Gynaecological Infections as Risk Determinants of Subsequent Cervical Neoplasia

Merja Viikki, Eero Pukkala, Pekka Nieminen and Matti Hakama

From the University of Tampere School of Public Health, Tampere, Finland (M. Viikki), Deutsches Krebsforschungszentrum, Epidemiologie, Germany (M. Viikki, M. Hakama), the Finnish Cancer Registry (E. Pukkala, M. Hakama), and the Department of Obstetrics & Gynaecology, Helsinki University Central Hospital (P. Nieminen), Helsinki, Finland

Correspondence to: Dr Merja Viikki, University of Tampere School of Public Health, Box 607, FI-33101 Tampere, Finland. Tel: +358 3 21 56 883. Fax: +358 3 21 56 057. E-mail: memekl@uta.fi

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A longitudinal cohort study was carried out to determine whether gynaecological infections other than human papillomavirus (HPV) are also related to the subsequent increased risk of cervical neoplasia. The study comprised 19114 women attending the organized mass screening in Finland in 1985–1990 with cytologically detected HPV, *Actinomyces*, herpes simplex, *Trichomonas vaginalis*, or yeast. The women were followed-up for subsequent preinvasive lesions and invasive cancers until the end of 1994 by linkage to the nation-wide Cancer Registry. Standardized incidence ratios (SIR) with rates for the whole of Finland as reference and 95% confidence intervals (CI) were estimated. *Trichomonas vaginalis* and HPV were associated with a high relative risk of cervical cancer, SIR 6.4 (CI 3.7–10, preinvasive lesion and invasive cancer combined) and SIR 5.5 (CI 4.2–7.2, preinvasive lesion and invasive cancer combined), respectively. Herpes simplex was rarely detected, but the highest and statistically most significant point estimate was observed (SIR 12, CI 2.4–34, preinvasive lesion and invasive cancer combined). Neither *Actinomyces* nor yeast was associated with a significantly increased risk of cervical cancer. None of these results could be accounted for by the confounding effect of the other infections. Our results, based on a prospective design, lead us to propose that *Trichomonas vaginalis* and herpes simplex virus are also predictors for cervical neoplasia.

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In many studies human papillomavirus (HPV) is reported to be a major risk factor for cervical neoplasias (1–8). It has been suggested that *Chlamydia trachomatis* (9, 10), herpes simplex virus (11, 12) and *Trichomonas vaginalis* (13–15) are also risk factors for cervical neoplasia. Often the experience stems from a cross-sectional study design, i.e., simultaneous diagnosis of the infection and cancer. The purpose of our study was to determine whether infections diagnosed cytologically (HPV, herpes simplex, *Trichomonas vaginalis*, *Actinomyces*, yeast) also precede the occurrence of preinvasive cervical lesions and invasive cancer of the cervix uteri. The highly developed Finnish healthcare infrastructure, which includes the linkable National Population Registry, the Finnish Cancer Registry and the Mass Screening Registry for organized screening programme of cervical cancer, makes it possible to carry out studies with such a longitudinal setting.

MATERIAL AND METHODS

An organized mass screening programme for cervical cancer was introduced in Finland in 1963. The target screening population consists of the entire Finnish female population aged 30–60 years; there is, however, some variation in the age groups because the cohorts to be screened are chosen by municipal authorities. Every woman is identified from the National Population Registry and invited every fifth year by personal letter to attend the screening programme, with place and individual time specified. The results including the infections diagnosed cytologically and result of the Papanicolaou smear are sent to the nation-wide Mass Screening Registry.

In our study we followed for cancer the cohorts of women registered in the Mass Screening Registry with cytological detection of HPV, *Actinomyces*, herpes simplex, *Trichomonas vaginalis*, or yeast at mass screening from 1 January 1985 to 31 December 1990. In addition to the combined cohort with all women with any of these infections, each of the infections diagnosed cytologically was analysed separately. In this paper the detection of a microbe and detection of an infection caused by that microbe are used synonymously. The Mass Screening Registry collects data only on women with different gynaecological infections, self-reported bleeding symptoms and abnormal smear (Papanicolaou classes II–V); these

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women are invited to attend the next screening round 1–2 years after the original screening instead of the routine 5-years' interval. Therefore, there are no records, at the level of individuals, on non-infectious women of the same period of observation.

