

HUMAN WART-VIRUS ANTIBODIES IN PATIENTS WITH GENITAL AND SKIN WARTS

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Abstract. A comparative follow-up study of the antibody response against human wart-virus was performed, using the immunodiffusion and complement fixation methods on patients with condylomas and skin warts. By the immunodiffusion method, 13% of the patients with skin warts and 3% of the patients with condylomas showed \geq fourfold increase of antibody titre during the follow-up of 2-35 months. The findings show some typical features of the weak antibody response of a chronic virus infection and suggest a serological overlapping between condyloma viruses and certain group of skin wart-viruses. The antibody prevalence in age-matched controls is shown to be significantly higher than that in the wart or condyloma patients' initial serum samples. This is indicative of the protective function of antibodies against warts and condylomas. Also the analysis of the history of warts in patients with condylomas suggests that protection against condylomas can be acquired from previous warts, evidently by immunological mechanisms. In a control group of medical students, human wart-virus antibodies were frequently (52%) found in subjects without any history of warts. This finding supports the view that human wart-virus can frequently induce latent or sub-clinical infections in human beings.

Key words: Antibody response; Chronic virus infection; Latent virus

The infective nature of genital warts or condylomas has been known for decades. However, the causative agent was not revealed to be a human papilloma virus (HPV) until the late 'sixties (1, 3, 9). Originally, based on transfer experiments, the agent was suggested to be the virus which induces skin warts. Subsequently, the virus has been shown to be electronmicroscopically similar to that isolated from skin warts (1, 3, 9), though there may be serological differences (1). Because of the small quantities of virus particles in genital warts and the lack of an in vitro propagation method, extensive serological studies on genital warts are lacking.

The present investigation was aimed at elucidating the antigenic relationship between skin and genital wart-virus and certain features of the immune response in these diseases by measuring antibodies from patients with genital and skin warts against skin wart-virus antigen.

PATIENTS AND METHODS

Patients

The patients with *condylomas* numbered 243: 149 were females who were treated as outpatients at the Department of Gynaecology; 94 were males, outpatients at the Department of Urology, University Central Hospital, Helsinki. The ages of the patients ranged between 15 and 56 years, the mean age for females being 25.4 years and for males 24.6 years, an average of 25.1 years. On admission to the clinic, the history of condylomas and skin warts and the number and the size of condylomas as well as skin warts were recorded for the male patients. Wart history was recorded only from those female patients who had high or increased titres of wart-virus antibodies, measured by immunodiffusion or measurable complement fixing antibodies. On admission to the clinic, serum samples were taken for serological studies. Serial serum samples were obtained over a 2-month observation period from 75 of the patients. The patients were treated primarily by surgical excision and electrocoagulation. When this procedure failed, other treatments, described previously (14), were also used.

The patients with *skin warts* were treated as outpatients at the Department of Dermatology, University Central Hospital, Helsinki. The first group of these patients (Table II) comprises 230 patients aged 15-56 years. Of these 155 were females and 75 were males. The mean age of the females was 27.2 years and of the males, 25.1 years (average 26.5 years). Thus this group does not differ in age and sex from the condyloma patients. Additionally, all the skin wart patients whose serum samples were available during the 2-month or longer observation period were collected. This group comprises 190 patients, varying in age from 6 to 70 years (mean 19.1 years). The results of antibody studies on the latter group of patients are presented in Table IV.

Control subjects

Part of the control group comprised 111 patients whose sera were sent to the Department of Virology, University of Helsinki, for determination of viral antibodies because of some acute infection. The ages of these patients varied from 15 to 50 years (mean 26.5 years). Another part of the control group comprised 81 medical students aged 20-28 (mean 22.3) years. The age and sex distribution of this collected control group did not differ significantly from that of the patients with condylomas or skin warts (see Table II).

Additionally the history of skin warts and condylomas was obtained from a total of 300 medical students, by

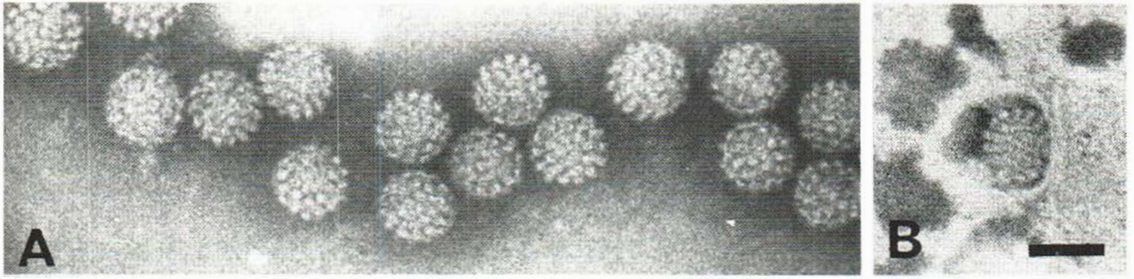


Fig. 1. Negatively stained preparation of virus particles located in a pool of skin wart tissue (A) and in a pool of genital wart tissue (B). The particles show similar

structures and the same size, about 50 nm. The bar represents 50 nm. $\times 200\,000$.

means of a questionnaire. Antibodies against the human wart-virus were measured in the sera of all those students who did not recall ever having had skin warts or condylomas.

