Prevalence of Serum IgG Antibodies for the E7 and L2 Proteins of Human Papillomavirus Type 16 in Cervical Cancer Patients and Controls

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Relationship between the prevalence of the antibodies to HPV16E7 and L2 proteins and the development of cervical cancer was examined. Sera from 57 patients with invasive cervical cancer and from 200 age-matched healthy blood donors (16 to 64 years old) were examined for antibodies against E7 and L2 proteins of human papillomavirus (HPV) type 16. Bacterially expressed fusion antigens were used in a Western immunoblot assay. Ten (18%) of the patients and 10 (5%) of the controls were positive for only E7. Each eighteen of the patients (32%) and of the controls (9%) were positive for only L2. Three of the patients and 2 of the controls were positive for both E7 and L2 antibody reactivity.

The patients' prevalence of antibodies for the E7 and L2 proteins was significantly higher than that of the controls (E7: \(x^2 = 14.3, p <0.01\); L2: \(x^2 = 23.8, p <0.01\)). On the other hand, neither sex specific difference in the antibody prevalence was observed, nor was there any difference in the antibody prevalence with age. Our findings indicate that antibodies to the HPV16L2 proteins could be a parameter for cervical cancer development as well as those to the HPV16E7 proteins.

Human papillomaviruses (HPVs) are a heterogeneous family of DNA viruses that are associated with a variety of epithelial lesions. The viruses are classified into types by genotyping, i.e., types are all defined as isolates that display at least 50% DNA homology in a liquid hybridization assay (Coggins and zur Hausen 1979). HPV type 16 is associated most frequently with malignant proliferative lesions, including uterine cervical cancer (zur Hausen and Schneider 1987).

Diagnosis of viral infection is generally based on the detection of serum antibodies that react with virus antigens. However, the efforts to detect specific
HPV antibodies were hampered by lack of suitable antigens, mainly due to the inability to propagate HPVs in culture and due to the scarcity of virions in clinical tissues (Galloway and Jenison 1990). Recently, proteins expressed from recombinant HPV DNAs or synthetic peptides have been used to detect antibodies to various HPVs. Serologic studies with these antigens have shown higher prevalence of antibodies to the early gene products of HPV 16, such as E2, E4, E6 and E7 proteins, among women with cervical cancer compared with controls (Dillner et al. 1989; Dillner 1990; Reeves et al. 1990; Hashido et al. 1991; Kochel et al. 1991a, b; Suchankova et al. 1991, 1992; Barber et al. 1992; Sasagawa et al. 1992). In addition, some studies with cervical cancer patients and age-matched controls have estimated an elevated relative risk of cervical cancer for women with antibody reactivity to the early proteins (Jochmus-Kudielka et al. 1989; Mann et al. 1990; Mandelson et al. 1992). The results of antibodies to the late proteins are controversial. Dillner et al. showed higher prevalence of antibodies to the 16L1, but not to 16L2 (Dillner et al. 1990). However, other studies showed no significant difference in the prevalence of antibodies to either 16L1 or 16L2 between cervical cancer patients and controls (Jenison et al. 1990; Barber et al. 1992; Kochel et al. 1991a, b; Mandelson et al. 1992).

We measured the prevalence of serum IgG antibody of cervical cancer patients to HPV16 antigens using E7 and L2 bacterial fusion proteins in the Western immunoblot assay. We show here that seroreactivity not only to the HPV16E7 protein but also to the L2 protein is more frequently found in cervical cancer patients than in controls, and also that there is neither sex-specific difference nor age-dependent variation of seropositivity among a healthy population, between 16 and 64 years of age.

**MATERIALS AND METHODS**

**Study population**

Sera were obtained from cervical cancer patients on the day they first visited the Department of Gynecology, Tohoku University Hospital, i.e., before they received primary treatment. The sera were stocked at –70°C until assay. All the patients were histologically and clinically diagnosed as having invasive cervical cancer of the uterus. Fifty-seven patients, who consisted of 51 with squamous cell carcinoma, 4 with adenosquamous carcinoma, and 2 with adenocarcinoma were subjected to this study. Three were in their twenties, 11 in their thirties, 17 in their forties, 11 in their fifties, and 15 over sixty. Age-matched control sera were obtained from healthy blood donors, 100 men and 100 women. There were 20 men and 20 women each in their teens (16 to 19), twenties, thirties, and forties, 14 men and 14 women in their fifties, and 6 men and 6 women over sixty (60 to 64).

