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The Study of HCV Antibody and Serologic Tests for Syphilis in Thai Patients with Gynecologic Disorders

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SUMMARY **Investigation for prevalence of antibodies to hepatitis C virus (HCV) and to *Treponema pallidum* was conducted in 883 females with gynecologic disorders who were admitted to the gynecological ward of the Department of Obstetrics and Gynecology, Siriraj Hospital during April to August 1991. The study population consisted of 678 patients with malignancies and 205 patients with benign diseases. Anti-HCV antibody was found in 3.1% of the cases with malignancies and 1.46% of those with benign diseases. Among the gynecologic malignant group, the patients with carcinoma of cervix had the highest prevalence of HCV antibody (3.6%). The positive serologic tests for syphilis in patients with carcinoma of cervix (9.8%) were significantly higher from those in patients with ovarian carcinoma (3.75%) ($p < 0.01$). There were 3 cases with carcinoma of cervix who were simultaneously sero-positive for both HCV and syphilis.**

Hepatitis C virus (HCV) is strongly associated with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.[1-3] The prevalence of anti-HCV antibody was found to be high in Southeast Asia.[4] In the past decade, patients with clinical manifestations of hepatitis will be categorized as A, B and non A - non B groups. Among the non A - non B hepatitis, HCV is one important causative organism.[5,6]

Blood transfusion is generally accepted as an important mode of transmission[7,8] of HCV while sexual transmission is considered as a rare possible route.[9]

In the present time, according to the standard treatment of gynecologic malignancies, especially ovarian carcinoma, radical surgical removal of the tumor mass plus chemotherapy is found to be the most successful treatment which will increase survival time of 2-5 years compared to the unfavourable result in the past decade.

During radical surgery of gynecologic malignancies, the surgeon tries to remove the tumor mass as much as possible for better prognosis. Massive hemorrhage usually follows such operation. Not infrequently, bood transfusion is needed. In such circumstance, the harmful diseases which can be transmitted by blood transfusion should be considered such as HIV, HBs Ag, HTLV-1 and also HCV.[10] As previously pointed out by some authors about the necessity of HCV antibody screening in blood donors, one would look for the prevalence of HCV antibody in blood donors and also in those high risk groups who will most likely receive blood transfusion, for example, patients with hemoglobinopathies or patients who will have a radical surgical treatment.[10,11]

The investigators wanted to know the prevalence of HCV antibody in patients with gynecologic malignancies as a baseline data with the awareness of preventive mind to compare it with the prevalence of blood donors. If the study population have already high prevalence

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of HCV antibody, the screening program in blood donors is not mandatory. Possibility of sexual transmission of HCV was also studied.

MATERIALS AND METHODS

Eight hundred and eighty three serum samples were collected from patients with gynecologic disorders in gynecologic ward, Department of Obstetrics and Gynecology, Siriraj Hospital, Bangkok, Thailand during 1 April and 1 August 1991. Two hundred and five serum samples collected from non-malignant gynecologic patients served as a control group while the rest 678 patients were gynecologic malignant cases. All blood samples were collected on admission before any treatment and were tested for anti-HCV antibody by particle agglutination (PA Serodia, Fujirebio, Japan), enzyme linked immunosorbent assay (EIA, Ortho) and radioimmunoassay (RIBA 2, Ortho). Moreover, all sera were tested simultaneously for antibody to *Treponema pallidum* by both VDRL and TPHA (*Treponema pallidum* Hemagglutination Absorption Test). The patients were also interviewed for history of blood transfusion, jaundice, drug abuse, sexually transmitted diseases among themselves and their partners.

Particle agglutination (PA)

Serodia-HCV reagent kit was purchased from Fujirebio, Japan. The procedures have been previously described elsewhere.[12] In brief, 50 μl of a two-fold serially diluted serum samples including negative and positive controls were prepared in a 96-well round bottomed microtitre plate. Twenty five microlitre of antigen solution containing c200 antigen and core antigen (c22-3) of HCV coated onto the surface of gelatin particles was added into each well. The mixtures were incubated for 2 hours at room temperature. The results of agglutination were visually read and interpreted as positive or negative.

Enzyme linked immunosorbent assay (EIA)

Anti-HCV EIA reagent kit was commercially obtained from Ortho Diagnostics, Japan. Detection of IgG antibodies against recombinant HCV peptide antigen (c100-3) in the serum samples was carried out in a 96-well flat bottomed microtitre plate according to the manufacturer's specification.[13] The optical density (O.D.) read at 450 nm was compared with the cut-off (C.O.) value of 0.495 and interpreted as cut-off index (C.I.). Specimens with O.D. values higher than the cut-off value (or C.I. $>$ 1) were considered positive.