The nation-wide Finnish Cancer Registry was founded in 1952, and since 1961 it has been obligatory for all physicians and hospitals under public as well as private administration to report all cases of diagnosed cancer to the Cancer Registry. Carcinoma in situ of the cervix uteri has been registered since 1964 and severe dysplasia since 1991. Because the classification of dysplasia gravis, CIN III and carcinoma in situ depends more on the time and place of recording the lesion than on its biological character, all these diseases were grouped together under the heading of preinvasive cervical cancer.

The linkage of data in the Mass Screening Registry to the Finnish Cancer Registry was done with personal identification numbers as the key. The dates of death and emigration were obtained from the National Population Registry. The follow-up for cancer of the women in the study cohort started at the beginning of the month following the date of mass screening when infection was diagnosed, and ended at death, emigration to a foreign country, or on 31 December 1994, whichever occurred first. The follow-up was complete.

The numbers of observed cases and person-years at risk were counted by time from the beginning of follow-up (<1, 1–4 and $5+$ years) and by 5-year age groups. The expected numbers of cancer cases were calculated by multiplying the number of person-years by the corresponding average age-specific cancer incidence rate in the whole Finnish female population during the same period of observation.

To calculate the standardized incidence ratio (SIR), the observed number of cases was divided by the expected number. The 95% confidence intervals were estimated assuming that the number of observed cases followed a Poisson distribution.

RESULTS

The total number of women with a cytological diagnosis of certain infections registered in the Finnish Mass Screening Registry in 1985–1990 was 19787, and the combined cohort of women with any of these infections consisted of 19114 women. The difference 673 is due to diagnosis of multiple infections in the same woman. The maximum follow-up was 10 years (mean 6.2 years) (Table 1).

Trichomonas vaginalis infection was associated with a high relative risk of both preinvasive and invasive cervical cancer combined (SIR 6.4) (Table 2). Infection with herpes simplex virus was rare and only three cancer cases were observed (expected 0.3). There was substantial increase in cervical cancer subsequent to diagnosis with HPV (SIR 5.5), which was greater for the preinvasive lesions than for invasive cancer (Table 2). The relative risk estimates for both preinvasive lesion and invasive cancer were increased in the total cohort of women with any of the microbial findings included. (Table 2). Neither cytological detection of *Actinomyces* nor yeast was associated with a significantly increased relative risk of cervical cancer or preinvasive lesion. The relative risk of cervical cancer after any infection was independent of age at screening (Table 3). However, there was wide random variation in the individual infections because of the small numbers of cases.

In the first year of follow-up after diagnosis of infection, the relative risk of cervical cancer was higher than during the subsequent years (Table 4), and the high relative risk of any infection during the first year of follow-up (SIR 6.7) went down to 2.1. The decrease in the SIR from <1 year to 1–10 years was statistically significant only for HPV, but the numbers were small and subject to wide random variation. The relative risks remained high and statistically significant after the first year of follow-up for *Trichomonas vaginalis* and HPV.

DISCUSSION

In our study 19114 women were registered in the Finnish Mass Screening Registry with gynaecological infection diagnosed cytologically in 1985–1990. These women were followed-up, and the subsequent incidence of preinvasive and invasive cervical cancer was estimated.

Ascertainment of personal identification numbers of the cohort as well as follow-up for death was complete for our study. Computerized record linkage procedure is precise in Finland (16) and does not cause bias in the results. Registration of invasive cancer in the Finnish cancer registration system is virtually complete (17). Although the registration of carcinoma in situ and of severe dysplasia of the cervix is probably somewhat underreported, this is not likely to be correlated with the infections studied, and therefore would not bias the relative risk estimates.

