Prevalence and Determinants of Human Papillomavirus (HPV) Infection in Kerala, India

Acta Universitatis Tamperensis 755

CHERIAN VARGHESE

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University of Tampere
Tampere 2000

ACADEMIC DISSERTATION
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Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 755
ISBN 951-44-4839-1
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 41
ISBN 951-44-4840-5
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2000

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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the auditorium of
the School of Public Health of the University of Tampere,
Medisiinarinkatu 3, Tampere, on May 26th, 2000, at 12 o'clock.

University of Tampere
Tampere 2000

To my wife
and daughter

Contents

1 INTRODUCTION

Human papillomavirus (HPV) is the major etiological agent for cervical cancer, the second most common cancer among women everywhere in the world (Munoz et al. 1994). This virus is also implicated in other anogenital cancers. HPV is among the most important viruses in the causation of cancer and a large number of epidemiological, biological and clinical studies are underway to get to know the nature of this infection and its outcomes. The prospects of HPV vaccine in preventing cervical cancer makes it the most suitable target for studies in low resource settings with high morbidity and mortality from cervical cancer.

Papillomaviruses are non-enveloped, double stranded DNA viruses that are included in the papaovaviridae family and multiple types have now been identified. More than 85 well characterised genotypes are recognised, and more than 120 have been tentatively identified (zur Hausen 1999). Human papillomaviruses are associated with benign and malignant lesions of the anogenital tract and cause genital and foot warts. These viruses show tissue specificity and variable malignant potential.

Sexual behaviour, mainly age at first intercourse and multiple sexual partners have been consistently reported as risk factors for invasive cervical cancer. These epidemiological characteristics are consistent with a sexually transmitted disease. Once HPV has been identified, most of the known risk factors for cervical cancer appeared as surrogates for this infection.

The prevalence and determinants for HPV infection in general populations has been addressed in a few studies (Becker et al. 1991, Van Den Brule et al. 1991). It is important to address HPV epidemiology in general populations especially in low resource countries with a high incidence of cervical cancer, as it will provide information for the prevention and control of this cancer.

1.1 Cervical cancer control in Kerala

The state of Kerala on the south western coast of the Indian Union has achieved considerable progress in the health sector which is reflected in the low infant and maternal mortality rates and higher life expectancy compared to the country as a whole (Table 1). The life expectancy at birth was 67.0 years among males and 72 years among

Table 1. Demographic data and vital statistics of Kerala and India in 1995.

	India	Kerala
Density of population/Sq Km	267.0	749.0
Annual popn. growth rate %	2.2	1.3
Birthrate/1000	27.2	17.9
Deathrate/1000	8.9	6.2
Infant mortality rate/1000 LB	71.0	12.0
Literacy male %	64.1	93.6
Literacy female %	39.3	86.2
Life expectancy male	60.6	67.0
Life expectancy female	61.7	72.4

LB live births, popn. population

females in 1995 in Kerala (Economic Review 1996). This demographic transition has already taken place in the Western countries of the world. Need-based planning within the limited resources is essential to control the emerging epidemic of chronic diseases like cancer and cardiovascular diseases in Kerala.

Lack of reliable data on the magnitude of chronic diseases in India has been a major limitation in assigning their priorities in health care and medical research. This prompted the Indian Council of Medical Research to launch the National Cancer Registry Programme (NCRP) in 1981, by which seven population based cancer registries have been established in various parts of the country so far. The pattern of cancer in India shows a predominance of tobacco related cancers in men. In women the cervix and the breast were the most common sites of cancer (NCRP 1992).

India is one of the few developing countries that have formulated a National Cancer Control Programme (NCCP 1984). The programme envisages control of tobacco related cancers, early diagnosis and treatment of uterine cervical cancer and distribution of therapy services and palliative care through augmentation of health infrastructure. Suggested surrogate outcome measures include change in tobacco use, the 'Knowledge, Attitude, Practice' (KAP) pattern, compliance to screening programmes, changes in referral practices and shift in stage distribution (Sankaranarayanan et al. 1992).

The Regional Cancer Centre, Trivandrum (RCC) has developed a feasible and sustainable community based cancer control programme in Kerala, and has been designated as the “WHO Collaborating Centre for Cancer Control in Developing Countries”. The RCC is the seat of a hospital based cancer registry, which is responsible for cancer registration from hospitals under Trivandrum medical college besides the RCC. These data have been the main resource for initiating cancer control programs in Kerala.

Population based cancer incidence data were available in Kerala from the Trivandrum and Karungappally registries (PBCR 1999, NBRR 1997). Besides these a

large number of epidemiological studies are being conducted by the RCC. Risk factors for common cancers in the hospital cancer registry were reported (Sankaranarayanan et al. 1994).

The age adjusted incidence rate for uterine cervical cancer in Trivandrum was 15.5 per 100,000 in the period 1991–92 (Parkin et al. 1997). Based on this, it is estimated that on an average 2,500 to 3,000 new cases of cervical cancer would occur in Kerala annually. Data from the Hospital Cancer Registry in Trivandrum reveal that more than 50% of cervical cancers present in FIGO stage III, and this is reflected in an overall 5-year survival rate of 47% (Sankaranarayanan et al. 1995).

Pap smear based cytology screening has been the recommendation for the prevention of invasive cervical cancer and several agencies and countries have accepted this strategy. The effectiveness of organised cytology screening programmes has been well demonstrated by the Finnish mass screening system. The incidence of cervical cancer in Finland decreased from 15/100,000 in 1963 when screening was introduced to 2.5/100,000 in the mid 1990's (Hakama et al. 1991). Countries with limited resources are finding it difficult to establish organised screening programmes and alternative strategies like 'visual examination' have been proposed. These approaches have not been found to be useful (Wesley et al. 1997). Improvements in living standards and empowering women are the prerequisites for cervical cancer control in India (Varghese et al. 1999).

Prevalence of HPV has largely been studied among cervical pre-cancerous lesions and in cervical cancer. It is important from the public health and preventive aspects to study the prevalence and identify the determinants of HPV infection, which will offer the potential for primary prevention and newer methods of screening and vaccination.

With this background a study was carried out in the suburbs of Trivandrum, the capital of Kerala State, to address the prevalence and determinants of Human Papillomavirus. This dissertation will present the organisation of the cohort, and prevalence and determinants of HPV infection and discuss the implications of these results.

2 AIMS OF THE STUDY

1) To study the prevalence of uterine cervical Human Papillomavirus infection among married women in a general population in Kerala, India.

2) To study the determinants of uterine cervical HPV infection in this population.

3) To consider the implications for control of HPV infection and cervical cancer.

3 REVIEW OF THE LITERATURE

3.1 Human Papillomavirus

Beral (1974) postulated that exposure to sexually transmitted infection is an important determinant of cervical cancer. zur Hausen (1991) suggested that HPV infection and HPV viral gene expression have emerged as necessary, but not sufficient factors for cancer induction. Reviewing the epidemiological evidence linking HPV to cervical cancer, Bosch et al. (1997) concluded that over 90% of cervical cancers could be attributed to certain HPV types. The central role of HPV in cervical carcinogenesis has far-reaching implications in the prevention of this cancer.

Molecular Virology of HPV

HPV is a small, double-stranded DNA virus that is a member of the papovavirus group. The subtypes of HPV are not serotype viruses but are genotypes in which the typing scheme is based on the similarity of one HPV type to the other known HPV types at the DNA level. The central HPV-DNA repository is in Heidelberg and this facility assigns a new type of HPV after adequate studies. The viral genome of HPV consists of approximately 7900 nucleotides, and all viral gene transcription occurs off one strand.

The HPV genome may be divided into three parts based on the function of the encoded genes: the early (E) region E6, E7, E1, E2, E4 and E5 and the late (L) region L1, L2 and L3 and a non coding region which harbours the origin of replication and transcription control signals essential for the regulatory functions of the genome (Fig.1).

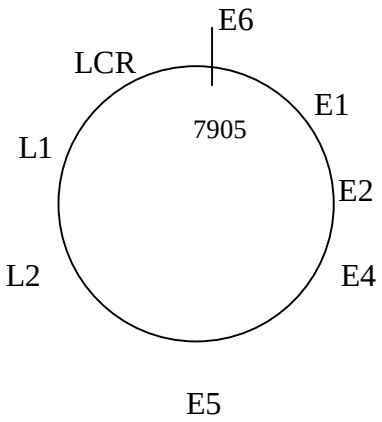

Figure 1. HPV genome – schematic representation.

HPV genome is approximately 7900 bp in length. The early (E) region of the genome is separated from the late (L) region by the long control region (LCR) that contains sequences involved in the regulation of expression of HPV proteins.

The viral genomic DNA in fully formed viral particles is surrounded by a protein coat known as the viral "capsid" that consists of the (L) regions L1 and L2. The (E) region proteins are associated with cell transformation and viral gene regulation and are most critical in the pathogenesis of invasive cancer. Between (E) and (L) lies the LCR, which contains promoter and enhancer DNA sequences critical to viral gene transcription by both viral and cellular genes.

The specific HPV types exhibit a degree of tissue tropism. Some types such as HPV 1 and 2 are most found in the keratinized skin of the palms and soles in the form of plantar and palmar warts. Types such as 6,11,16 and 18 are most often found in the keratinized skin and mucosal surfaces of the anogenital region, including the cervix. Types 16 and 18 are considered to have a 'malignant' phenotype as they exhibit a strong association with invasive cancer (Palefsky and Holly 1995).

Detection of HPV infection and HPV genotypes

Reliable and reproducible measurement of 'exposure' is an important aspect of epidemiological investigations. In studying the relation between HPV and cervical neoplasia, it is not always possible to obtain tissue biopsies. A cytological finding of koilocytosis represents HPV infection but cytology cannot detect the large percentage of infections at the DNA level. A major limitation of earlier epidemiological studies was the lack of an appropriate method for assessing type specific HPV infection.

Serological methods were also used to study the HPV infection. Sero-reactivity to HPV is a a reflection of the cumulative exposure to HPV. It is also useful to use serological methods to study the transmission of HPV. High risk of seropositivity to HPV 16 with multiple sexual partners has been shown in studies (Oslen et al. 1997).

The techniques of Southern, dot-blot and 'filter in situ' hybridisation have suffered from problems of sensitivity and specificity, particularly when DNA extracted from cervical smears was examined (Munoz et al. 1988). The Polymerase Chain Reaction (PCR) is an *in vitro* method for primer directed enzymatic amplification of specific target DNA sequences (Saiki et al.1988). PCR generates millions of copies of a specific DNA fragment in a few hours by *in vitro* enzymatic synthesis. HPV–PCR consists of amplification of a targetted portion of the viral DNA and identification of the amplified product. PCR has been extensively used for HPV identification from exfoliated cells and biopsy tissues and for facilitating cloning and sequencing of HPV

genomes. Stringent measures have to be adopted to avoid contamination of the specimen for PCR diagnosis. PCR has been shown to be more sensitive than Filter in situ Hybridisation and Southern Blot analysis in the detection of HPV in cervical scrapes (Melchers et al. 1989).

3.2 Risk factors for HPV infection and persistence

The prevalence rates of HPV in the general population is available mainly from the Western world. A study in New Mexico has found a prevalence of 9% among the general population. The prevalence rates were 13.7% for non-Hispanic white women, 9.7% for Hispanics and 6.6% for Native American Women (Becker et al. 1991). The International Agency for Research on Cancer (IARC) surveys of HPV prevalence in general populations have been presented recently. The prevalence varied from 8% in Thailand to 17% in Colombia. The overall prevalence was found to correlate roughly with the risk of cervical cancer in the population (Munoz et al. 2000).

The risk factors for cervical cancer may be those that are risk factors for HPV infection as well. Kataja et al. (1993) reported a case-control study of risk factors for HPV infection in Kuopio, Finland. The risk of infection varied with age, being the highest in the age group 20–29 years, and thereafter declining over the years. Number of sexual partners in the past two years was the most significant independent risk factor (adjusted OR 12.1). Current smoking, warts in sexual partner and frequency of sexual intercourse were identified as independent risk factors for HPV infection. This study also suggested that sexual intercourse was the main form of HPV transmission.

Risk factors for HPV infection were studied (Sexually Transmitted Disease clinic based study) in two areas with different cervical cancer incidence (Greenland and Denmark) by Svare et al (1998). The HPV prevalence among women declined after the age of 20 years in Greenland compared to Denmark, where the decline was after the age of 30 years. Age at first intercourse was not associated with HPV infection in either region. In Greenland those subjects who had had their first intercourse within 4 years prior to the study had a risk of 139.2 (95% CI 5.3–367.6) compared to those whose first sexual intercourse had occurred 15 years previously. The risk of HPV increased with increasing number of sexual partners. The number of sexual partners within the last year showed a strong association with HPV infection. Ley et al (1991) have reported that HPV infection was strongly and independently associated with increasing number of sexual partners in a lifetime, use of oral contraceptives, younger age and black race. Positive associations with lifetime number of sexual partners, socio-economic status, and Chlamydia trachomatis and HPV infection has been reported (Munoz et al. 1996).

A strong association of high risk HPV DNA and HIV-1 sero-positivity has been reported suggesting the role of immunosupression in the occurrence of HPV (Piper et al. 1999).

Most of the HPV infections are transient and the persisatnt infections are important events. It has been shown that type-specific persistent HPV infection, particularly with a high viral load, produces chronic cervical dysplasia (Ho et al. 1995). Hildesheim et al. (1994) had reported type specific persistance of HPV in 41% over a median follow up of 14.9 months. Age above 30 years and infection with high risk types were found to be more likely to persist. Factors associated with persistence of HPV infection were addressed by Brisson et al. (1996). Among 179 women who were HPV positive, 50.8% had persistent infection after a mean follow-up of 11 weeks. The factors associated with persistence of HPV were young age (< 25 compared to >25 years), lifetime number of sexual partners, and use of oral contraceptives. Among women who had had more than 8 partners, HPV infection persisted in 66.7% compared to 17% among those who reported one partner. Infection among those with high-risk HPV types (16,18,31,33,35) and high viral load at initial examination was more likely to persist than low risk types. Age at first intercourse, number of pregnancies, history of gynaecological infection, condom use, smoking and alcohol drinking were not associated with persistence of HPV infection.

3.3 Aetiology of cervical neoplasia

A review of epidemiological studies by Rotkin (1973) revealed that the incidence of carcinoma of the uterine cervix was higher in women of lower social class, those with many pregnancies, with a young age at first marriage, and in those with multiple sexual partners. These factors point towards the likelihood for a sexually transmittable agent in the aetiology of carcinoma of the cervix.

An increase in the incidence of cervical cancer among the younger age groups in certain regions had been linked to HPV and some concern was raised about the emergence of adenocarcinoma of the cervix (Zheng et al. 1996).

