status, whereas the residents of the other 8 non-maritime villages are of higher status. A subsequent screening study in Tzukuan among adults aged 45 or more showed that the prevalence of HCV in the maritime areas (61·4%) was twice that of non-maritime areas (29·1%) [23]. The geographical difference of prevalence exists in Tzukuan according the data obtained from the studies among both adults and teenagers. It might therefore be related to different environmental factors. Further epidemiological studies to identify environmental factors will be performed in the future.

Most patients with chronic HCV infection are asymptomatic [32–34]. In this study, all the anti-HCV-positive students were asymptomatic and all but one (97·4%, 37/38) of them had never been diagnosed before the screening. Among these, only 8 students had elevated ALT levels during the initial screening. Meanwhile, none of the HCV-RNA-negative students had elevated ALT levels, while only 32% (8/25) of HCV-RNA-positive students had elevated ALT levels. Our results suggest that many cases of HCV infection might be overlooked or underestimated during childhood and adolescence [34]. Therefore, people living in endemic areas should be screened for this clinically silent disease since early treatment may be beneficial.

From the viewpoint of clinical management, it is important to perform the HCV RNA assay on every anti-HCV-positive subject [32]. If the HCV RNA assay was not affordable or not available, sequential anti-HCV and ALT tests might be surrogates.

In the past decade, several HCV endemic areas have been discovered following the widespread use of anti-HCV tests. In this large-scale epidemiological study, we used the second generation EIA test because of its low cost, high specificity and sensitivity. Whether the OD value of anti-HCV is related to infectiousness of HCV is controversial [20, 35–37]. It has been postulated that the OD value of anti-HCV might decrease in the late stage or remission of chronic infection [20, 37]. Host factors of young ages may play some role in the natural process of HCV infection, leading to such responses [38]. Our longitudinal results demonstrated that initial anti-HCV OD value $> 2\cdot0$ did not fall to $< 2\cdot0$. Among the students whose OD values of sequential anti-HCV tests were $> 2\cdot0$ during the short-term and long-term follow-up, HCV RNA was positive in $95\cdot2\%$ (20/21) and $88\cdot2\%$ (15/17) of the students, respectively. In our previous study among children of 3–15 years of age in another HCV-endemic area, only 4 of 11 anti-HCV-positive children had OD values $> 2\cdot0$, and 3 of the 4 cases were HCV-RNA-positive [20]. Therefore, anti-HCV OD values $> 2\cdot0$ is associated with a higher probability of persistent HCV-RNA-positive rates.

All the 5 students in group B (i.e. initial OD values $< 2\cdot0$, then elevated to $> 2\cdot0$ at 1 month follow-up study) were HCV-RNA-positive, and three of them had elevated ALT levels. Meanwhile, the ALT levels were significantly higher than those of the initial screening. These 5 students might be cases of acute HCV infection. This finding was supported by the results of long-term follow-up study that shows three of these cases remained HCV-RNA-positive.

During the short-term follow-up study among 6 students of group C, none of them was HCV-RNA-positive. Although anti-HCV-positive, none had elevated ALT levels. All the students of this group remained HCV-RNA-negative during long-term follow-up. It suggests that students of this group might have already recovered from chronic HCV infection. Among them, 2 cases had risk factors of HCV acquisition (transfusions in one case, anti-HCV-positive mother in one case). Low ALT levels, viral load and mild histological changes characterize chronic hepatitis C infection in children. Meanwhile, low chronic carrier rates of HCV infection have been reported in children, indicating that age at time of infection could be a factor in determining whether chronic infection occurs [20, 39, 40–42].

Those students in group D were demonstrated to be anti-HCV-negative during both follow-up studies. This result might be possibly due to non-specific reaction of anti-HCV test or laboratory errors. It raised the importance of sequential follow-up studies by using anti-HCV test, ALT and HCV RNA in order to avoid the possible false-positive presentation of a single anti-HCV test.

In conclusion, our findings suggest that young teenagers living in this HCV-endemic area had higher prevalence of HCV infection. The maritime part of this township was more endemic. In such a highly prevalent and high-incidence area, various patterns of HCV infection were observed. The natural history of community-acquired HCV infection among this age population may be more benign than that of adults. Identification of this higher prevalence of HCV acquisition in these teenagers will stimulate measures to identify further and reduce the impact of these risk factors.

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