In other studies the association between history of HPV-associated genital condylomas and cervical neoplasia has

Table 1

The number of women (N) with different infections (microbes) in the Finnish mass screening 1985–1990, and person-years of follow-up to 31 December 1994

Infection	N	Person-yrs
Trichomonas	1 544	10 417
Herpes	138	927
HPV	5 407	36 217
Actinomyces	3 225	20 528
Yeast	9 473	55 358
Any infection	19 114	119 100

Table 2

Observed (Obs.) numbers of cervical cancer and standardized incidence ratios (SIR) with 95% confidence intervals (CI) among women with different infections (microbes) in the Finnish mass screening 1985–1990, and followed-up to 31 December 1994

Infection	Preinvasive			Invasive			Total		
	Obs.	SIR	CI	Obs	SIR	CI	Obs.	SIR	CI
Trichomonas	13	6.8	3.6–12	3	5.0	1.0–15	16	6.4	3.7–10
Herpes	3	14.3	2.9–42	–	0.0	0.0–76	3	12	2.4–34
HPV	54	6.4	4.8–8.3	3	1.6	0.3–4.8	57	5.5	4.2–7.2
Actinomyces	6	1.4	0.5–3.0	3	2.7	0.6–7.8	9	1.6	0.7–3.1
Yeast	12	1.0	0.5–1.8	1	0.3	0.0–1.8	13	0.9	0.5–1.5
Any infection	83	3.2	2.6–4.0	9	1.4	0.6–2.7	92	2.9	2.3–3.5

varied: adjusted odds ratios were 1.4–4.9 for invasive cervical cancer (18–20) and 1.0–3.9 for preinvasive lesions (14, 19, 21). Few studies have been prospective, i.e., studying the association between infection and subsequent risk of cervical neoplasia, and in these studies the strength of the association has been dependent on the method of detecting the HPV: with cytological diagnosis of HPV the relative risk was reported to be 3.5 (14), and with serological diagnosis specific for HPV 16 the risk was 12.5 (6). Modern methods (PCR) have given much higher relative risks for HPV 16 (22). Cytological diagnosis of HPV is usually based on dignosis of condylomatous lesions, which are known to express preferentially low-oncogenic-risk HPV types (23, 24). Despite the diluting effect of the rather low sensitivity and specificity of cytological HPV diagnosis, we found a 5.5-fold risk of cervical cancer among women with a diagnosis of HPV, which is consistent with other studies with a similar design.

Zhang & Begg (15) analysed 24 studies for the association between *Trichomonas vaginalis* and cervical neoplasia (including both cervical intraepithelial neoplasia and cervical cancer) and found a significant positive association with large variation in the strength of the association. This meta-analysis included only two cohort studies for which the summary relative risk was 1.9 (95% CI 1.2–2.6). Both of these studies were based on cytological detection of *Trichomonas vaginalis* and thus comparable to our study. In our study, the cervical cancer risk, subsequent to trichomoniasis was six-fold after excluding the first year of follow-up, with even the lower confidence limit being above the mean relative risk of 1.9 derived from the other studies. The *Trichomonas vaginalis* parasite is sometimes difficult to distinguish from particles of inspissated mucus and degenerating cellular material (23–25), resulting in a poor validity of the cytological diagnosis, which perhaps explains the low relative risk rates in other studies.

The role of herpes simplex type II virus in cervical intraepithelial neoplasia was considered to be important in the 1970s (11, 12), but subsequent prospective studies based on serology have shown no association between herpes simplex virus and cervical carcinogenesis (6, 26, 27). However, some studies indicate the importance of herpes simplex virus as a factor leading to cervical carcinoma in the presence of HPV (3, 28, 29). In our study, herpes simplex type II virus was only rarely detected, partly because of poor validity of cytology in detecting herpes simplex virus. However, loss in validity tends to reduce the estimates of relative risk rather than to enhance them. In our study, the risk estimate was high and statistically significant, which is consistent with herpes simplex virus being a risk factor for cervical cancer.