Murphy et al. (1992) reported that the social class difference in incidence of cervical cancer was present in all age groups and that this could not be explained by the gradient of social class on the attendance rate of cervical screening. It is possible that factors other than compliance with screening are operating in the lower social class. The religious practice of circumcision of males was considered as a protective factor among Jews and Muslims (Brinton and Fraumeni 1986).

Sexual behavioural characteristics, particularly young age at first intercourse and multiple sexual partners have been consistently reported as risk factors for invasive cervical cancer and pre-cancer (Cuzick et al. 1989, Jussawala et al. 1971, Parazzini et al. 1992, Bjorge and Kravdil 1996, Kvale et al. 1988). The role of a male factor in cervical cancer was noted from the studies showing geographic clustering of cervical cancer and penile cancer (Li et al. 1982). Risk of cervical cancer was found to be increased significantly in women whose male partners have reported multiple partners (Brinton et al. 1989). Beral et al. (1988) reported a significantly higher incidence of cervical cancer among oral contraceptive users compared to never-users in a cohort study. Juneja et al. (1995) have observed age below 18 years at consummation of marriage and multiplicity of sexual partners as the independent risk factors in Indian women.

Smoking also had been considered as a risk factor for cervical cancer (Winkelstein 1977). However in India, which is a high incidence region for cervical cancer, tobacco smoking in women is extremely rare.

Bacterial vaginosis is a common problem in un-screened women. In bacterial vaginosis, the lactobacilli-dominated flora is replaced by an abundant complex flora, constituted by gardenella, micrococci, streptococci and staphylococci. The abnormal vaginal flora can produce carcinogenic nitrosamines and may contribute to cancer (Frega et al. 1997).

Etiological role of HPV

Experimental studies have provided strong evidence that HPV is the long sought venereal cause of cervical neoplasia. A series of epidemiological studies with adequate exposure measurement in different settings has confirmed this finding (Munoz et al. 1994).

Prevalence of HPV DNA ranging from 22% to 100% have been reported in case series from different settings (IARC 1995). This broad range is considered to be due to variations in the methods used for HPV detection. Bosch et al. (1995) carried out a worldwide prevalence study of HPV in cervical cancer in 1995. More than 1000 specimens from 22 countries were studied. HPV DNA was detected in 93% of tumours, with no significant variation in HPV positivity among countries. HPV 16 was present in 50% of the specimens and HPV 18 in 14% of specimens. These results confirmed the role of genital HPV in the causation of cervical cancer. The etiological role of HPV and other risk factors in precancerous lesions of the cervix has been reviewed by Murthy and Mathew (2000).

Case-control studies showed that the prevalence of HPV infection among cases was very high, which resulted in high odds ratios not usually seen in epidemiological studies. The classical risk factors have lost their significance in most studies when adjusted for HPV status (Hernandez et al. 1997, Eluf Neto et al. 1994, Munoz et al. 1992, Peng et al. 1991, Reeves et al. 1989).

The majority of the prospective studies have established a substantial excess risk for progression to higher grades of cervical precancerous conditions among those who were HPV positives and more so for high risk HPV types (Campion et al. 1986, Ho et al. 1998, Remmink et al. 1995).

Presence of HPV antibodies in serum was associated with an odds ratio of 13.2 for invasive cervical cancer and this was significant (Lehtinen et al. 1996). After adjustment for smoking and antibodies to other sexually transmitted agents the risk of HPV infection remained significant with an odds ratio of 12.5. Ferrera et al. (1997) have reported similar findings in a study in Honduras. After adjusting for other sexually transmitted infections, presence of HPV16 anti E7 emerged as the significant risk factor for cervical cancer with an odds ratio of 3.6. In a prospective sero-epidemiological study, Dillner et al. (1997) reported an excess risk for past infection with HPV 16 for invasive cervical cancer.

3.4 HPV in the natural history of cervical neoplasia

Cervical carcinogenesis passes through well-defined entities as the changes occur in the epithelium. The age specific incidence of cervical dysplasia shows that it was most often diagnosed among women in their 20’s, carcinoma in situ (CIS) among those aged 30 to 39 years and invasive cancer after the age of 40 years (Canadian Task Force 1976). Each non-invasive type can regress to normal, progress to the next level of dyplasia or progress to invasive cervical cancer. The age specific incidence rates of preinvasive lesions are not usually collected. This information was available in Denmark on a population level, which showed that the distribution of mild, moderate and severe dysplasias and of CIS have the same shape as a function of age. The sequence in time at which they attain the peak supported the successive progression (Storm et al. 1989).

Koutsky et al. (1992) did a prospective study on 241 women with baseline HPV status and other risk factors. The cumulative incidence of cervical intraepithelial neoplasia grade II or III was 28% among women with a positive test for HPV and 3% among those without detectable DNA. The risk was highest among those with HPV type 16 or 18 infection. In four years, the infection progressed among 56% of those who

were HPV positive compared to 25% of the negative subjects. In a prospective study of 95 women with cervical intraepithelial neoplasia (CIN I). Downey et al. (1994) did not find any excess risk of progression in those who were positive for HPV. They suggested that a histological diagnosis of low-grade cervical disease was a better long term predictor of disease progression than HPV positivity. Rozendaal et al. (1996) have tested the value of a PCR based test for high risk HPV types in a cohort of 1622 women with a follow-up of 40 months. The outcome was CIN III. Those women with high risk HPV types and normal Pap smear were 116 times more at risk of developing CIN III compared to women without high risk HPV. In a follow up study of Finnish females, Syrjanen et al. (1990) have reported a prevalence of HPV infection of 3% at the beginning of the follow up and 7% one year later. They estimated that up to 79% of the Finnish females would contract at least one HPV infection between ages 20 and 79 years. They suggested that the progression of precancer to invasive cancer was regulated by factors not clearly delineated at that time.

The prevalence of HPV infection (detected in cervical epithelium) declines with age, while cervical cancer prevalence rises with increasing age. However the HPV antibody level in the serum shows a steady increase. The role of HPV in the natural history of cervical cancer has been suggested to be mediated through the proteins E6 and E7. The protein E6 transforms cells by binding the cellular tumour suppresser and regulatory protein p53, leading to rapid degradation of p53. This results in chromosomal instability and aneuploidy. The E7 protein binds and phosphorylates the retinoblastoma protein (Rb) and leads to activation of mitosis (Werness et al. 1990).

3.5 Prospects of HPV testing in screening and vaccination against HPV

Anogenital warts are caused by HPV and many methods are available for their treatment. Patient administered topical therapies and provider-administered cryotherapy, interferon and surgery are the current practices. In the uterine cervix the HPV infection is treated as part of the treatment of cervical precancerous lesion (Beutner and Ferenczy 1997). Even in countries with organised screening programmes and adequate resources, treatment of asymptomatic HPV infection remains an important issue.

Cuzick et al. (1995) tested for HPV in women who were attending a screening programme in England. They found that 44% of the cervical intraepithelial neoplasia of grade II/III proven by HPV typing had negative cytology. The positive predictive value for HPV testing was 42%. HPV types 16 and 31 were found to be the most specific.

They suggested that HPV testing could usefully augment but not replace the conventional cytology.

Galloway (1998) presented the features that make HPV amenable to vaccination. The predominant genital types are conserved globally, and the viral genomes are not prone to mutation because HPV uses the cellular replication machinery. There are only eight viral genes and this limits the complexity of the immune response. Animal models of therapeutic vaccines have been found to be feasible.

Luostarinen et al. (1999) have shown that women who harbour both HPV 6/11 (low risk types) and HPV 16 do not have an increased risk for cervical cancer. The HPV specific immunity provided by the coexisting low risk types might have provided the protection from the high risk types. This finding supports the prevention of squamous cell carcinoma of the cervix by vaccination. A study of eight patients with advanced cervical cancer vaccinated for HPV was reported by Borysiewicz et al. (1996). Vaccination resulted in no significant clinical side effects in the women. HPV specific antibody response (HPV specific cytotoxic T lymphocytes) was detected in one out of three evaluable patients. Clinical trials are in progress for therapeutic vaccines.

The availability of a vaccine for HPV might help to prevent cervical cancer in the future. Meanwhile established methods of cervical control have to be provided for the women at risk, especially for the poor and vulnerable sections of society in low resource countries. Epidemiological studies are needed in these countries to study the occurrence and determinants of HPV infection, which is the most important etiological agent for cervical cancer.

4 SUBJECTS AND METHODS

Prevalence of HPV was studied in the general population in a defined geographical area by establishing a cohort of married women. This cohort provided the framework for addressing the determinants of HPV infection.

The study was conducted in two suburban regions of Trivandrum District viz. Kazhakuttom Panchayat (one of the administrative units) and Maryandu village, (15 Kilometres away from the Regional Cancer Centre) (Figure 2). Kazhakuttom Panchayat was selected for operational and logistic convenience as well as for the fact that this area had a good representation of the three major religious groups in Kerala. Maryanadu village is an area where we had conducted field studies earlier and hence had a logistic advantage. The Panchayat consisted of 10 wards, which were subjected to repeated changes in their composition, based on the definitions for the electoral constituencies (Figure 3). The addresses of all the households in these regions were available at the Panchayat office (administrative office), the reliability of which was not certain. It was known from the official records that there were about 6000 households.

Pilot phase

A pilot study was conducted between March and August 1993 to assess the feasibility. The list of houses maintained in the Panchayat office was utilised to identify the eligible population during the pilot phase. Clinics were held at the field office, in Kazhakutam. Attendance at these clinics was not restricted to any defined population and women from outside the study area also visited the clinic. One thousand six hundred samples were collected during this period, of which 1295 samples were from the eligible subjects (20% were from outside the study area). It was realised from the pilot phase that the list of addresses in the administrative office was not adequate and unique identification of an individual was not possible. Hence, considering the logistic aspects, the study was conducted in the following way.

Figure 2. Notional map of study area in relation to Kerala and India.

Figure 3. Notional map of Kazhakuttam Panchayat with wards.

4.1 Organisation of a cohort

A team of staff was recruited (medical officer, sociologist, cytotechnologist, cyto-technician, field workers, data entry clerk, office clerk, driver and helpers) in November 1993. The various questionnaires, household form, individual form, consent forms in local language and cytology forms to be used in the study were finalised, incorporating the experience of the pilot phase.

A complete survey of the households was undertaken in the study area to establish a cohort. Information was collected on a household survey form (Appendix 1), which contained details of the ownership of the house, members of the household, their age, marital status, and relationship to the head of the household. Maryanadu, a Catholic fishing village, is a settlement in makeshift, thatched huts on the beach. Women of this village were identified by the local field worker through the parish list, and the list of the social services organisations in the village.

The enumeration of the entire study area took 9 months to complete and details of all the households were entered into the computer. From this database, all married females who were permanent residents of the area were identified as the potentially eligible population. Each of them was given a unique identification number, which consisted of the ward number, house number and the individual number within the household. This unique identification number was used in all the forms and data entry programmes for linkage purposes. Women were given an identity card with this number (Appendix 2).

4.2 Awareness programmes

There had been no previous screening programmes for cervical cancer in the study area and the local health programmes were focussed on maternal and child health and immunisation. The help of voluntary organisations and women's groups was solicited to conduct awareness programmes in the community. Thirteen community seminars were organised in the study area. Pamphlets describing the study and the procedures were distributed through various local groups (Appendix 3).

All religious organisations and political parties were approached to gain the confidence of the community. Both women and men were invited to the awareness programme. Men's participation was essential, as women have to get their permission to attend the clinics. The content of the awareness programmes included information on cancer, its warning symptoms in general and cervical cancer prevention in detail. The female medical officer explained about the procedure for smear taking. The community

was assured that appropriate treatment and follow-up for those with abnormalities would be provided at the RCC, Trivandrum.

4.3 Field clinics

Clinics were initially held in Government health centres. These centres were sometimes situated at places remote from where the people resided and women were not happy to walk long distances to attend the clinic. In order to make it convenient, field clinics were organised. Two weeks before the clinic, the sociologist would identify the women to be invited from the computerised list. The social workers would then visit these households and personally invite the women.

Women were more likely to attend a programme in their vicinity without missing their daily household chores. Government buildings and private households were used for conducting the clinics. The team from RCC would reach there sufficiently early and arrange the furniture and electricity supply. Transportation was arranged to bring the older women who did not care to walk.

Medical consultation

The medical officer of the team provided prescriptions for the women and sometimes for the family members who accompanied these women. The medical officer was available in the field office every day and women were asked to see the doctor if they had any complaints.

Data collection

Women who attended the clinics with their identification cards were matched with the computer list. The rest of them had to be checked by name and address through the lists in the clinic to make sure that they belonged to the cohort. An informed consent form in vernacular (Appendix 2) was given which explicitly mentioned the biological sample collection. After obtaining an informed consent, the social worker interviewed them to collect data on socio-demographic details, marital and reproductive factors and gynaecological symptoms (Appendix 4). Gynaecological symptoms and temporary contraceptive measures were asked over a reference period of one year.

A questionnaire on sexual behaviour was introduced after the completion of the first year of study. This was mainly due to logistic reasons as such a questionnaire in the beginning would have been difficult to administer. The female medical officer and the sociologist interviewed women in the privacy of the clinic. Information on multiple sexual partners and other sensitive questions were included in this interview schedule (Appendix 5). Questions on genital hygiene were asked to reflect the usual practise. Men were not available to be interviewed and their behaviour was also enquired from their wives.

Data collection from the field was through interviews and interviewers assessment of the responses were considered for data quality. Given the nature of the details collected, repeated questioning was not feasible. The HPV status was not known at the time of the interview. Range checks were introduced at the time of data entry.

4.4 Biological sample collection

A female cytotechnician took the Pap smear using an Ayer's spatula from the cervix. The first sample from the spatula was dissolved in a phosphate buffered saline solution and placed in ice packs immediately. The next sample was smeared onto glass slides, which were marked with the unique identification numbers. The samples were taken to RCC. The cell samples were separated into three aliquots, numbered and stored in -40^{o} Centigrade freezers. Cytology smears were received in the cytopathology division for staining and reading.

HPV testing

The cell samples in deep freezers were transported to the UK for HPV testing. The samples were carried in dry ice to preserve the cells. However a few of them perished in transit. PCR analysis of cervical cell samples was undertaken in the dedicated laboratory facilities at the Institute of Cancer Research, set up according to recommended anti-contamination procedures (Bauer et al. 1992). The results of these analyses were then linked to the database on interview schedules using unique identification numbers.

Samples were thawed at room temperature and resuspended. The cell pellets obtained from centrifugation of 250µl of each sample were resuspended and digested by incubation at $55^{\circ}C$ for 1 hour in 25µl of 50mM Tris-HCl buffer (pH8.5) containing 1mM EDTA (TE buffer), 1% (v/v) Tween-20 and 200µg/ml proteinase K. The protease

was inactivated by incubation at 95°C for 10 minutes and the crude digests were made up to 250µl in TE buffer. 5µl aliquots of the digests were then used for HPV L1 consensus PCR amplification in 100µl reactions using the MY09/MY11 primers (Bauer et al. 1992, Manos et al 1989). A 286bp human β-globin fragment was amplified simultaneously in all samples to act as an internal PCR control. PCR-negative controls, and HPV-positive (SiHa cells) and negative controls were run in each experiment.