**Expression plasmid constructs**

Expression plasmids, p16E7NP1 and p16L20B1 (Jenison et al. 1990), which contained the entire HPV16E7 and L2 open reading frames, respectively, were kindly provided by Dr. Galloway. The HPV16E7 and L2 proteins were expressed in Escherichia coli as fusion proteins with tryptophan E synthetase (trpE) through an expression vector, pATH11
Antibodies to Human Papillomavirus Type 16 E7 and L2 Proteins

Synthesis of fusion proteins, SDS-polyacrylamide gel electrophoresis, and Western immunoblot assays.

The expression of fusion proteins, sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, and western immunoblot assays were performed as described previously (Yaegashi et al. 1991, 1992). Serum specimens were tested for IgG antibody reactivity to HPV16E7 and L2 fusion proteins in a Western immunoblot assay at a dilution of 1:200. The staining intensity of the Western blot bands resulting from the presence of human IgG antibodies was qualitatively graded as 0 (=negative), +1 (faint band or broad band), or +2 (definitely positive). Only +2 samples were counted as positive in this study. Staining intensity was graded by two different readers. Laboratory personnel were blinded to the case/control status of the serum samples.

Statistical analysis

Differences between proportions of seropositivity in different groups were evaluated with the use of chi-square test. A p-value below 0.05 was considered to indicate a significant difference.

RESULTS

Sera from the patients and controls were examined by a Western immunoblot assay for IgG antibodies for the HPV16E7 and L2 proteins. Fig. 1 shows some representative results of Western blot assays. As shown in Fig.1A, the fusion proteins of HPVE7 and L2 products are 60KDa and 120KDa, respectively, the

![Fig. 1. Four representative Western immunoblots. Each contains the trpE protein encoded by the expression vector pATH11 (lane 1), the HPV E7 fusion protein (lane 2) and L2 fusion protein (lane 3). Each filter was allowed to react with a single human serum, preabsorbed with blocking agent, as described in materials and methods. (A) An amidoblack stained nitrocellulose filter. Each expressed protein is indicated by arrows. (B) Reactivity to both E7 and L2 fusion proteins. (C) Reactivity to the L2 alone. (D) Reactivity to the E7 alone. (E) No apparent reactivity to either E7 or L2. Molecular size standards in kilodaltons on the left.](image-url)
parental expression vector, pATH11, generates the 37KDa molecule of trpE. Some sera reacted with both E7 and L2 products (Fig 1B), some were reactive to either L2 and E7 (Figs. 1C and 1D). Specificity of positive sera was tested by preabsorption of sera with bacterial lysates of HPV16E7, L2 and pATH fusion proteins. We confirmed that there was no difference in antibody titers between positive sera of patients and normals with representative samples in our previous study (Jenison et al. 1990).

Ten (18%), 18 (32%), and 3 (5%) of the patient sera were found to be positive for antibodies against the HPV products, E7 alone, L2 alone, and both E7 and L2, respectively (Table 1). On the other hand, 10 (5%), 18 (9%), and 2 (1%) of the control sera contained antibodies positive for E7 alone, L2 alone, and both E7/L2, respectively (Table 1). Statistical analysis indicated that the difference in the prevalence of the antibodies to E7 alone and L2 alone between the patient and

| Table 1. Summary of prevalence of the antibodies against HPV16 E7 and L2 proteins |
|-------------------------------|-------------------|-----------------|-----------------|
| Group                        | Number of tested sera | only E7 (%)     | only L2 (%)     | both E7 and L2 (%) |
| Cervical cancer              | 57                 | 10 (18%)        | 18 (32%)        | 3 (5%)             |
| Control                      | 200                | 10 (5%)         | 18 (9%)         | 2 (1%)             |

![Graph showing the prevalence of E7 and L2 antibodies in patients and controls](image-url)

**Fig. 2.** Prevalence of E7 (bottom panel) and L2 (top panel) antibodies in patients and controls. Numbers of tested sera of patients and controls are indicated in parentheses under age. Patients (■). Controls (■). **p < 0.01.
control groups was significant with values of $\chi^2 = 14.3$, $p < 0.01$ for E7, and $\chi^2 = 23.8$, $p < 0.01$ for L2 (Fig. 2).

Comparisons of the prevalence of E7 and L2 antibody between patients and controls in each age group indicated a higher rate in patients of each age and statistical analysis showed that the difference was significant only in the group of forties ($\chi^2 = 9.1$, $p < 0.01$ for E7; $\chi^2 = 11.8$, $p < 0.01$ for L2) (Fig. 2).