Methods

Human wart-virus antigen was prepared from surgically removed skin warts as described previously (10, 11). The antigen was prepared from a pool of 2 g wart tissue from more than 10 patients. Immunodiffusion (ID) tests and complement fixation (CF) tests were performed as previously described (10, 11).

For electron microscopic studies, 2 g of condyloma or wart tissue was minced with scissors and ground with sand in 10 ml of phosphate-buffered saline solution, as for the preparation of wart-virus antigen. The resulting suspension was clarified twice at 600 g for 30 min. The supernatant was used for electron microscopy.

Statistical analysis

The significance of various differences in the results was evaluated by χ^2 -test.

RESULTS

Electron microscopic findings

Four separate antigen specimens prepared from 2 g of condyloma tissue pooled from 10 or more con-

dylomas from various persons were studied by mean of electron microscopy. Three of these specimens could be shown to contain virus particles when at least 30 well stained squares out of 200 squares of a grid were studied by EM at $\times 40\,000$ magnification. The particles were structurally similar to those found in skin wart tissue (Fig. 1). However, the number of particles was approximately 10^4 times less in condyloma specimens than in specimens prepared similarly from skin warts, as the latter specimens could be diluted 10^4 times and yet viral particles were still detectable at the same frequency as in undiluted condyloma specimens (Table 1).

Prevalence and history of skin warts in patients with condylomas and in controls

When attending the clinic, 12% (11/94) of the male patients with condylomas also had skin warts. The mean age of these patients was 24.6 years. The prevalence of skin warts in male medical students of mean age 22.0 years was 16% (31/188). When analysing the history of warts in these subjects, only 29% (27/94) of the male patients with condylomas

Table 1. Number of HPV particles in skin wart and condyloma antigen specimens

| Skin wart specimen | | | | | | Condyloma specimen | | | | | |
|-------------------------|-------------------|-----------|-----------|-----------|-----------|-------------------------|----------|-----------|-----------|-----------|-----------|
| Antigen pre-preparation | Dilution | | | | | Antigen pre-preparation | Dilution | | | | |
| | 10^0 | 10^{-1} | 10^{-2} | 10^{-3} | 10^{-4} | | 10^0 | 10^{-1} | 10^{-2} | 10^{-3} | 10^{-4} |
| 1 | ++++ ^a | ++++ | +++ | ++ | + | 1 | + | - | - | - | - |
| 2 | ++++ | ++++ | +++ | + | - | 2 | + | - | - | - | - |
| 3 | ++++ | ++++ | +++ | + | - | 3 | + | - | - | - | - |
| 4 | ++++ | ++++ | ++++ | + | + | 4 | - | - | - | - | - |

^a The marks denote the numbers of particles counted by electron microscopy at a magnification of 40 000. +++++: >100 particles/grid square; ++++: 50-100 particles/grid square; ++: 10-50 particles/grid square; +: <10 particles/grid square.

Table II. Prevalence of wart-virus antibodies in patients with condylomas, or skin warts, in the control group and in medical students without any history of warts

| Titre | Patients with condylomas ^a | Patients with skin warts ^b | Control group ^c | Medical students with no history of warts |
|-------|---------------------------------------|---------------------------------------|----------------------------|---|
| <1 | 78 (32%) | 62 (27%) | 34 (18%) | 31 (48%) |
| 1-2 | 88 (36%) | 92 (40%) | 94 (49%) | 17 (27%) |
| 4-8 | 58 (24%) | 53 (23%) | 54 (28%) | 10 (16%) |
| 16-32 | 13 (5%) | 15 (7%) | 10 (5%) | 6 (9%) |
| ≥64 | 6 (2%) | 8 (3%) | - | - |
| Total | 243 | 230 | 192 | 64 |

^a Condyloma patients aged 15-56 (mean 25.1) years, antibody titre on admission to the clinic.

^b Skin wart patients aged 15-56 (mean 26.5) years, antibody titre on admission to the clinic.

^c Control group is composed of 81 medical students aged 20-28 (mean 22.3) years and 111 patients, whose sera were sent to a routine virus laboratory. The ages of the latter were 15-50 (mean 26.5) years.

had ever had skin warts, whereas 78% (146/188) of the male medical students had then or had previously had warts, which is significantly more than in patients with condylomas.