10µl aliquots of PCR product were run on agarose gels, vacuum blotted onto nylon membranes and immobilised by UV cross-linking. Membranes were hybridised with a β-globin oligonucleotide probe, then with a generic HPV probe in order to determine HPV positivity. The probes used were biotin-labelled and positive hybridisation was detected using enhanced chemiluminecence. Samples found to be HPV positive were dot blotted onto new membranes and hybridised with a series of biotinylated type-specific probes including 6/11/42 (mixed), 16, 18, 26, 31, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 73 (PAP238A),ME180, PAP88, PAP155, PAP291 and W13B. Samples giving a positive signal with the generic probe but which were negative on all dot blots were considered positive but untyped.

4.5 Data management and analysis

The study was completed in 1997 and the flow chart presents the number of women available at various levels of the study (Figure 4).

A descriptive analysis of the baseline data of the area and the study population was carried out. Representativeness of the compliant and non-compliant subjects was studied with regard to known variables.

Prevalence of HPV was studied by variables grouped under the following categories: socio-demographic factors, marital and reproductive factors, contraceptive practices, symptoms at presentation, genital hygiene and sexual behaviour. The factors considered under the socio-demographic category were age group, religion, monthly income of the whole family, type of house, education of woman, occupation of husband and tobacco chewing.

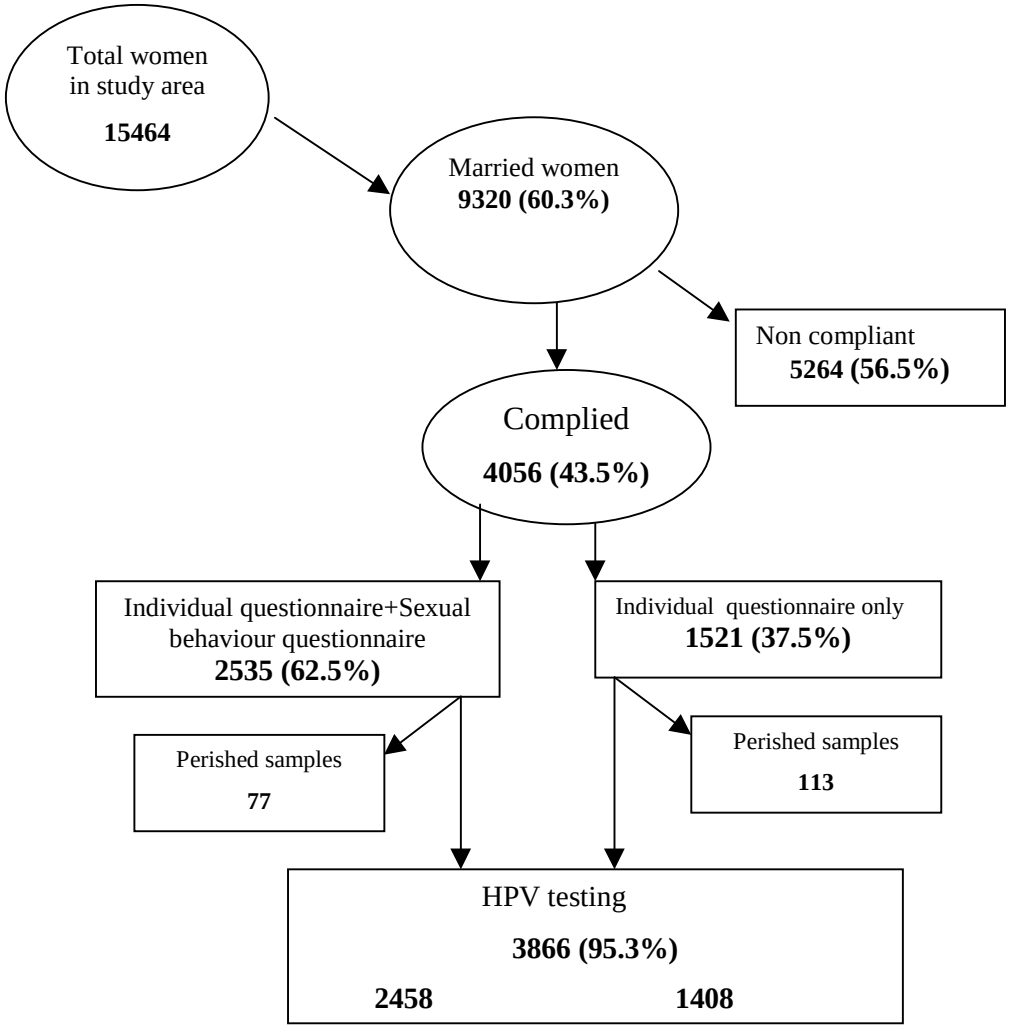

Figure 4. Flow chart – HPV prevalence study in Trivandrum, Kerala, India, 1995–1997.

Socio-economic status of the woman was not clearly evident from these variables. An index of socio-economic status (SES) was generated by combining the different levels of the factors, 'monthly income', 'education of woman' and 'husband's occupation'. Based on these combinations, three levels of (low, middle and high) social class were generated. This variable 'SES' was used for further analysis.

The category of marital and reproductive factors included age at menarche, age at marriage, marital status (status of marriage at the time of interview), age at first childbirth, total number of pregnancies, contraceptive methods and history of vasectomy in husband. To obtain the time between the onset of menarche and age at marriage, a new variable was generated by calculating the difference in years between the age at marriage and age at menarche. This variable had three levels, <6 years difference, 6–10 years and >10 years. Instead of age at menarche and age at marriage, this variable (menarche-marital difference) was used for further analyses.

Contraceptive methods of woman and husband's vasectomy were combined to generate a new variable which had three levels; no contraception for husband and wife, spacing methods and permanent sterilisation for husband and/or wife. This variable 'contraception' was used for further steps.

The factors included in the category, genital hygiene were 'washing after coitus by woman (W)' washing after coitus by husband (H) and 'intercourse during menstruation'. The variables for husband and wife were considered together and the new variable 'genital hygiene (H&W)' was used for further steps. This variable is a representation of the hygienic practices of the couple.

The category sexual behaviour included the variables, husband's premarital partnerships (H), extramarital partnerships (H), husband's visit to commercial sex workers, wife's premarital partnerships (W) and extra marital partnerships. These variables reflected the sexual behaviour and hence were combined to generate two variables. Promiscuity for wife, which classified the women as having had no premarital or extra marital partners versus having had extra or premarital, partners (promiscuity W). Similarly for men, the variable promiscuity (H) classified them as having had no premarital, extra marital partners or visits to commercial sex workers versus those who had any one of these contacts. These two variables were considered for further steps.

In each category the prevalence of HPV positivity is given along with the total and HPV positive numbers in each level of the factor.

The determinants of HPV infection were studied by applying the logistic regression model (Breslow and Day 1980). Logistic regression analysis have been employed in similar situations to study the determinants (Coutlee et al. 1997, Jaakkola et al. 1993, Makela et al. 1993). HPV infection was taken as the binary outcome. Univariate analyses were carried out for each factor and the odds ratios and corresponding 95% confidence intervals were presented. A multivariate analysis was done within each category and adjusted for the variables within that category and for age. The adjusted odds ratios and 95% confidence intervals are also presented in the tables. All the analyses were done with the software SPSS (SPSS for windows 9.0).

The variables which were statistically significant, or those which were considered relevant, were carried forward. The final model was constructed with the variables thus selected from each category and they were adjusted against each other and for age in a multivariate analysis. Interactions among the variables in this model were tested and there were no significant interactions and hence are not presented. The final model identified the independent determinants of HPV infection.

5 RESULTS

5.1 Total population

The study area had a total (men 14,966 and women 15,464) population of 30,430, spread over eleven wards in Kazhakuttom Panchayat and Maryanadu village. From the analysis of the data from the household questionnaire, age and gender distribution, and distribution by religious groups of the community were generated.

Age and gender distribution

The age structure of Kerala State (Figure.5) and the study area (Figure.6) are given below. The younger ages (base of the pyramid) are receding with a trend towards an expansion in the middle.

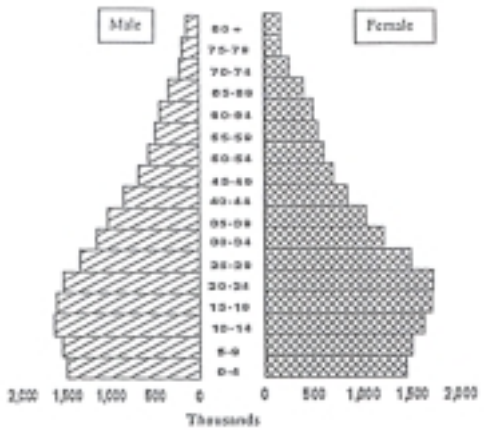

Figure 5. Kerala population by age and sex (1996).

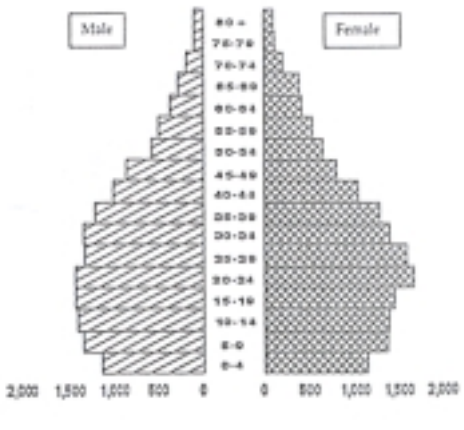

Figure 6. Kazhakuttom & Maryanadu population by age and sex (1996).

The male to female ratio was 1033 women for 1000 men. The sex ratio for the State of Kerala was 1036 women for 1000 men (Economic Review 1996)

Distribution by religion

The distribution (%) of religious groups in the study area and in the State of Kerala is presented in Figure 7. The majority of the population belong to Hinduism and there was an almost equal proportion of Christians and Muslims.

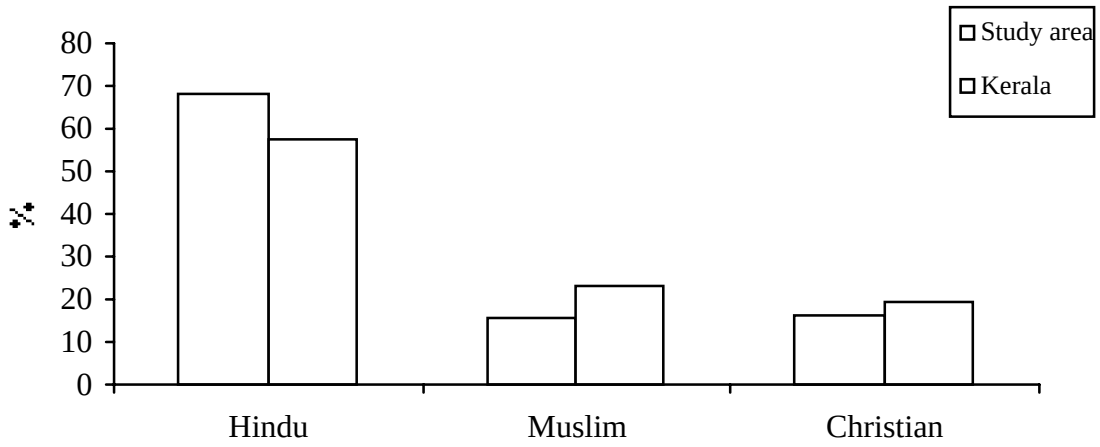

Figure 7. Distribution (%) by religion, Study area and Kerala State, 1995.

5.2 Eligible women

From the baseline survey, all married women who were permanent residents of the area were identified and were considered as eligible women (n = 9320). Table 2 presents the female population, number of eligible women and those who participated in the study by age group.

Table 2. Age distribution of all women, eligible women and those who complied in the Trivandrum HPV study 1995–97.

Age group(yrs)	Female population	Eligible women n	(%)+	Compliant women n	(%) ++
0–4	1136	–		–	
5–9	1370	–		–	
10–14	1372	–		–	
15–19	1441	9	(6.3)	16	(17.6)
20–24	1660	1000	(60.2)	306	(30.6)
25–29	1583	1440	(91.0)	668	(46.4)
30–34	1394	1355	(97.2)	728	(53.7)
35–39	1281	1266	(98.8)	690	(54.5)
40–44	1037	1024	(98.7)	546	(53.3)
45–49	794	781	(98.4)	359	(46.0)
50-54	644	635	(98.6)	275	(43.3)
55-59	524	520	(99.2)	197	(37.9)
60-64	416	409	(98.3)	134	(32.8)
65+	812	799	(98.3)	137	(17.1)
Total	15464	9320	(60.3)	4056	(43.5)

+ Percentage to the total female population, ++ percentage to eligible population

5.3 Compliance with the study

The field study was carried out over two years. During this period 4056 women were interviewed using the individual questionnaire and biological samples were collected. The overall compliance rate was 43.5%.

Compliance by age

The mean age of the compliant women was 38.5 yrs (SD 11.53) and non compliant women was 41.6 yrs (SD 16.25). The distribution of compliant women by age group is presented in Table 2. Women in the younger age groups and the very old were found to be less likely to comply to the invitation.

Compliance by ward of residence

Compliance rate by ward (units within the panchayat) of residence varied from 56.3% in ward 3 to 31.2 % in ward 9 (Figure 8).

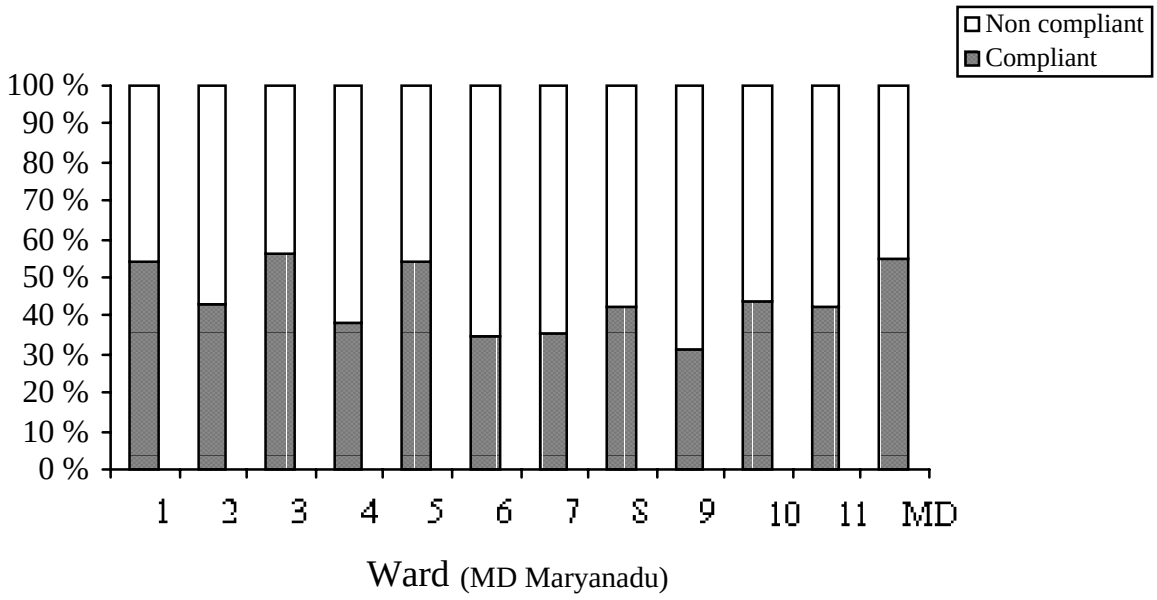

Figure 8. Compliance with screening by area of residence, Trivandrum HPV study.