Radioimmunoassay (RIBA 2)

The second generation RIBA immunoblot kit for the detection of anti-HCV antibody against 4 recombinant HCV antigen (c100-3, 5-1-1,

Table 1. Age characteristics of the study population

Studied population	Number tested (case)	Percent	Mean age ± SEM (years)
Gynecologic malignancies			
1. Cervix	306	45.13	50.06 ± 0.64
2. Ovarian	267	39.38	42.53 ± 0.92
3. Corpus	79	11.65	53.52 ± 1.14
4. Others	26	3.84	50.00 ± 3.38
Subtotal	678	100.0	47.65 ± 0.51
Gynecologic non-malignancies			
1. Myoma uteri	72	35.12	41.96 ± 0.92
2. Ectopic pregnancy	44	21.46	27.68 ± 0.84
3. Acute pelvic inflammatory disease	18	8.78	24.53 ± 1.29
4. Others	71	34.64	36.92 ± 1.74
Subtotal	205	100.0	36.62 ± 0.77
Total	883	100.0	44.67 ± 0.46

c33c[NS3 region] and c22-3 [virus core]) was obtained from Ortho Diagnostics, Japan. The results were interpreted according to the manufacturer's recommendation as negative(−), indeterminate(Ind) or positive(+).[14]

VDRL and TPHA

VDRL and TPHA were carried out by the standard and routine procedures in the hospital.[15]

RESULTS

Among 678 gynecologic malignant cases, carcinoma of cervix, carcinoma of ovary and carcinoma of the uterine corpus were the 3 most common malignant diseases. On the other hand, the three most common disorders in 205 non-malignant cases of the control group included myoma uteri, ectopic pregnancy and acute pelvic inflammatory disease.

The gynecologic malignant patients significantly older ($p = 0.016$) than the control (47.65 ± 0.51 *vs* 36.62 ± 0.77 years) as demonstrated in Table 1.

A total of 24 out of 883 cases showed positive anti-HCV antibodies by PA method. Nevertheless some of these 24 cases showed negative results when determined by ELISA and/or radioimmunoassay (Table 2). There was no statistical correlation of the results from different assays. The PA test appeared to be the most reliable test according to its high sensitivity and specificity,[16] so the number of positive samples counted was of those who were PA positive.

Table 2. Comparative results of anti-HCV antibody screening by particle agglutination, enzyme-linked immunosorbent assay and radioimmunoassay

	Sample No.	PA (Fujirebio)	EIA (Ortho)	C.I	RIBA 2 (Ortho)
1	12	+	−0.112	0.2	Ind
2	81	+	−0.113	0.2	Ind
3	92	+	+0.684	1.4	+
4	94	+	−0.096	0.2	+
5	96	+	−0.073	0.1	Ind
6	115	+	−0.259	0.5	Ind
7	126	+	−0.102	0.2	−
8	153	+	−0.156	0.3	Ind
9	163	+	+>2.5	5.1↑	+
10	220	+	+>2.5	5.1↑	+
11	241	+	−0.172	0.4	+
12	247	+	−0.106	0.2	Ind
13	254	+	−0.178	0.4	−
14	257	+	−0.094	0.2	Ind
15	424	+	−0.135	0.3	−
16	438	+	+>2.5	5.1↑	+
17	463	+	+1.153	2.3	+
18	789	+	−0.101	0.2	Ind
19	825	+	−0.114	0.2	Ind
20	836	+	+>2.5	5.1↑	+
21	870	+	−0.128	0.3	−
22	873	+	−0.111	0.2	−
23	880	+	−0.100	0.2	−
24	883	+	−0.145	0.3	Ind
			(C.O. =0.491)		

PA = Particle agglutination
EIA = Enzyme-linked immunosorbent assay
RIBA = Recombinant immunoblot assay
C.I. = Cut-off index
C.O. = Cut-off value
↑= Equal to or more.

Of all the 24 seropositive for HCV antibodies by PA test, anti-HCV antibody was found in 3.1% in the gynecologic malignant group compared to 1.46% in the control group (Table 3). The difference was not statistically significant ($p > 0.05$). Subgroup analysis comparing between carcinoma of cervix with carcinoma of ovary, carcinoma of ovary with carcinoma of corpus, carcinoma of cervix with carcinoma of corpus, and also with the control group, the results had no significant differences ($p > 0.05$) between each pair using Chi-square test, two tail analysis.