Wart-virus antibodies in patients with condylomas versus in patients with skin warts and in controls

The prevalence of antibodies in patients with condylomas and skin warts on admission to the clinic and in the respective controls was measured by the immunodiffusion method (Table II). In the control group, antibodies could be detected more frequently than in the patients with skin warts or condylomas. The difference is significant (condyloma patients versus controls, $p < 0.005$; wart patients versus controls, $p < 0.05$). Antibodies in a high titre (≥64) were detected in a small proportion (2-3%) of patients with condylomas or skin warts but in none of the 192 controls. No significant difference in the antibody prevalence or in titres was detected between males and females, either in the patients or in the controls.

Altogether 64 of the 300 medical students (21%) did not remember ever having had any kind of skin wart or condyloma. In most of these cases their "negative" wart history was checked. About half of these students (33/64) had human wart-virus antibodies, 6 of them at fairly high titres of 16-32 (Table II).

To analyse the influence of skin wart history on the antibody levels in condyloma patients, the prevalence of wart-virus antibodies was studied in separate groups of the 94 male patients with condylomas (Table III). No significant difference was detected in the antibody prevalence between those

patients who had never had skin warts and those who currently had, or had previously had, skin warts.

From 190 patients with skin warts and 75 patients with condylomas, at least two consecutive serum samples were available for antibody determinations during a 2-month (or longer) observation period. Of the patients with condylomas only 3% (2/75) developed a ≥ fourfold or greater increase in antibody titres (mean observation period 4.8 months), whereas 13% (24/190) of the patients with skin warts (mean observation period 5.6 months) showed a significant increase in antibody titres ($p < 0.02$). None of the condyloma patients with increases in titre had detectable skin warts. On analysing the antibodies in skin wart patients, the frequency of titre change was observed to be related to the initial antibody level (Table IV). 24% (14/59) of those patients who were initially without antibodies developed measurable antibodies, whereas only 8% (10/131) of all the patients with antibodies on admission developed increased titres

Table III. Wart-virus antibodies in male condyloma patients grouped according to their history of warts

| Titre | Number of patients | | |
|-------|--------------------------|---------------------|---------------------------------------|
| | No history of skin warts | Skin wart currently | With history of skin warts previously |
| <1 | 22 (33%) | 4 (36%) | 5 (31%) |
| 1-2 | 27 | 2 | 6 |
| 4-8 | 13 | 5 | 4 |
| 16-32 | 4 | - | 1 |
| ≥64 | 1 | - | - |
| Total | 67 | 11 | 16 |

Table IV. Changes in wart-virus antibody titres compared with initial antibody titres in the sera of skin wart patients

| Initial antibody titre | No. of patients (total no. 190) | No. with four-fold increase in titre | Mean observation period (months) | % of patients with fourfold increase of a.b. titre/month |
|------------------------|---------------------------------|--------------------------------------|----------------------------------|--|
| <1 | 59 | 14 (24%) | 5.12 | 4.6% |
| 1-2 | 70 | 2 (3%) | 5.66 | 0.5% |
| 4-8 | 45 | 4 (9%) | 5.62 | 1.6% |
| 16-32 | 11 | 3 (27%) | 6.64 | 4.1% |
| ≥64 | 5 | 1 (20%) | 7.00 | 2.9% |

during observation. The percentages of these antibody changes per month were respectively 5% and 1%, such a difference being statistically significant ($p < 0.005$). The changes in titre were especially few when the antibody titre was initially one or two; only 2 out of 70 (3%) such patients later developed an increased antibody titre (0.5% per month).

Differences were also found in the pattern of CF-antibodies from genital and skin wart patients. 7 out of 202 patients with condylomas had measurable CF-antibodies in their initial serum samples and none of the 4 patients, followed-up over at least 2 months, developed increased titres, nor did any of the other 71 patients followed-up for at least 2 months develop CF-antibodies. Only one of the 5 patients with CF-antibodies had ever had detectable skin warts (according to the questionnaire). Of the patients with skin warts, 10% (19/190) initially had CF-antibodies and an additional 10% (19/190) developed CF-antibodies during the observation period.

Correlation of the serological findings with the clinical course of condylomas

The mean duration of condylomas after admission to the clinic was 6.1 months for those with wart-virus antibodies and 5.1 months for those without antibodies. Even a comparison of antibody titres with the duration of the disease failed to show any significant correlations. The size and the numbers of condylomas showed no significant correlations with the antibody findings and the antibodies had no prognostic value for healing.