Compliance by religion

Compliance to the invitation was compared among the different religious groups. The compliance among Hindus was 46%, Muslims 38% and 36% among Christians.

5.4 Characteristics of the study participants

Characteristics of the four thousand and fifty six (4056) women who had complied with the study through the data collected by the personal interview are presented.

5.4.1 Socio-demographic variables

Type of house

Twenty percent of people lived in concrete houses and 49.7% were in thatched houses. Thatched houses are built with mud and the roof is made of coconut leaves. The rest were living in tiled houses.

Educational status

The distribution (%) of educational status of the 4056 women is given in Figure 9. Only 15% of the study population were illiterate. A good proportion of women had been to high school (23.9%) and university (6.2%).

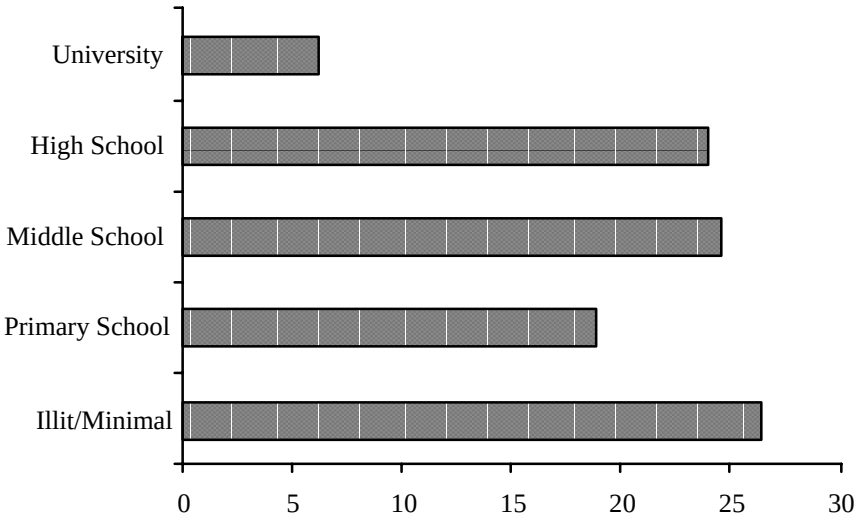

Figure 9. Women's educational status in Trivandrum HPV study, 1995–1997.

Occupation of women

The women were mostly housewives even though they were educated. Ninety percent of the women were housewives, 6.9% manual labourers and 2.6% had office jobs. In Maryanadu, the fishing village, 78% were housewives and 22% were manual labourers.

Occupation of husband

Occupation of the husbands of the participants was asked and categorised (Figure10). Forty five percent of the husbands were manual labourers. Working in countries around the Persian Gulf is becoming increasingly common and 8.8% of women had husbands working in one of the Gulf countries.

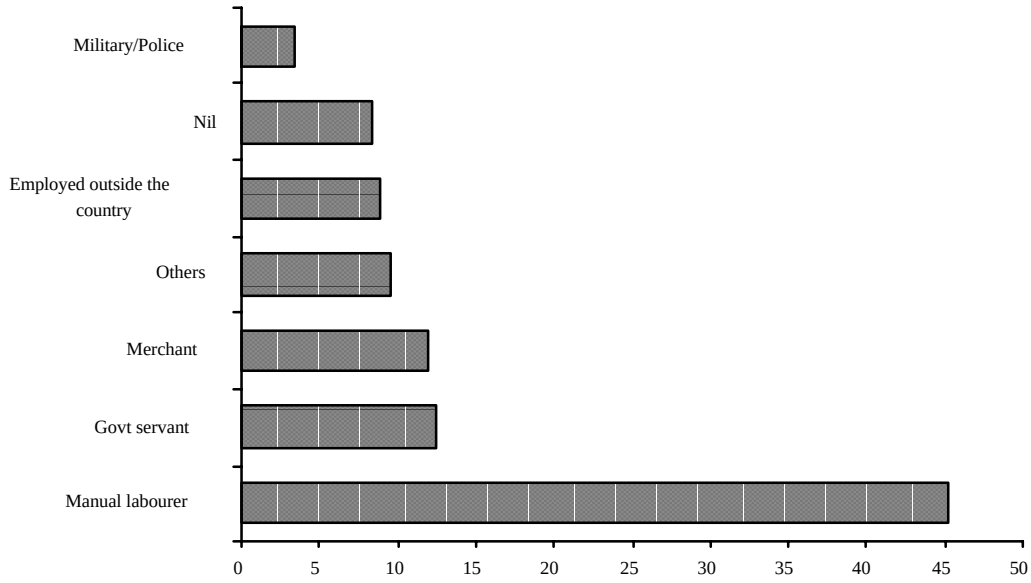

Figure 10. Occupation of husbands of study subjects in the Trivandrum HPV study 1995–97.

Monthly income

Many men were not on fixed salaried jobs and an overall income of the family per month was collected in Indian Rupees (1US $ = 43 Indian Rupees). There was some reluctance to give the correct amount and the information from the wife may not always have been correct. Majority of the women (64.9%) said that they were in the low income category of less than 1000 Rs per month. Only 7.3% women were in the high income category.

Tobacco and alcohol habits

Tobacco habits were enquired in the questionnaire. Information on tobacco chewing, smoking and alcohol consumption were collected. Thirty one women (0.7%) reported tobacco smoking and this was mainly seen in the older age groups. The mean age of tobacco smokers was 58.2 yrs (SD 8.62). All women who smoked tobacco, used bidi (a locally made cigarette consisting of tobacco rolled in a dried leaf). Table. 3 presents the distribution by chewing habit of women.

Tobacco chewing was practised as pan chewing, which consists of betel leaf, tobacco, shell lime and areca nut. The median number of pan chewings per day was 4 with a range from 1 to 25 times. Only 3 women chewed 25 times a day. The average duration of pan chewing was 11.2 years. None of the 4056 women reported alcohol consumption.

Table 3. Tobacco chewing habits among the study subjects in the Trivandrum HPV study 1995–1997.

Tobacco chewing	n	%
Not habituated	3388	83,5
Current habituees	625	15.4
Past habituees	43	1.1
Total	4056	

5.4.2 Reproductive and marital history

Marital status

Status of married life at the time of the study was asked and information was available on 4032 women (Figure11).

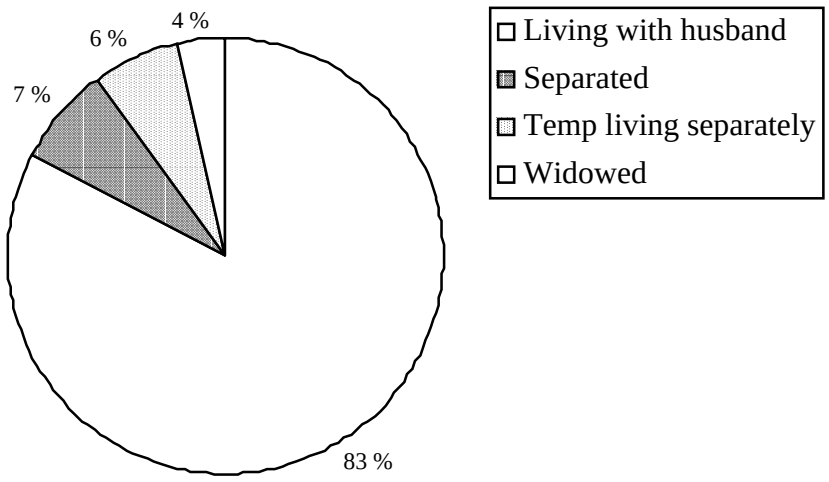

Figure 11. Status of marriage of the women in the Trivandrum HPV study.

Age at menarche

The median age at menarche was 14 years. (Mean 14.4 and SD 1.5). Sixty four percent of the women had menarche between the ages 14 and 16 years.

Age at first marriage

The median age at marriage was 20 years for the women who came into the study. Information was not available on 18 women.

Age at first childbirth

Of the total of 4056 women, 3911supplied information on age at first child birth, while it was unavialable in 28 women. One hundred and seventeen women had only three years of married life and were not considered for this variable. The median age at first childbirth was 21 years and this represents the usual practice of having the first child within one year after marriage.

Total pregnancies

Ninty nine women had no live birth at the time of interview (18 women did not provide the information). Forty six percent of the women had 3 to 5 pregnancies.

Age at menopause

Eight hundred and twenty six women (20.3%) had attained menopause at the time of the study. Exact age at which menopause attained was available for 401(48.5%) women. The median age of attaining menopause was 46 years.

The distribution of the study subjects in the various strata by these variables are presented in Table 4.

Table 4. Distribution by marital and reproductive factors of women in the Trivandrum HPV study 1995–97.

Marital and reproductive factors	n	%
Age at menarche		
< 14 yrs	1100	27.1
14–16 yrs	2602	64.2
> 17 yrs and above	354	8.7
Total	4056	
Age at first marriage		
< 20 yrs	1964	48.4
20–24 yrs	1689	41.6
25+	385	9.6
Not available	18	0.4
Total	4056	
Age at first childbirth		
< 20 yrs	1126	28.8
20–24	2126	54.4
25+	659	16.8
Total	3911	
Age at menopause		
30–39 yrs	19	2.3
40–49 yrs	290	35.1
50–59 yrs	92	11.1
Age not known	425	51.5
Total	826	
Total pregnancies		
0	99	2.4
1–2	1660	41.1
3–5	1877	46.4
5+	402	10.0
Total	4038	

Contraceptive practices

Contraceptive practices of the women were collected through the interview. Information was available on 4026 women. Women were also asked about vasectomy for husbands. Temporary contraceptive practices among the 1131 women in the age group below 50 yrs is presented in Table 5.

Permanent sterilisation was the most common method among women. Two thousand three hundred and forty four women had tubectomy. The prevalence of permanent sterilisation was studied by year of birth. The exact age at sterilisation was not available. Figure 12 presents the proportion of women with permanent sterilisation by year of birth.

Ninety three (2.3%) women reported that their husbands had had a vasectomy, of these 88 women had no contraception, one had a coil (intrauterine contraceptive device) and 4 had sterilisation.

Table 5. Contraceptive (spacing) practices in women < 50 years in the study subjects in the Trivandrum HPV study 1995–97.

Age group	None		Husband's use condom		IUD		OC Pill		Diaphragm		Total
	n	%	n	%	n	%	n	%	n	%	
15–19	15	3.7		–	1	6.3		–			16
20–24	177	77.6	23	10.1	25	11.0	3	1.3			228
25–29	224	77.8	22	7.6	37	12.8	4	1.4	1	0.4	288
30–34	146	78.5	13	7.0	6	8.6	9	4.8	2	1.1	186
35–39	136	87.8	9	5.8	8	5.2	1	0.6	1	0.6	155
40–44	121	94.5	2	1.6	5	3.9		–		–	128
45–49	123	94.6	2	1.5	4	3.1		–	1	0.8	130
Total	942	83.3	71	6.3	96	8.5	17	1.5	5	0.4	1131

OC Pill- Oral contraceptive pills

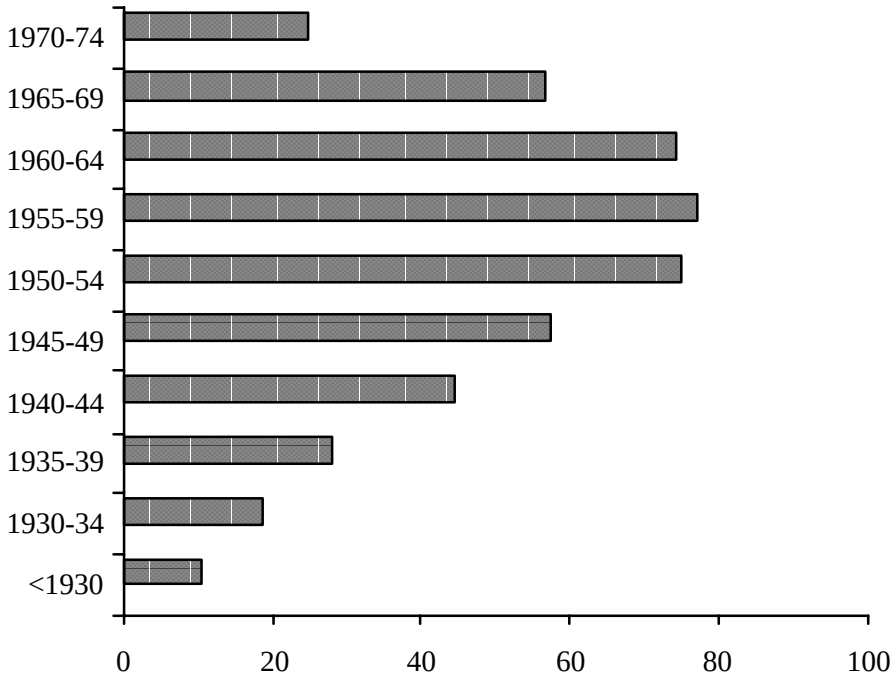

Figure 12. Proportion of women with permanent sterilisation by year of birth in the Trivandrum HPV study.

5.4.3 Gynaecological symptoms

Gynaecological and related symptoms were elicited before the smear test. White discharge per vaginum was the most common symptom in the women. Post coital and post menopausal bleeding were reported by a very small proportion of the women. The main symptoms at presentation are given in Figure 13.