In the control group no difference in the prevalence of the antibodies between men and women was seen (Fig. 3). The anti-E7 antibody was detectable in 6 men (6%) and 6 women (6%). There was no variation of age-dependent prevalence of the E7 antibody (Fig. 3). Anti-L2 antibodies were detectable in 12 men (12%) and 8 women (8%) of the control. There was neither significant difference between men and women, nor variation of age-dependent antibody prevalence of L2 (Fig. 3).

Some previous reports of DNA-based experiments indicated that the difference of histological type in cervical cancer tissues could be due to type of HPV infection, i.e., HPV 16 is mainly related to squamous cell carcinoma of the cervix, while HPV18 is mainly associated with adenocarcinoma (Tase et al. 1988a, b). A study showed a higher incidence of serum IgG antibody to HPV16E2 in the adenocarcinoma of the uterine cervix (Lehtinen et al. 1992). Thus, we
classified patients by histological types and examined the type specificity of antibodies of the patients. As shown in Table 2, 28 of the 51 patients with squamous cell carcinoma possessed antibodies to the E7 and/or L2 proteins (10 cases for the E7 alone, 15 for the L2 alone and 3 for both the E7 and L2), and 3 patients with adenosquamous carcinoma had antibodies to the L2. On the other hand, no patients with adenocarcinoma had antibodies to either E7 or L2. However, statistical analysis did not show any significant difference in the prevalence of the antibodies among histological groups.

Some studies have revealed the relationship between the clinical stage of uterine cervical cancer and HPV infection (Hashido et al. 1991; Sasagawa et al. 1992), that is, progression of the stage is related to a higher incidence of HPV antibody. In our study, E7 and L2 antibodies were positive in 16% (8/49) and 33% (16/49) of early stage (stage I and II) patients, and 25% (2/8) and 25% (2/8) of late stage (stage III and IV) patients, respectively. There was no statistical difference of positive cases between patients of early stage and late stage.

### DISCUSSION

The aim of this study was to establish the relationship between the prevalence of the antibodies to HPV16E7 and L2 proteins and the development of cervical cancer.

We examined the prevalence of the antibodies in the cervical cancer patients and the normal population. Our findings clearly demonstrated that the patient group exhibits a higher prevalence of the antibodies to the HPV16E7 product than the control, as shown by previous studies (Hashido et al. 1991; Mandelson et al. 1992; Sasagawa et al. 1992).

The L2 protein encodes a minor capsid protein (Yaegashi et al. 1991) that induces a higher response of antibody than other HPV encoded proteins (Jenison et al. 1990), probably because the primary response may be induced by the L2 molecules of exogenous infected virions. The results of previous studies about antibodies for the proteins encoded by the HPV late genes were controversial. Dillner et al. showed a higher prevalence of antibodies to the 16L1, but not to 16L2. However, other studies showed no significant difference in the prevalence of antibodies to either 16L1 or 16L2 between cervical cancer patients and controls (Jenison et al. 1990; Kochel et al. 1991a, b; Barber et al. 1992; Mandelson et al. 1992). Our data show that 37% of the patients and 10% of the controls are
positive for the L2 antibodies and there was a significant difference between the two groups. The discrepancy between our findings and those reported by others might be due to the difference of specimens or methodology. As antibody response to a capsid protein of a virus, such as the L2, is usually thought to be a sign of host immune response to viral infection, coupled with the fact that there is currently no data showing the L2 protein as an oncogenic protein, more research will be needed to confirm the relationship between the presence of the serum L2 antibody and cancer progression.

We did not find any age-dependent variation of antibody prevalence or sex-specific difference. Approximately 10% of the blood donors between 16 and 19 years old were positive for HPV E7 or L2 antibody, indicating that primary exposure to HPV type 16 is possible in a young generation. Genital HPVs are thought to be sexually transmitted (Galloway and Jenison 1990). A survey of the prevalence of HPV antibodies in a younger population is in progress to determine whether or not the main transmission route of the virus is sexual.

Previous reports have shown that HPV18DNA is most frequently found in adenocarcinoma and HPV16DNA in squamous cell carcinoma (Tase et al. 1988a). Lehtinen et al. (1992) reported that the prevalence of both IgG and IgA antibodies to the HPV18E2 peptide was associated with adenocarcinoma, which suggested a specific role of HPV18 in adenocarcinoma of the cervix. However, our findings did not show any difference in the prevalence of the antibodies between two histological groups probably because of the small number of tested sera. Further investigation is needed, such as examining more sera of adenocarcinoma or examining the antibody reactivity to the HPV18 encoded proteins.

In summary, our findings indicate that antibodies to the HPV16L2 products could be a parameter for cervical cancer development as well as those to the HPV16E7 products.

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References