Table 3

Observed numbers of cervical cancer cases (Obs.) and standardized incidence ratios (SIR) with 95% confidence intervals (CI) among Finnish women with different infections (microbes) in mass screening 1985–1990 and followed-up to 31 December 1994, by age at screening

Infection	Age <45			45+		
	Obs.	SIR	CI	Obs.	SIR	CI
Trichomonas vaginalis	10	6.4	3.1–12	7	7.4	3.0–15
Herpes simplex	1	4.8	0.1–27	2	40	4.8–140
HPV	47	5.7	4.2–7.6	10	4.7	2.3–8.7
Actinomyces	6	1.5	0.5–3.2	3	2.1	0.4–6.2
Yeast	11	1.0	0.5–1.8	2	0.5	0.1–1.8
Any infection	69	2.9	2.3–3.7	23	2.8	1.8–4.2

Table 4

Observed numbers of cervical cancer cases (Obs.) and standardized incidence ratios (SIR) with 95% confidence intervals (CI) among Finnish women with different infections (microbes) in mass screening 1985–1990 and followed-up to 31 December 1994, by follow-up time

Infection	Years of follow-up					
	<1			1–10		
	Obs.	SIR	CI	Obs.	SIR	CI
Trichomonas vaginalis	3	8.1	1.7–24	13	6.1	3.2–10
Herpes simplex	2	50	6.1–180	1	4.5	0.1–25
HPV	24	16	10–24	33	3.7	2.6–5.3
Actinomyces	3	3.4	0.7–9.9	6	1.3	0.5–2.8
Yeast	4	1.5	0.4–3.9	9	0.7	0.3–1.4
Any infection	35	6.7	4.7–9.3	57	2.1	1.6–2.7

There are controversial reports on the association between altered vaginal flora and cervical neoplasia (30, 31). The association between yeast and cervical dysplasia has in different studies been either non-existent (32) or positive (33). Fungal organisms can be a sign of good genital hygiene, which makes the lack of a positive association credible. In our study, yeast was not associated with cervical cancer. Nor did we observe any association between *Actinomyces* and cervical cancer; this association has not to our knowledge been studied in other prospective studies in the general population. The cervical smear is quite insensitive in detecting fungal organisms, i.e., yeast, compared to culture. *Actinomyces*, on the other hand, is usually detected with a high degree of validity in smears even though a number of other organisms, including *Candida* and bacterial aggregates, and even foreign substances can resemble *Actinomyces* (23–25).

The increased risk of cervical neoplasia subsequent to a given infection may also be due to factors other than the infection itself, because women prone to one gynaecological infection, especially to a sexually transmitted one, are also prone to other gynaecological infections because of their sexual habits. In our study, however, less than 4% of women were diagnosed with multiple infections. There was only one woman with cervical cancer who had a prevalent diagnosis of both trichomoniasis and HPV, and not a single woman with cervical cancer who had a prevalent diagnosis of both herpes simplex virus and HPV. Therefore, confounding with HPV is not likely to account for the positive association between cervical cancer and *Trichomonas vaginalis* or herpes simplex virus.

Within a short lag between detection of infection and diagnosis of cancer, the cause and effect relationship may be in either direction: a woman with an infection may be at an increased risk of cancer, and a women with an occult undiagnosed cancer may be more susceptible to an infection. In many studies (8, 15) infection and cervical cancer were diagnosed simultaneously, and thus the direction of causality could not be studied. A prospective study like ours provides a strong aetiological tool; the infection always precedes cancer. We found the relative risk of cervical cancer to be significantly increased during the first year of follow-up, consistently with the results of cross-sectional studies. The relative risk estimates became smaller with a longer follow-up but remained higher than unity after detection of *Trichomonas vaginalis*, herpes simplex and HPV. The increased relative risk during long-term follow-up probably indicates a causal relationship from infection to cancer. Therefore, the association between *Trichomonas vaginalis* and herpes simplex, and cervical cancer is unlikely to account for the general susceptibility to infection among cancer patients.

Cervical cancer and preinvasive lesions are likely to have many causes and their infectious aetiology is well documented (34). Cytology is poor in diagnosing past infections, and the validity of cytology for present infection varies by infection. Therefore, it is impossible to rank different infections on the basis of their aetiologic importance. However, our results suggest that not only HPV but also *Trichomonas vaginalis* and herpes simplex virus are predictors of cervical cancer.

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