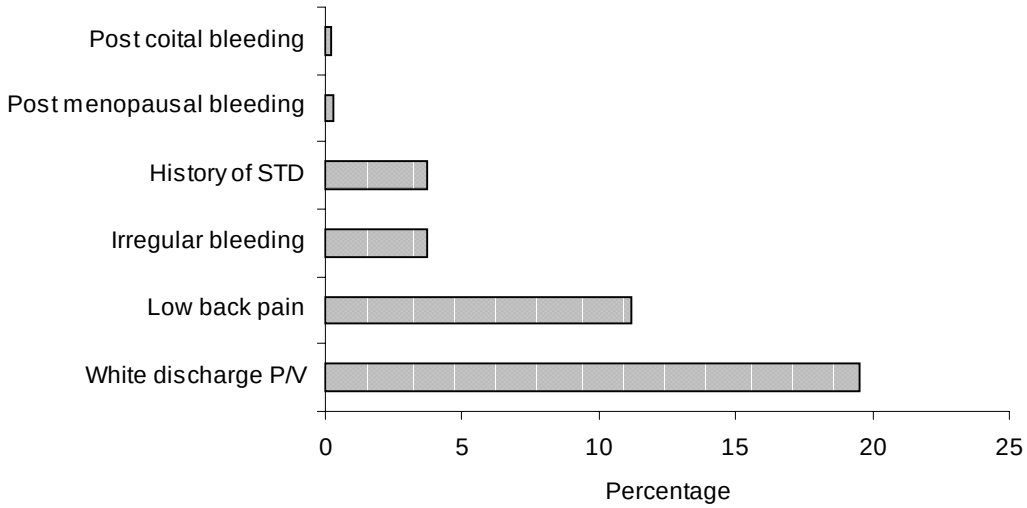

Figure 13. Prevalence of gynaecological symptoms in the Trivandrum HPV study 1995–97.

5.4.4 Genital Hygiene and sexual behaviour

Two thousand five hundred and thirty five women (62.5% of the compliant women) were interviewed for sexual behaviour and genital hygiene. The questionnaire for these variables were introduced one year into the study and the interviewed do not form a sample. Hence the chance factor is not tested between the groups. Moreover, with such large numbers even very small differences will emerge siginificant. A comparison of those who were interviewed against those who were not interviewed, by selected variables is given in Table 6. The groups are comparable with respect to the variables studied.

Table 6. Comparison of women who were interviewed (n = 2535) for sexual history and those who were not interviewed (n = 1521) by selected factors.

Factor	Interviewed		Not interviewed	
	n	%	n	%
Age group (yrs)				
<30	593	23.4	397	26.1
30–44	1277	50.4	687	45.2
45–59	524	20.6	307	20.2
60+	141	5.6	130	8.5
Income (Rs)				
Low	1581	62.4	1051	69.1
Middle	745	29.4	381	25.0
High	209	8.2	89	5.9
Religion				
Hindu	1937	76.4	1082	71.2
Muslim	377	14.9	198	13.0
Christian	221	8.7	241	15.8
Type of house				
Concrete	568	22.4	255	16.8
Tiled	767	30.3	452	29.7
White discharge P/V				
No	2124	83.8	1143	75.1
Yes	411	16.2	378	24.9
Post coital bleeding				
No	2531	99.8	1516	99.7
Yes	4	0.2	5	0.3
Pregnancies	(n = 2519)		(n = 1519)	
0	57	2.3	42	2.8
1–2	1046	41.5	614	40.4
3–5	1195	47.4	682	44.9
>5	221	8.8	181	11.9

Genital hygiene

Genital hygiene was elicited by enquiring about private washing facilities at home and practices of personal cleanliness. Table 7. presents the proportion of men and women who practised genital hygiene in the households with private washing facilities. Fifty two women had no private washing facilities.

Table 7. Genital hygienic practices (Yes/No) among men and women in the Trivandrum HPV study

Woman	Husband	n	%
Yes	Yes	2344	94.4
Yes	No	36	1.5
No	Yes	3	0.1
No	No	100	4.0
Total		2483	

Pre marital and Extramarital relationships

Extramarital and premarital sexual behaviour of the women and their spouses were enquired. Out of the 2535 women who answered the sexual history questionnaire, the information could not be gathered from 56 women on extra marital relationships of husband, leaving 2479 in the Tables 8 and 9.

Six hundred and ninenty six (28%) women reported that their husbands had partners prior to marriage. Five hundred and fifty seven (22.4%) women reported that their husbands had had extra marital partners. Five hundred and twenty three (21.1%) women reported that their husbands visited commercial sex workers. The responses given by the wives were compared (Table 8). Association between the statements of the wife regarding premarital and extramarital partners for husband was high, ($\kappa = 0.82$).

Table 8. Association between premarital and extramarital partners for husband.

Extramarital partner	Premarital partner		
	No	Yes	Total
No	1768	154	1922
Yes	15	542	557
Total	1783	696	2479

Table 9. Association between husband's extramarital partners and visiting commercial sex workers

Extramarital partner	Commercial sex worker		
	No	Yes	Total
No	1879	43	1922
Yes	77	480	557
Total	1956	523	2479

There was a high association between the responses of the wife regarding extramarital partners and visit to commercial sex workers by husbands ($\kappa = 0.85$) (Table 9).

Premarital and extramarital partners for wives were asked and the association between the responses are given in Table 10.

Sixty two (2.4%) women reported having had partners prior to marriage. Thirty four (1.3%) women reported having extra marital partners. The association of the responses for premarital partner and extramarital partner of the wife was moderate ($\kappa = 0.59$).

Table 10. Association between premarital and extramarital partner for wife

Premarital partner	Extra marital partner		
	No	Yes	Total
No	2468	5	2473
Yes	33	29	62
Total	2501	34	2535

5.5 Prevalence and determinants of HPV infection

HPV prevalence was studied from the 3866 women whose samples were analysed by PCR. The results of the PCR tests done in the Institute of Cancer Research Laboratories in Sutton, UK, were linked to the database and prevalence by various characteristics of the women were generated. The prevalence for HPV all types has been described in all the tables. The characteristics were broadly grouped as socio-demographic, marital and reproductive factors, contraceptive practices, symptoms at presentation, genital hygiene and sexual behaviour.

5.5.1 Prevalence by age

The age specific prevalence of HPV is given in Figure 14. The age specific prevalence at 4 points and the 95% confidence intervals for the prevalence rates are plotted. The overall prevalence of all ages (234/3866) was 6.1% (95% CI 5.3–6.8). The tests for linear trend ($p = 0.7$) and for heterogeneity ($p = 0.84$) were non significant.

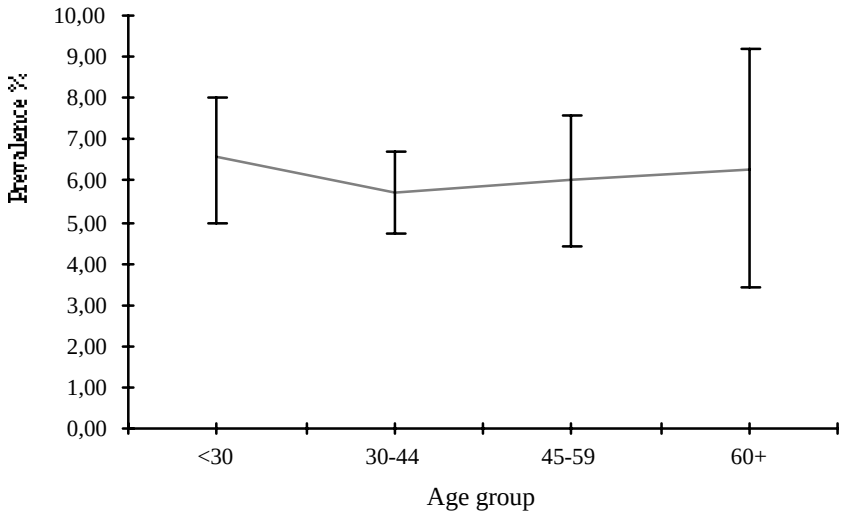

Figure 14. Prevalence rate & 95% CI of HPV (all types) by age group in the Trivandrum HPV study, 1995–97.

5.5.2 Socio-demographic variables.

Prevalence of HPV, crude and adjusted odds ratios for the various socio-demographic factors are presented in Table 11.

Muslim women had the least prevalence of 4.1% compared to Christian and Hindu women. Low income group and low socio-economic groups had a higher prevalence compared to lower income groups. When adjusted for each other, socio-economic status emerged as significant and religion had borderline significance (p = 0.06). However Muslims had a significantly reduced risk.

5.5.3 Marital and reproductive factors and contraceptive practices

Prevalence of HPV, crude and adjusted odds ratios and corresponding 95% confidence intervals for marital and reproductive factors and contraceptive practices are presented in Table 12.

The prevalence was very low (2.9%) in those who practised barrier contraceptives. History of vasectomy for husband resulted in a prevalence of 5.5%. Permanent sterilisation of either husband or wife did not have an effect on the prevalence rates. Age at first childbirth was correlated with the variable menarche-marriage difference (Spearman correlation coefficient 0.7) and hence was not included in the multivariate model. None of the factors attained statistical significance in this group.

Table 11. HPV prevalence, crude and adjusted odds ratios and 95% confidence intervals by socio-demographic variables in the Trivandrum HPV study.

Factor	Total	HPV+		Crude		Adjusted[1]	
		%	(n)	OR	95% CI	OR	95% CI
Age group							
<30	956	6.6	(63)	1.00		1.00	
30-44	1879	5.7	(108)	0.86	0.62–1.19	0.78	0.56–1.09
45-59	779	6.0	(47)	0.91	0.61–1.34	0.71	0.46–1.09
60+	252	6.3	(16)	0.96	0.54–1.69	0.66	0.35–1.22
Religion					(p = 0.08)		(p = 0.06)
Hindu	2867	6.5	(187)	1.00		1.00	
Muslim	555	4.1	(23)	0.61	0.39–0.96	0.63	0.40–0.99
Christian	444	5.4	(24)	0.81	0.52–1.20	0.70	0.45–1.09
Income (Rs)				#		n.i.	
<1000	2503	6.9	(173)	1.00			
1000–2500	1078	4.8	(52)	0.68	0.49–0.93		
2501+	285	3.2	(9)	0.43	0.22–0.86		
Education						n.i.	
Illit./Minimal	1003	7.1	(71)	1.00			
Middle	1669	6.2	(103)	0.86	0.63–1.18		
College/Uni	1194	5.0	(60)	0.69	0.48–0.99		
Occupation (H)				#		n.i.	
Nil	313	9.3	(29)	1.00			
Manual labr.	1751	6.9	(121)	0.72	0.47–1.10		
Military/Police	140	7.9	(11)	0.83	0.40–1.72		
Merchant	459	3.3	(15)	0.33	0.17–0.62		
Govt Employ.	483	3.7	(18)	0.37	0.20–0.69		
Gulf countries	346	4.9	(17)	0.50	0.27–0.94		
Others	374	6.1	(23)	0.64	0.36–1.13		
Type of house				#			
Concrete	789	4.8	(38)	1.00		1.00	
Tiled	1168	5.3	(62)	1.10	0.73–1.67	0.87	0.56–1.34
Thatched	1909	7.0	(134)	1.49	1.03–2.16	1.06	0.70–1.60
Occupation (w)							
Housewife	3499	6.0	(210)	1.00		1.00	
Manual labr.	264	7.6	(20)	1.28	0.79–2.06	1.06	0.70–1.60
Office job	103	3.9	(4)	0.63	0.23–1.73	0.99	0.34–2.86
Tobacco chewing				#			
Non habituee	3231	5.8	(187)	1.00		1.00	
Habituee	635	7.4	(47)	1.30	0.93–1.81	1.18	0.80–1.76
SES				#		#	
Upper class	531	4.0	(21)	1.00		1.00	
Middle class	948	4.0	(38)	1.01	0.58–1.74	0.94	0.53–1.66
Lower class	2387	7.3	(175)	1.92	1.20–3.05	1.82	1.37–3.07

[1]Adjusted for variables in the table (Socio-economic status was included instead of husband's occupation, income and educational status of women)
Significant ($p<0.05$); n.i. – not included

Table 12. HPV prevalence, crude and adjusted odds ratios and 95% confidence intervals by marital and reproductive factors and contraceptive practices in the Trivandrum HPV study.

Factor	Total	HPV+		Crude		Adjusted[1]	
		%	(n)	OR	95% CI	OR	95% CI
Age at menarche							n.i.
<14 yrs	1058	7.5	(79)	1.00			
14-16 yrs	2476	5.5	(136)	0.72	0.54–0.95		
17+ yrs	332	5.7	(19)	0.75	0.44–1.26		
Age at marriage							n.i.
<20 yrs	1870	6.4	(120)	1.00			
20–24 yrs	1610	5.8	(94)	0.90	0.68–1.19		
25+ yrs	371	5.1	(19)	0.78	0.47–1.29		
Marital status							
With husband	3200	5.8	(187)	1.00		1.00	
Separated	274	8.4	(23)	1.48	0.94–2.32	1.65	0.99–2.75
Temp sep.	252	6.7	(17)	1.17	0.69–1.95	1.11	0.65–1.86
Widowed	140	5.0	(7)	0.85	0.39–1.84	0.80	0.34–1.90
Age at FCB							n.i.
<20 yrs	1065	6.4	(68)	1.00			
20–24 yrs	2033	6.2	(126)	0.96	0.71–1.31		
25+ yrs	633	4.9	(31)	0.75	0.48–1.16		
Pregnancy							
0	92	5.4	(5)	1.00		1.00	
1–2	1590	6.4	(102)	1.19	0.47–3.00	1.23	0.48–3.17
3–5	1788	5.9	(106)	1.09	0.43–2.75	1.17	0.44–3.11
5+	380	5.0	(19)	0.91	0.33–2.52	0.93	0.31–2.80
Method							n.i.
None	1421	6.2	(56)	1.00			
Barrier	69	2.9	(2)	0.46	0.11–1.96		
Others	112	8.0	(9)	1.37	0.65–2.85		
Sterilisation	2236	5.9	(122)	1.00	0.72–1.38		
Vasectomy (H)							n.i.
No	3775	6.1	(229)	1.00			
Yes	91	5.5	(5)	0.90	0.36–2.23		
Contraception							
None	1251	6.2	(83)	1.00		1.00	
Spacing	181	6.1	(11)	0.97	0.50–1.86	0.92	0.46–1.80
Permanent (H/W)	2323	5.9	(137)	0.94	0.71–1.25	0.95	0.70–1.36
M-M interval							
< 6 yrs	2497	6.0	(150)	1.00		1.00	
6–10 yrs	1007	6.4	(64)	1.06	0.78–1.63	1.04	0.76–1.42
>10 yrs	339	5.3	(18)	0.87	0.53–1.65	0.87	0.52–1.46

Adjusted[1] for variables in the Table and age.
Age at menarche and age at marriage was considered together in the variable menarche-marital interval and hence were not included in the multivariate model. n.i not included
FCB- First child birth; M-M- Menarche-marital interval

5.5.4 Symptoms at presentation

Prevalence of HPV, crude and adjusted odds ratios and corresponding 95% confidence intervals for symptoms at presentation are presented in Table 13. Women who had postmenopausal bleeding had a prevalence of 15.4% compared to 6.0% for those who did not have this symptom. However, this was based on only 2 HPV positive women. Presence or absence of symptoms of white discharge p/v and irregular bleeding p/v did not show any appreciable change in the prevalence. Women with history of any sexually transmitted disease had 7.1% prevalence compared to 6% among those who did not have such a history. None of the variables studied under this category attained statistical signficance.