DISCUSSION

Fourfold or greater increases in antibody titres against pooled antigen of skin wart-viruses measured by ID were detected in 13% of the patients

with skin warts, compared with 3% in patients with condylomas, during the observation period. Such a difference could be due to a weak antibody response in patients with condylomas, or to the viruses in genital warts being antigenically so divergent from skin wart-viruses, that the antibody response cannot be measured by using the skin wart-virus antigen. The EM-studies show that the number of viral particles in condylomas tissues is, on average, at least ten thousand times less than in skin warts. Thus the antibody response against the virus must be weak in contrast to skin warts.

No remarkable correlations between the clinical features of condylomas and the serological findings were found. This differs from the previous findings for skin warts (10) and suggests certain serological differences between the majority of skin wart and genital wart viruses—or it may be due to the smaller amount of viral antigens in condylomas. 78% of the male medical students, compared with only 29% of the male patients with condylomas, remembered having had skin warts at some time. Although such figures are not very reliable, the large difference suggests that some immune reactions elicited against skin warts offer protection against condylomas. Comparison of the antibody prevalence in condyloma and skin wart patients with the prevalence in age-matched controls shows that the controls have antibodies significantly more frequently than either group of patients. This is suggestive of a protective effect of wart-virus antibodies against induction of skin warts as well as of condylomas.

Studies by Almeida and her co-workers (11) using immune electron microscopy and the CF-method suggested that there is a one-way antigenic cross-reactivity between genital and skin wart-viruses. Recent studies on the DNA structure of wart-virus by Gissmann et al. (4) show that at least four subgroups of skin wart-viruses exist. In addition to

DNA structure, structural proteins and antigenicity are similar in three sub-groups, but the fourth group seems to be entirely different. The antigen used in the present study was a collected pool of numerous warts from more than 10 patients. It might thus have included any or all of these sub-groups. It is known that the viruses of the fourth sub-group are found only in small amounts in wart tissues (4). As development of the precipitation line in microimmunodiffusion requires about 10^{10} viral particles/ml, it is evident that antibodies presently detected are against virus of sub-groups 1-3. All the precipitation lines made by the sera of condyloma patients formed a continuous line, with a control serum of a wart patient.

In conclusion, the following findings suggest that there is overlapping between condylomas and skin warts inducing viruses. Firstly, in transfer experiments (12) skin warts have been induced by injecting condyloma extracts into the skin of volunteers. Secondly, increases in antibody titre against the skin wart-viruses are sometimes detected in condyloma patients, as shown here. Thirdly, antibodies against skin wart-viruses are detected in some patients with condylomas and without skin warts at titres (≥ 64) higher than those detected in age-matched controls (Table II). Fourthly, in contrast to Almeida's findings, CF-antibodies against skin wart virus antigen were detected in patients with condylomas but without any history of overt warts in this study. From the circumstantial evidence presented it may be concluded that some group(s) of HPV are able to induce skin warts as well as genital warts. The genital area might be the site of preference for some groups of HPV infections, whereas other cutaneous areas are preferred by other groups of human papilloma viruses. The existence of an additional "pure" condyloma virus which has received support from some epidemiological findings (8) cannot, of course, be excluded.

As shown in this and previous studies, wart diseases of months' or years' duration is a good example of a typical chronic viral infection with peculiar features in that: changes in antibody titres are not frequently observed and many of the patients have IgM-type wart-virus antibodies. I have observed detectable levels of such antibodies in wart patients' sera, sometimes over years. It seems highly probable that subclinical wart infections also occur. Medical students in the present series were questioned about their past wart history. Many of them did not

remember ever having had skin warts, although they had measurable wart-virus antibodies, in some cases at fairly high titres. I have also found a patient with a history of bladder papillomas who had wart-virus specific IgM antibodies for 18 months but without overt skin or genital warts. Observations suggesting subclinical wart infections have also been reported by Ogilvie (7) and Cubie (2) from their series of students and Morison (6) from patients with sarcoidosis and recently by Lee et al. (5) in their studies on cell-mediated reactions against warts. The appearance of warts during immunosuppressive therapy (13) is also suggestive of the frequent existence of latent virus in humans.

The present findings show that even low levels of wart-virus antibodies may effectively inhibit an additional antibody response. In patients who initially had no antibodies, increases in titre were about ten times more frequent than in those with low antibody levels (titres of one to two). The antibody response described here for warts evidently is generally valid also for other chronic viral infections. Thus in diagnostics the registration of changes of antibody titres in infections such as warts seems to give very poor results, especially when there are pre-existing antibodies. This knowledge is important because of the increasing likelihood of the association of papilloma viruses with human malignancies. The association of condyloma virus with cervical cancer seems especially important (15, 16). The present findings show that measurement of viral antibodies will not give any conclusive results, because changes in antibody titres are not usually expected and latent or subclinical HPV infections can disturb the interpretation of results. The application of delicate protein analysis methods to the identification of virus coded proteins from tumours could constitute an important approach for future studies.

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