The rate of seropositivity for syphilis, STS +, positive VDRL and TPHA, was 7.08% in gynecologic malignant group as compared with 5.36% in the control group. There was no statistical difference ($p > 0.05$) in seroconversion among the 2 groups (Table 3). However, subgroup analysis in the malignant patients showed that patients with cervical carcinoma had a significantly higher ($p < 0.01$) seropositive rate than those with ovarian carcinoma (9.8% *vs* 3.75%).

The relationship of malignancies anti-HCV antibody and serological test for syphilis (STS) was assessed

Table 3. The relationship of malignancies, HCV antibody and serological test for syphilis

Studied population	No. tested (case)	No. of Anti-HCV + (%)	No. of STS + (%)	No. of both HCV + & STS + (%)
Gynecologic malignancies				
1. Cervix	306	11 (3.6)	30 (9.8)	3 (0.98)
2. Ovarian	267	9 (3.4)	10 (3.75)	0 (0)
3. Corpus	79	1 (1.3)	4 (5.06)	0 (0)
4. Others	26	0 (0)	4 (15.38)	0 (0)
Subtotal	678	21 (3.1)	38 (7.08)	3 (0.44)
Gynecologic non-malignancies				
1. Myoma uteri	72	0 (0)	3 (4.17)	0 (0)
2. Ectopic pregnancy	44	0 (0)	2 (4.54)	0. (0)
3. Acute pelvic inflammatory disease	18	0 (0)	1 (5.55)	0 (0)
4. Others	71	3 (4.2)	5 (7.04)	0 (0)
Subtotal	205	3 (1.46)	11 (5.36)	0. (0)
Total	883	24 (2.72)	59 (6.68)	3 (0.34)

as shown in Table 3. Only 3 cases with carcinoma of the cervix simultaneously were seropositive for both anti-HCV antibody and STS. This represented very low prevalence of only 0.98% of the patients with carcinoma of cervix and 0.34% of the whole study population. The history of risk factors of HCV transmission was negative in all 24 cases.

DISCUSSION

The prevalence of seropositivity for HCV antibody in the patients with gynecologic disorders was 2.72% which was not so different from that (1%) in blood donors.[11,16] A higher percentage of positive HCV antibody has been found to be positively correlated with age as reported by Japanese Red Cross in 1989. It is possible that the higher prevalence in this study population is due to an older age range compared with blood donors whose average age was 30-39 years.[17]

The subgroup analysis of HCV antibody, as demonstrated in Table 3, showed no significant differences among those subgroups. The only striking result was that there was no single case of anti-HCV antibody positive among the control subgroup of myoma uteri, ectopic pregnancy and acute pelvic inflammatory disease, all of which were accounted for 134 cases. In contrast, those with carcinoma of cervix, carcinoma of ovary and carcinoma of the corpus uteri were HCV positive ranging from 1.3-3.6% in each subgroup. This may also be explained by the higher mean ages of the carcinoma group than those of the control.

There was no difference in the prevalence of STS seropositivity between the malignant and the non-malignant groups as shown in Table 3 (7.08% and 6.68% respectively). However when one considers each subgroup separately, it is apparent that patients with carcinoma of the cervix had the highest prevalence of STS positivity of 9.8% either compared with the other malignant subgroups or the control group. The STS positive rate in the carcinoma of cervix group was statistically different ($p < 0.01$) from that of carcinoma of ovary, carcinoma of the uterine corpus or the control group as shown in Table 3. It sugguests that the patients with carcinoma of cervix were more heavily infected by sexually transmitted diseases in the past than the other malignant subgroups and the control group.

Although it cannot be concluded that STS positivity is the direct cause of carcinoma of cervix, it is certainly a risk factor.[18] Simultaneous HCV antibody and STS seropositivity found in only 3 cases of carcinoma of cervix further supports the role of sexual transmission of HCV in this carcinoma of cervix group. However, the percentage of HCV seropositivity by sexual transmission route in this study was relatively low (0.98%). Similar findings have been reported in Europe.[9] Definite conclusion of the correlation requires more epidemiological

studies in the future.

All 24 patients who were HCV antibody positive reported no risk factors for example: previous history of blood transfusion, intravenous drug abuse, jaundice in the patients and their family members, sexually transmitted diseases in sexual partners. The only evidence of sexual transmission of HCV in this study was simultaneous HCV and STS seropositivities in 3 cases of carcinoma of cervix.

In conclusion, the prevalence of HCV seropositivity was found to be a little higher in patients with gynecological disorders than in blood donors. It was less likely that HCV was transmitted via sexual route. The question for further research is what is the route of HCV transmission in such cases without any defined risk factors.[19]

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