Table 13. HPV prevalence, crude and adjusted odds ratios and 95% confidence intervals by symptoms at presentation in the Trivandrum HPV study.

Factor	Total	HPV+		Crude		Adjusted[1]	
		%	(n)	OR	95% CI	OR	95% CI
White discharge p/v							
No	3111	6.1	(191)	1.00		1.00	
Yes	755	5.7	(43)	0.92	0.65–1.29	0.88	0.62–1.27
P.M bleeding p/v							
No	3853	6.0	(232)	1.00		1.00	
Yes	13	15.4	(2)	2.83	0.62–12.87	3.13	0.67–14.46
Irregular bleeding p/v							
No	3725	6.1	(226)	1.00		1.00	
Yes	141	5.7	(8)	0.93	0.45–1.92	0.90	0.43–1.89
History of any STD							
No	3725	6.0	(224)	1.00		1.00	
Yes	141	7.1	(10)	1.19	0.61–2.30	1.29	0.65–2.56

Adjusted[1] for variables in the table and for age. The variable contraception is generated out of the variables contraceptive methods for wife and vasectomy for husband and hence these two variables were not included.
P.M.-Post menopausal

5.5.5 Genital hygiene

Prevalence of HPV, crude and adjusted odds ratios and corresponding 95% confidence intervals for genital hygiene related variables are presented in Table 14. The two variables, washing habits of husband and wife were highly correlated [Correlation

between washing habit of wife and husband – Spearman Correlation Coefficient 0.872 ($p<0.0001$)]. A variable genital hygiene for husband and wife (H&W) was generated by combining the variables collected in separate questions. The prevalence was very high in women who did not practise genital hygiene (20.3% compared to 7.8%). Women when they themselves and their husbands did not practice genital hygiene had a prevalence of 24% compared to 7.3% for those who practised hygiene. The variable genital hygiene of husband and wife was considered as the representative variable and used in the multivariate model. The adjusted odds ratio was significant for genital hygiene (H&W) when adjusted for age and coitus during menstruation.

Table 14. HPV prevalence, crude and adjusted odds ratios and 95% confidence intervals by genital hygiene in the Trivandrum HPV study (n = 2458).

Factor	Total	HPV+		Crude		Adjusted[1]	
		%	(n)	OR	95% CI	OR	95% CI
Washing after coitus (W)				#			
Yes	2315	7.8	(181)	1.00		n.i.	
No	143	20.3	(29)	2.90	1.94–4.63		
Washing after coitus (H)				#			
Yes	2281	7.3	(167)	1.00		n.i.	
No	177	24.3	(43)	4.06	2.78–5.92		
Genital Hygiene (H&W)				#		#	
Yes	2278	7.3	(167)	1.00		1.00	
No	180	23.9	(43)	3.96	2.72–5.78	3.97	2.60–6.10
Coitus during menstruation				#			
No	2306	8.1	(187)	1.00		1.00	
Yes	152	15.1	(23)	2.02	1.26–3.22	1.37	0.83–2.27

Adjusted[1] for variables genital hygiene of H&W, coitus during menstruation and age.
Significant ($p<0.05$); n.i – Not included

5.5.6 Sexual behaviour

Prevalence of HPV, crude and adjusted odds ratios and corresponding 95% confidence intervals for sexual behavioural factors are presented in Table 15. Women whose husbands had pre marital sexual partners had a prevalence of 10.2% compared to 7.9% for those who did not have that history. Promiscuity for wife resulted in a prevalence of 24.2% and women whose husband's were promiscuous had a prevalence of 10.2%. Univariate analysis produced odds ratios which were significant for husband's and wife's promiscuity. These two variables and age were studied in a multivariate model and promiscuity for wife emerged as the significant factor.

Table 15. HPV prevalence, crude and adjusted odds ratios and 95% confidence intervals by sexual behavioural factors in the Trivandrum HPV study (n = 2458).

Factor	Total	HPV + %	(n)	Crude OR	95% CI	Adjusted[1] OR	95% CI
Pre marital partner (H)							
No	1781	7.9	(141)	1.00		n.i.	
Yes	677	10.2	(69)	1.32	0.97–1.78		
Extra marit. partner (H)							
No	1864	8.0	(150)	1.00		n.i.	
Yes	538	9.6	(52)	1.22	0.87–1.70		
Husband been to CSW							
No	1949	8.6	(199)	1.00		n.i.	
Yes	509	8.3	(32)	0.95	0.66–1.35		
Pre marital partner (W)				#			
No	2398	8.3	(199)	1.00		n.i.	
Yes	60	18.3	(11)	2.48	1.26–4.84		
Extra marit. partner(W)				#			
No	2425	8.3	(202)	1.00		n.i.	
Yes	33	24.2	(8)	3.50	1.56–7.90		
Promiscuity (H)				#			
No	1693	7.8	(132)	1.00		1.00	
Yes	765	10.2	(78)	1.34	1.00–1.80	1.31	0.96–1.71
Promiscuity (W)				#		#	
No	2393	8.3	(198)	1.00		1.00	
Yes	65	18.5	(12)	2.51	1.32–4.77	2.25	1.16–4.34

Adjusted[1] for variables Promiscuity H and promiscuity W and age.
Significant ($p<0.05$)
n.i. not included

5.6 Cytological findings

The cytological findings of the 3866 women who had HPV testing is presented in Table 16. The findings are classified by broad age groups. Inflammation was found to be very common in the smears among all the age groups

The prevalence of HPV by cytological findings is presnted in Figure 15. The prevalence increased with the grade of the cytological findings and was 100% for moderate and severe dysplasia. Among carcinomas HPV was negative in one case.

Table 16. Cytological findings by age groups in the Trivandrum HPV study.

Cytological finding	Age group (yrs)				Total
	<30	30–44	45–59	60+	
	n (%)	n (%)	n (%)	n (%)	
Normal	156 (16.3)	250 (13.3)	173 (22.2)	103 (40.9)	682
Inflammation	672 (70.3)	1321 (70.3)	457 (58.7)	112 (44.4)	2562
TV+Fungal	63 (6.6)	151 (8.0)	48 (6.2)	5 (2.0)	267
Koilocytosis	21 (2.2)	29 (1.5)	6 (0.8)	2 (0.8)	58
Mild Dysplasia	39 (4.1)	114 (6.1)	75 (9.6)	11 (4.4)	239
Mod Dysplasia	1 (0.1)	4 (0.2)	4 (0.5)	–	9
Severe Dysplasia	–	3 (0.2)	–	–	3
Ca in situ	–	1 (0.1)	1 (0.1)	1 (0.4)	3
Carcinoma	–	1 (0.1)	1 (0.1)	2 (0.8)	4
Others	4 (0.4)	5 (0.3)	14 (1.8)	16 (6.3)	39
Total	956 (100)	1879 (100)	779 (100)	252 (100)	3866

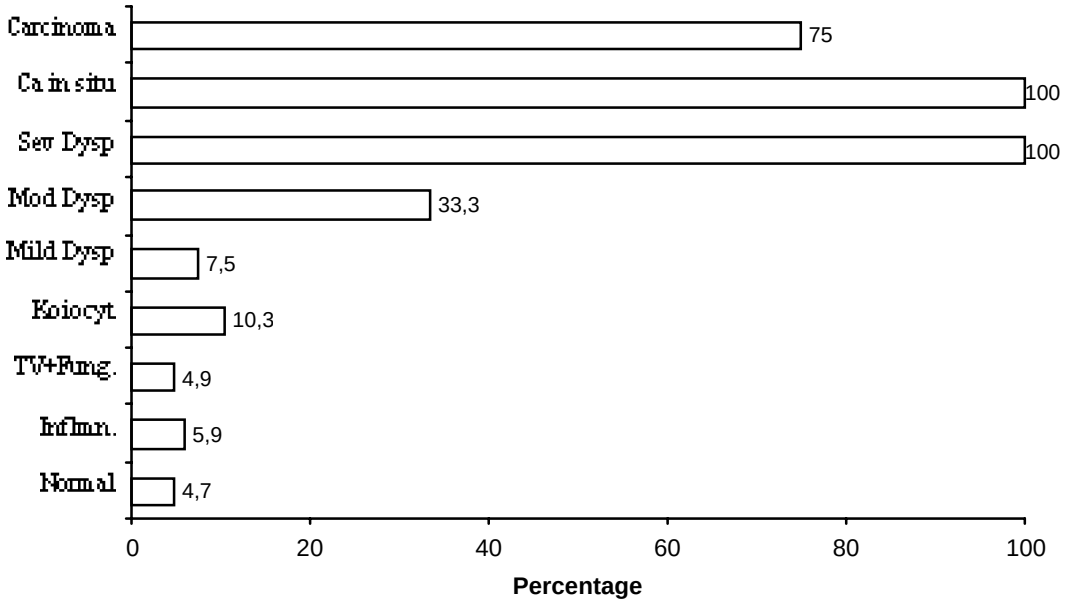

Figure 15. Prevalence of HPV (all types) by cytological diagnoses, Trivandrum HPV study.

5.7 HPV subtypes

Biological samples of all women who came into the clinics were sent for HPV testing. The results of HPV positivity (234 positives) were available first and they were linked

to the database of 4056 women and were used for this analysis. Results of the subtyping were available later. The subtypes of HPV in the 234 positives are presented in Table 17.

Based on these results, the frequency of HPV 16 and 18 was 32.9% among those with known HPV types, in this study.

Table 17. Frequency of HPV subtypes among the 234 HPV positive women in the Trivandrum HPV study, 1995-97.

HPV type	%	(n)
16	29.5	(69)
18	3.4	(8)
31	3.4	(8)
33	5.5	(13)
39	0.4	(1)
45	2.6	(6)
52	0.9	(2)
53	1.7	(4)
54	1.3	(3)
56	0.4	(1)
58	3.0	(7)
59	0.4	(1)
850	10.3	(24)
6	0.9	(2)
806	0.9	(2)
66	1.7	(4)
68	0.4	(1)
715	0.4	(1)
768	0.4	(1)
801	7.3	(17)
X	23.5	(55)
Untyped	1.7	(4)
Total	100.0	(234)

850 –any of the 50's, 806-6/11/42, 715-Pap155, 768-ME 180, 801-26/40/clinical types, X-no recognised type.

5.8 Independent determinants of HPV

To identify the independent determinants of HPV, factors which emerged as either significant or which were considered important from each group were identified. They were adjusted for each other and for age in the model. Socio-economic status, genital hygiene of H&W and promiscuity of wife were considered as they were significant ($p<0.05$) when adjusted for each other within their category. Age, religion, history of any STD and 'coitus during menstruation' were also added as they were considered

relevant, even though they did not attain significance in its group. In the final model these variables were adjusted against each other.

In order to be comparable, the analysis for the factors in the final model is done on the 2458 women who had information on all the variables. In comparison to the results from all the data, the crude and within group adjusted results of the variables age, SES, religion and history of any STD have very slightly altered, but the direction and the significance remains the same.

Odds ratios and 95% confidence intervals for individual effects (crude) and adjusted effects for the independent predictors of HPV are presented inTable 18. Socio-economic status of the women, genital hygiene of husband and wife and sexual promiscuity of women emerged as significant when adjusted for the variables in the table.

Interactions were tested between genital hygiene and socio-econmoic status and genital hygiene and sexual promiscuity of women. These interactions were not significant and hence were not considered further.

A backward logistic regression model was applied with all the variables in Table 18. The categories of the variable socio-economic status was collapsed to two from three, as the middle and upper class were behaving the same. The backward elimination process also resulted in the independent predictors of HPV infection and they were 'socio-economic status of women', 'genital gygiene of husband and wife' and 'sexual promiscuity of women' (Table 19).

A goodness of fit test (Hosmer and Lemeshow 1989) was performed and it showed that the model predicted the outcome. The Chi square value was 4.61 (8df) and the p value = 0.79.

Table 18. Odds ratios and 95% confidence intervals for individual effects (crude) and adjusted effects of the final model for predictors of HPV in the Trivandrum HPV study, 1995–97.

Factor	Crude		Adjusted[1]		Adjusted[2]	
	OR	95% CI	OR	95% CI	OR	95% CI
Age group	(p = 0.48)		(p = 0.43)		(p = 0.21)	
<30	1.00		1.00		1.00	
30–44	0.76	0.54–1.07	0.71	0.50–1.01	0.72	0.51–1.03
45–59	0.83	0.54–1.25	0.65	0.41–1.02	0.67	0.42–1.04
60+	0.87	0.45–1.67	0.60	0.30–1.28	0.60	0.29–1.27
SES	(p<0.001)		(p = 0.001)		(p<0.0001)	
Upper class	1.00		1.00		1.00	
Middle class	1.06	0.59–1.89	1.01	0.54–1.83	0.99	0.55–1.71
Lower class	2.19	1.34–3.5	2.02	1.16–3.52	2.06	1.25–3.09
Religion	(p=0.14)		(p=0.06)		(p=0.24)	
Hindu	1.00		1.00		1.00	
Muslim	0.65	0.41–1.03	0.64	0.40–1.03	0.67	0.42–1.07
Christian	1.13	0.70–1.82	1.07	0.66–1.75	1.04	0.63–1.70
H/o any STD	(p = 0.59)		(p = 0.45)		(p = 0.33)	
No	1.00		1.00		1.00	
Yes	1.19	0.61–2.30	1.29	0.65–2.56	1.45	0.69–3.05
Coitus during menstruation	(p = 0.003)		(p = 0.23)		(p = 0.33)	
No	1.00		1.00		1.00	
Yes	2.02	1.26–3.22	1.35	0.81–2.23	1.28	0.76–2.14
Genital hygiene (H&W)	(p<0.001)		(p<0.001)		(p<0.0001)	
Yes	1.00		1.00		1.00	
No	3.96	2.72–5.78	3.96	2.60–6.10	3.84	2.61–5.64
Promiscuity (W)	(p<0.005)		(p = 0.01)		(p = 0.02)	
No	1.00		1.00		1.00	
Yes	2.51	1.32–4.77	2.25	1.16–4.34	2.15	1.10–4.18

Adjusted[1] Adjusted for variables within its own group and age
Adjusted[2] Adjusted for variables in this table H/o any STD History of any sexually transmitted disease

Table 19. Independent predictors for HPV infection in the Trivandrum HPV study, 1995–97.

Factor	Adjusted[1]	
	OR	95% CI
SES	(p = 0.0002)	
Upper class	1.00	
Lower class	2.16	1.54–3.02
Genital hygiene (H&W)	(p<0.0001)	
Yes	1.00	
No	3.76	2.56–5.51
Promiscuity (W)	(p = 0.02)	
No	1.00	
Yes	2.17	1.12–4.22

[1] Result of the backward logistic regression model with age

Population attributable risk percentage (PAR%)

The population attributable risk provides an estimate of the fraction of the new cases of disease observed in the study population that is attributable to exposure to a risk factor (Isabel dos Santos Silva 1999). It is more appropriate in a scenario where an etiological exposure is studied. However in the present study the proportion attributed to the independent determinants can be estimated. Since the approach is more like a case-control analysis using the proportion of exposure among the 'controls' (HPV negatives), population attributable risk percentage is calculated for the independent determinants.

Socio-economic status

$$PAR\% = \frac{Pe\,(OR-1)}{Pe\,(OR-1)+1} * 100$$

Pe = 0.59 (proportion of women in the low SES among the HPV negatives)
OR = 2.16

$$PAR\% = \frac{0.59(2.16-1)}{0.59\,(2.16-1)+1} * 100 = 40.6\%$$

Poor genital hygiene of the couple

Pe = 0.06, OR = 3.76

$$PAR\% = \frac{0.06(3.76-1)}{0.06\,(3.76-1)+1} * 100 = 14.2\%$$

Promiscuity among women

Pe = 0.02, OR = 2.17

$$PAR\% = \frac{0.02(2.17-1)}{0.02\,(2.17-1)+1} * 100 = 2.3\%$$

If the above factors are responsible for HPV prevalence in the population, then 40.6% of the infection can be prevented by improving the socio-economic status of the women, 14.2% can be prevented by improving the genital hygiene of the couple and 2.3% can be prevented by women avoiding sexual promiscuity. The population attributable risk percentage thus provides an indication of the preventive potential of the various predictors. The preventive potential is related to the level of exposure of the factor in the population and the relative risk associated with it. Low socio-economic status is more prevalent and can have the maximum effect if corrected.

6 DISCUSSION

Cervical cancer is the leading cancer in women in many Indian registries and there have not been many studies on the epidemiology of HPV in this region. HPV has been blamed as the major etiological agent for cervical cancer (Munoz et al. 1994).

6.1 General population based studies in India

This is the first study from India to report general population based prevalence of uterine cervical HPV infection. This study included married women only, but 92% of the women above the age of 20 years were married in this population and hence this is a good reflection of the adult female population as a whole. Hospital based studies have been conducted in Delhi (Das et al. 1982, Das et al. 1989). In India the lack of unique identifiers of individuals and incomplete and inadequate notification and registration of health related events present many limitations.

In western countries registration with the general medical practitioner, membership in insurance schemes, motor vehicle licence, the telephone directory, etc. can be used as the sampling frame with unique identification features (Klatsky et al. 1974). No such systems could be used reliably in India even in 1995. Electoral lists and wards were changing every 5 years and cannot be considered reliable.

The extended family system in India adds to the difficulty in identifying an individual uniquely. There are large families with many small units and the head of the household will be listed everywhere. Identification of smaller units in the household is not easy in such situations. Hence, fresh enumeration was the only way to ensure completeness, validity and reliability of the base population.

Conducting a house to house survey was expensive and time consuming. The relatively small study area enabled the personnel to do a house to house survey in order to ascertain the baseline demographic data.

Computerisation was realised to be an essential element in data management from the beginning. Accordingly, all the data collected on every individual were computerised with unique identifiers. Once the baseline computer database is generated, listings of populations can be made in different formats.

Ensuring adequate compliance in the study was the second major difficulty. The compliance achieved in this study cannot be compared to a study conducted in a western population. Women had to attend to the daily chores of their households and in many instances were helping out to make both ends meet. Their list of priorities starts with food and shelter and it was difficult to drive home the message that they can benefit by attending the clinics.

This was the first organised programme of its kind in the region and we hope that as time goes by more and more people will participate in the study. Only the demonstrable benefits attained by those who participated in the programme could increase the participation rate. A sustained campaign and dissemination of the benefits should be a continuous process. Women might have looked at this as a study instead of a health care delivery programme. It would be easier if an ongoing screening programme was in place so that women could be recruited from the attendees.

Since Kerala is a state with a very high literacy rate, it was possible to use the print media to make the community aware of the study. The health benefits of participation were explained in small group discussions. Clinics were held as close to the homes of the eligible women as possible. The medical officer of the study offered health care to other members of the family and this provided an incentive for the women to come to the clinic. Subjects with higher levels of education and middle income categories were more likely to participate.

Biological sample collection, processing and storage of samples and sophisticated laboratory analyses have severe limitations in the economic and cultural scenario under which this study was conducted. Biological sample collection is viewed as a medical procedure and is always related to illness and is not perceived as a sign of well being. The visits of the women to the field clinics were mainly affected by the information that they would be subjected to biological sample collection. The clinic had a medical officer at all times and this instilled some confidence into the women.

Organising the entire infrastructure in makeshift clinics was an obstacle. Simple things like availability of electricity for sterilisation of specula and other materials posed major challenges. There were days when the clinic had to be cancelled after inviting women for want of uninterrupted electricity supply.

Once the samples were collected they had to be stored in cold temperatures and transported to the centre. Storage and transportation from the field in dry ice in a tropical country like India is difficult. The samples were transported to the RCC, labelled and stored in deep freezers.

The analyses with PCR were carried out at the Institute of Cancer Research Laboratories in UK. Shipping the samples to UK involved many difficulties, as they had to be transported through domestic airports in India without adequate facilities for

cooling in transit. Some samples perished in the transportation and given the difficulty in collecting them from the field this was a major loss. There were no methodological controls to demonstrate that the sensitivity of the HPV test used was not hampered by the sample transportation.

Epidemiological studies with biological sample collection are required to be done in low resource settings and amidst all the infrastructure deficiencies this study has addressed the problems, recorded a fair coverage after surmounting several obstacles and evolved strategies to overcome the limitations.

6.2 Prevalence of HPV infection

The prevalence of HPV among women in this study was 6.1%. Gjooen et al. (1996) reported a prevalence of 15% in a general population sample in Norway. The prevalence of HPV was 5% in women with normal cytology findings in a study from Bangkok (Siritantikorn et al. 1997).

Recent results from an IARC study has reported population prevalence of HPV and age adjusted incidence of cervical cancer from different countries. The age adjusted incidence rate per 100,000 of cervix cancer was 44.4 in Mexico and the HPV prevalence was 15%. The corresponding figures in Thailand were 23 and 8 (Munoz et al. 2000). The age adjusted incidence of cervical cancer in this study area is 15 per 100,000 and the prevalence rate of HPV of 6% corresponds with this.

HPV infection is considered to be very transient and many studies have shown the disappearance of this infection. In a study from Sweden, 276 women were studied for two years and the prevalence of HPV infection decreased from 21% to 8.3% (Evander et al. 1995). In a study in England, 93% of sexually active young women initially infected with HPV shed their infection in 4 months time (Hinchcliffe et al. 1995).

6.2.1 Prevalence by Age

The lack of a decline with age in this study may be a reflection of the sexual behaviour of the population. Premarital sex is relatively uncommon. This study population comprised only married women and hence the prevalence of HPV infection in the premarital period was not studied. It was not possible to invite unmarried women to participate in the study and usually cervical smears were not taken from them unless they presented with a complaint at a hospital. The exclusion of unmarried women may be a reason for the lack of age related decline of HPV in this study.

A study from Italy has reported a prevalence of HPV of 23% in the age group 17-25 years, 10% in the group 26 to 35 years, 6% in the group 36 to 50 years and 3% in the group 51 to 70 years (Gradiolone et al. 1996). The age related decline in HPV prevalence was also shown in a study from the Netherlands. The prevalence of HPV declined from 19% in the age group 15–25 years to 5% in the group 40–49 years (de Roda Husman et al. 1995).

A study among sexually transmitted disease (STD) clinic attendees in Jamaica showed an effect of age independent of sexual activity. A reduction in prevalence with advancing age was observed and attributed to an increased immunity to HPV with age (Figueroa et al. 1995). The present study showed some reduction in risk of HPV with increasing age but this was not found to be significant. The odds ratio for women >60 years was 0.60 (0.29–1.27) compared to those who were less than 30 years of age. In the Jamaican study, more than the age related decline it is possible that the younger age groups had higher levels of infection and among them a subgroup had persistent infection even in older ages. The Jamaican study was among STD clinic attendees and this may be one of the reasons for the very high rates in young women, 39% in the age group 15-19 years, which came down to 17% in the age group 30 years and above. The transient nature of the high levels of infection seen in younger ages is well documented.

6.2.2 Religion and socio-economic factors

Muslim women had a prevalence of 4.1% compared to 6.5% among Hindus. Genital hygienic practices of Muslim men may be the reason for this reduction. The reduced prevalence of HPV among women living in concrete houses (4.8%) compared to thatched houses (7.1%) was suggestive of an association between the HPV prevalence and living standards. Thatched houses are usually situated in a cluster and lack many personal facilities.

The prevalence among the highest income category was 3.2% compared to 6.9% in the lowest income category. A similar variation is also seen with education and occupation. Here again living standards and hygienic practices associated with these variables may be different and these differences could have manifested as the difference in the prevalence rates.

Tobacco habituees had a higher prevalence (7.4% compared to 5.8% among non-habituees) of HPV in this study. Thirty eight percent of women in the lowest educational category were tobacco chewers compared to 3% in women with high school education and above. Tobacco chewing was not significantly associated with HPV in this study. Sikstrom et al. (1995) reported that smoking, alcohol use and drug abuse

were risk markers for HPV infection and the odds ratio associated with smoking was 1.4 (95% CI 0.8–2.4).

The prevalence among women whose husbands were in the military or police force was 7.9% compared to 3.7% for women whose husbands were government employees. Husbands who are away from their homes and travel far are likely to have different behaviour and hence the difference in prevalence.

The prevalence among subjects with lower socio-economic status was 7.3% compared to 4% among the middle and upper classes. The prevalence was consistently high in women with low standards of living. Since the risk factors for carcinoma cervix could well fit with the risk of HPV, these findings are corroboratory. Most of the reported prevalence figures in other studies are from hospital clinic attendees and in women with cytological abnormalities.

6.2.3 Marital and reproductive factors and contraceptive practice

Cervical neoplasias and infections of the genital tract are usually addressed in the context of marital and reproductive factors. This study also attempted to describe the prevalence of HPV among different strata of marital and reproductive factors.

The prevalence of HPV did not show any appreciable change among the different categories of ‘age at menarche’, ‘age at marriage’, ‘age at first childbirth’ and ‘number of pregnancies’. Women in the upper social class and from educated families are likely to continue with their education and may marry later than those from relatively poor families. However, the length of time between menarche and age at marriage did not influence the prevalence of HPV. This is a window period for premarital sexual activity and if there was a high occurrence of multiple partners during this period, then the prevalence in those who spent more time in this window should have been high.

Those who were separated from their husbands had a prevalence of 8.4% compared to 5.8% in those living with their husbands. Living with husband is the social norm and husband moving to a distant place for work usually brings about temporary separation. Divorce is not common and couples live separately if the marriage fails. Of men separated from their wives 45% had extramarital partners compared to 19% for those living together (considering the women who were interviewed for sexual behaviour).

Infection with HPV seems to take place only after coitus. In a study from Sweden, girls in the age group 15 to 17 were sampled and followed up. Only the sexually experienced girls harboured HPV-DNA in the cervix with a cumulative prevalence of 37% (Ellstrom et al. 1996). Given the very low level of sexual activity among girls

before marriage in Kerala, it is likely that the HPV infection in the women in the study was mostly acquired after marriage. If this is the case, then the infection is likely to persist as continued sexual transmission takes place from the husband. Poor genital hygiene and lack of cervical cytology screening are also likely to make the infection persist in the genital tract. It has been suggested that cervical cancer arises in women with persistent HPV infection (Hildesheim et al. 1994).

Fife et al. (1999) studied a cohort of pregnant women in the USA. In the first trimester of pregnancy 31% of the patients had positive test results and comparison with postpartum results showed a decline to 26.5%. It was observed that the increased prevalence observed during pregnancy persists at a similar level throughout pregnancy but declines in the postpartum period. The authors suggested that the activation of the virus occurs through the physiological changes of pregnancy and leads to higher persistence. The present study excluded pregnant women and hence there is no data to compare with these figures.

Permanent sterilisation by tubectomy was the most common form of contraception in these women. Spacing methods and condom use were relatively rare. Women whose husbands used condoms had a prevalence of 2.9% compared to 6% in those who had sterilisation. This is based on only two cases in the condom use category and hence may not be reliable. However, it is a suggestion that barrier contraceptive use by men can protect women from the infection. In a study of male Finnish conscripts, it was shown that 16.5% of men had HPV infection demonstrated from penile swabs. Multiple sexual partners, previous history of sexually transmitted diseases and the practice of not using condoms were the significant risk factors for the infection (Hippelainen et al. 1993). Condom use was not very common among men in this study population, but with the awareness programmes on Acquired Immune Deficiency Syndrome (AIDS), condom use is being promoted through the media. History of husband having a vasectomy did not seem to affect the prevalence.

Among the different contraceptive methods, post partum sterilisation was the most common followed by intrauterine contraceptive device for women in Kerala (Economic Review, 1996). Family planning as a choice is still not available to the majority of women. Sterilisation was much publicised earlier and many camps were held. A large number of women were sterilised and slowly it has been accepted as the contraceptive method for women. It was observed in this study that 70 to 80% of women who were born in the 1950's and early 1960's were sterilised by the 1990's. It is possible that permanent sterilisation might alter the sexual behaviour, but this study did not observe any such trends. A more liberal approach to contraception with more choice has to be provided to the population especially to women.

6.2.4 Symptoms at presentation

There is always a possibility that women who were symptomatic came in to the study in a large proportion. The prevalence of symptoms in the study group cannot be compared with the general population prevalence, as we do not have this information on the general population. However, there were no great differences in the prevalence of HPV among those who had symptoms and in those who did not report any symptoms.

The prevalence of HPV by symptoms reported at the clinic showed that those who had postmenopausal bleeding had a prevalence of 15% (based on small numbers) compared to 6% for those who did not. The presence of a history of sexually transmitted diseases resulted in a prevalence of 7.1% compared to 6% among those in whom it was absent. The minimal difference may be due to the possibility that the question on the history of sexually transmitted disease was not totally understood by the subjects and hence they may not have provided the required information.

6.2.5 Genital hygiene

Washing of genitalia after coitus and the practice of coitus during menstruation were considered as markers of genital hygiene. Lack of use of sanitary pads was found to be a risk factor for cervical dysplasia in Trivandrum (Varghese et al. 1999). Sterile sanitary pads are relatively expensive and the poor women usually resort to clothes, which they continue to use after washing. It is likely that these cloths are not sterile and lead to infections each time they are used.

Women who were classified as not practising genital hygiene had a prevalence of 23.9% compared to 7.3% for those who practised hygienic behaviour. Genital hygiene is likely to be a reflection of the living conditions, amenities etc. However, it seems that in itself it is important. Infections of the lower genital tract are very common in this population and 60–70% of women in all the age groups had ‘inflammation’ on their cervical smears. It is possible that these women harbour many bacterial and viral infections and chronic cervicitis and progression to various grades of dysplasias are likely in them.

6.2.6 Sexual behaviour

Sexual behaviour is a very sensitive area to address in this society, given the cultural practices. A trained sociologist interviewed the women after establishing rapport with

each woman in the privacy of the clinic. Responses to questions on sexual behaviour of husband and wife were elicited from the women. It was not feasible to interview the men. The agreement between the responses of the wives regarding the premarital and extramarital relationships of their husbands was very high. This is an indication that the responses were true and that those who had premarital partners were likely to have extramarital partners also. Eighty three percent of men who had extramarital partners visited commercial sex workers. It is possible that their wives reported these visits as 'extramarital partners'. Even though there was agreement between the reported pre and extra marital sexual contacts of husbands, women may not be aware of their husbands pre marital and extra marital partners and the reported sexual behaviour of husbands is likely to be an underestimate. However the sexual promiscuity of husband lost its significance when adjusted with wife's promiscuity.

Regarding their own sexual behaviour, the answers to premarital and extramarital partners were less strongly agreed upon by women. Only 1.3% women reported extramarital partners. This is likely to be true, as the majority of men who had extramarital partners had been visiting commercial sex workers. The prevalence of HPV was marginally greater among those women whose husbands had extramarital and/or premarital partners compared to those having none. In the case of women, those with extramarital partners had a prevalence of 24% compared to 8% in those who did not have partners. This was a three-fold difference in prevalence of HPV and suggests the significant role of sexual behaviour of the woman.

Sexual behaviour is a very difficult exposure to measure in conservative societies. This study attempted to interview women who participated in the study to collect some information on sexual behaviour. Sexual matters are not openly discussed and sex outside marriage is considered forbidden. Sexual behaviour is considered personal and confidential. Carael et al. (1995) studied the sexual behaviour in 18 countries of Asia and Africa with face to face interviews. The extramarital contacts of men ranged from 4% to 47%. Women were much less likely to report non-marital sex than men. The authors suggest that broad generalisations about sexual behaviour, especially multiple partners can be misleading.

Fifteen percent of husbands were reported to have contact with commercial sex workers in the Trivandrum study. Carael et al. (1995) reported that contacts with commercial sex workers for men ranged from 1 to 25% in their 18- country study. There was a predominance of this behaviour among the lower income groups, 21% of men in the lowest income group had contacts with commercial sex workers compared to 7% in the highest income group. de Sanjose et al. (1997) reported similar observations in studies from Columbia and Spain.

Extramarital partnerships for women were very low in this study, but were highly significant in predicting HPV infection. Only 1.3% (34) of women had extramarital partners. Of those who reported having extramarital partners 91% were in the lowest income group and 38% were in the group with the lowest level of education.

6. 3 Cytological findings

All women in the study had a cervical smear which was read at the RCC, Trivandrum. Inflammation was very high in all the age groups and for almost all women this was their first cervical smear. The prevalence of HPV was studied by cytological findings. The prevalence was found to increase from 7.5% among normal smears to 33.3% among mild dysplasia and 100% for moderate dysplasias and carcinoma in situ. Among the four invasive cancers, three had a positive HPV. HPV, especially the high risk types are found to be present in almost all cervical precancerous conditions (Van Den Brule et al. 1991).

6.4 HPV subtypes

HPV 16 was the most common type (29.5%) in this series. Recent results from IARC studies have now grouped HPV types 16,18,31, 33,35,39,45,51,52,56,58 and 59 as high risk types (Munoz et al. 2000a). Considering all the high risk types together their frequency was 49.5% in Trivandrum. In a study from Columbia, Munoz et al. (1992) reported that 69% of the HPV positives among general population based control women had HPV 16 subtype. In their world-wide study Bosch et al (1995) reported a prevalence of 42% for HPV 16 and 31% for HPV18 among cervical cancer cases from Southeast Asia. The prevalence of HPV 16 or 18 was 2.9% among controls (women with normal cytology) and 33.5% among cases (women with CIN) in a case-control study reported from USA (Schiffman et al. 1993). In a population based case-control study in Copenhagen, the prevalence of HPV 16/18/31/33 was 8.2% among control women and 49.5% among cases (Kjer et al. 1996). The frequency of HPV 16 and 18 tested by PCR was 58% among pregnant women and 27% among non-pregnant women in a hospital based study in Delhi, India. The prevalence of high risk HPV types was found to be increased during pregnancy (Gopalakrishna et al. 1995). Hospital attendees in India may be very different from the general population and regions within India are not strictly comparable in many aspects.

6.5 Independent determinants of HPV

A stepwise process was followed to identify the independent determinants of HPV infection. Collinearity was controlled to a certain extent by forming factors which are representative by combining correlated variables. Socio-economic status, genital hygiene of the couple and promiscuity of wife emerged as the independent determinants of HPV infection. There were no significant interactions among the predictors.

Munoz et al. (1996) identified lifetime number of sexual partners and socio-economic status as determinants of HPV prevalence in Spain, Columbia and Brazil. de Sanjos et al. (1997) reported that the difference in cervical cancer incidence by social class might be partly explained by differences in the prevalence of HPV. There was a 60% reduction in the prevalence of HPV among the upper social class women compared to the lower social class in this study.

Socio-economic status exerted its effect independent of the variables, genital hygiene and sexual behaviour in this study. The odds ratio associated with low socio-economic status was 2.16 (95% CI 1.54–3.02). However there could still be residual confounding. Low socio-economic status could be a reflection of many of the living conditions and behavioural patterns. It is unlikely to be the conceptually relevant exposure responsible for HPV, but could explain majority of the HPV infection in the population. Improvement in soico-economic status might lead to better living conditions, adequate availability of water and access to health care. If there is a non-sexual route of transmission of HPV then improved living conditions could reduce the infection rates as well.

The hygienic practices of both the husband and the wife were important and emerged as significant. There are not many studies on genital hygiene and HPV, but studies have addressed genital hygiene as a factor for cervical cancer and precancer. A case control study of cervix cancer in Nagpur, India, has shown poor genital hygiene as a significant risk factor after adjusting for other variables (Gwande et al. 1998). Varghese et al. (1999) also reported that poor genital hygiene is a risk factor for cervical dysplasia in Kerala. Dutta et al. (1990) have also shown poor genital hygiene as a risk factor for cervical cancer in a case control study in Pune, India. A case-control study of carcinoma cervix in China has also revealed the role of daily genital washing in the aetiology of cervical cancer (Zhang et al. 1989). In a study from Greece, frequent washing of genitalia during the menstrual period was found to be a risk factor for HPV (Agorastos et al. 1995). The behavioural pattern of asymptomatic Greek women and that of women in South India are obviously not comparable. Moreover, frequent washing of genitalia during menstruation may not be a hygienic practise and might lead to infections.

Genital hygiene is an important factor in low resource countries, as the living standards of poor women leave much to be desired. Persistent infection and inflammation of the genital tract is a common event in women in the low socio-economic groups and it is possible that prolonged inflammation reduces local immunity leading to infection with HPV. There was a significant increase (odds ratio 3.76, 95% CI 2.56–5.51) in the HPV prevalence in those who do not practise genital hygiene compared to those practise hygiene.

In a study from Sweden, lifetime number of male sexual partners emerged as the major risk factor for HPV infection (Karlsson et al. 1995). This study was among adolescent girls in Sweden who are not comparable to the housewives of Kerala, but the common factor of multiple partners suggest that there is a biological basis for this association. Figueroa et al. (1995) reported that sexual behaviour, especially having more than one partner per month was a significant determinant of HPV infection in STD clinic attendees in Jamaica. Munoz et al. (1996) reported a fourfold increase of HPV infection in women who had six or more partners. Kenny (1996) reported risk factors for HPV as initiation of sexual activity before the age of 15 years, more than four lifetime sexual partners and history of male partners having multiple partners. Venuti et al. (1994) reported multiple lifetime sexual partners as the most relevant risk factor for HPV 16/18 in an Italian population. They suggest that other factors like smoking and use of oral contraceptive pills were associations with clinically manifested HPV infection.

There were only very few studies on sexual behaviour and cervical neoplasia from India and one such study in rural India reported extramarital partners for women as an independent risk factor for cervical cancer (Biswas et al. 1997).

History of multiple sexual partners had an odds ratio of 2.17 (95% CI 1.12–4.22) for HPV infection in this study. It is possible that sexual behaviour was underreported by women in this study, but the high risk in those who reported multiple partners suggests that they have come out with the truth.

6.5.1 Non-sexual route of HPV acquisition

The notion that high risk HPV types are exclusively sexually transmitted is being reconsidered. The supporting facts for this consideration were, HPV DNA detection among virgins (Pao et al. 1993), low concordance of genital HPV types among hetero-sexual partners (Hippelainen et al. 1994) and presence of high risk genital HPV infection among children.

Transmission from mother to infant at birth has been suggested as the non-sexual mode of transmission of HPV. Pakarian et al. (1994) demonstrated HPV DNA by PCR on the external genitalia and/or in buccal cavities of 50% of 24 hour old infants delivered to HPV positive mothers. It is conceivable that transmission occurred through contact as infections in infants have been in the oral cavity and nasopharynx. External genitalia also have been identified as the site of infection in infants and virgins. However, for the infection to reach the uterine cervix epithelium there may have to be co-factors. The lack of genital hygiene identified in the present study has a potential role in facilitating the spread of the infection.

Considering the less than optimal conditions of childbirth in low resource settings it is possible that non-sexual transmission of HPV occurred in this study population. The socio-economic and hygiene related variables might be a surrogate for their poor circumstances during labour. Improvements in living standards of women can reduce the prevalence of HPV infection in these women even when considering the possibility of non-sexual transmission of HPV.

6.6 Limitations of the study

The study area was selected on the basis of it being a suburban region and the fact that the three major religious groups are well represented there. The area was not randomly selected and thus the results have a limitation in generalising to other areas. However it is not biased in any way and the age distribution, socio-economic profile and the distribution by religion are comparable with the rest of the State of Kerala.

The overall compliance was 43.5% in this study. The women in the younger age groups and the very old were less compliant. However it is unlikely that a bias has occurred due to the low compliance. Women were not aware of the HPV testing when invited and there were no misconceptions that would have affected the compliance in relation to their HPV status.

The sexual behaviour questionnaire was administered only on a section of the compliant women. The difficulties of eliciting this sensitive information have been discussed in detail and the two groups were compared with regard to selected variables to show their similarity. Except for small differences they were comparable. However the final model and the identification of independent determinants of HPV were made on 2458 women who were interviewed for sexual behaviour and had all the data. This might affect the generalisability to a certain extent, but the validity of the findings are not affected. The sexual promiscuity reported in this study is likely to be an underestimate of the true behaviour.

The HPV prevalence is assessed from a single smear and it is possible that some infections are missed. Repeated sampling would have provided better estimates. However majority of the studies in the literature is based on single smears and the transient nature of the infection is well known. Type specific comparisons were not made as the subtypes were made available later only. However 50% of the HPV's were high risk types and the results of this study are applicable to the high risk and low risk types. There was a concern that the samples may have been damaged during transport thus affecting the sensitivity of HPV testing. The identification of HPV in 75% to 100% of precancerous lesions and cancers suggest that the sensitivity of HPV detection in the samples was not affected by transportation.

6.7 Implications for control of HPV infection and cervical cancer

HPV has been considered as the major etiological agent for cervical cancer and the main risk factor is identified as sexual behaviour. Prevention strategies are based on the control of sexual transmission and the possibility of vaccination. Vaccination against HPV infection is currently under experimentation and might offer a better option for cervical cancer control. Sherman et al. (1998) suggested the potential of prophylactic vaccination. Phase I and II clinical trials in patients with advanced HPV related cancer have shown that vaccination is feasible (van Driel et al. 1999). If an efficient and cheap vaccine is made available it can be considered for mass vaccination, but no such options are available at present.

This study shows three independent determinants of HPV, low socio-economic status, poor genital hygiene and sexual promiscuity of women. The findings of the present study has shown that some of the risk factors for genital HPV infections seems very similar to those described as risk factors for CIN and invasive cancer.

Poor socio-economic status is a much more prevalent factor and it seems to exert its effect independent of poor genital hygiene and sexual promiscuity. It is possible that it is a reflection of other factors which were not directly elicited in this study. However, in view of the potential for control of HPV infection it suffices to say that the living conditions of women have to be improved. The population attributable risk percentage was 40% for low socio-economic status making it the most feasible intervention. Improvement in socio-economic status of women can have many other outcomes like reduced infant and maternal mortality rate in addition to reducing the prevalence of HPV infection.

Genital hygiene is an important aspect of the reproductive health of the women and special emphasis has to be placed on this aspect. Poor genital hygiene is likely to lead to chronic cervicitis, infertility and pelvic inflammatory disease. Improvement in

living standards and health education can improve the genital hygiene. The use of barrier contraceptive methods might help towards primary prevention of HPV.

Sexual behavioural modification is a difficult task in conservative societies. The reported promiscuity is an underestimate of the true figure and hence the low population attributable risk percentage for this factor. However since it is a major determinant of HPV infection as well as for other sexually transmitted diseases, health education with special emphasis on sexual behaviour is needed in these populations. It may be difficult in the older generations but certainly has to be considered for the younger generations who are exposed to the emerging social and cultural changes.

An organised Pap smear based cytology screening programme has been shown to be the best strategy for the control of cervical cancer. Introducing this in countries like India has many limitations. One lifetime selective screening as a feasible strategy has been suggested for India (Prabhakar 1992, Juneja et al. 1997). HPV testing as part of cervical cytology screening has been suggested (van Ballegooijen et al. 1997). Rozendaal et al. (1996) tested HPV screening in the Netherlands and reported that the interval between successive smears in cervical cancer screening can be increased considerably for women with cytomorphologically normal and high risk HPV negative cervical smears as determined by PCR. Routine cytology screening itself has not been feasible in low resource countries and it might take many years before HPV based screening can be undertaken in these countries. More than screening the facilities for optimal treatment of screen detected lesions is essential for the screening programme to be successful and this is a major limitation in many low resource countries with a high incidence of cervical cancer.

An alternative strategy like ‘visual inspection’ was tested and was not found to be a good option in our settings (Varghese et al. 2000). Health care workers in the community, whose mandate is to provide health education, need to be made aware of the changing disease patterns and the importance of primary prevention strategies for chronic diseases. The services of the print and electronic media should also be utilised for providing health education messages to the community.

Public health interventions and control strategies for improving genital and reproductive health need to be planned for controlling HPV infection in this population. It was appreciated that the price of sanitary pads was reduced in the last budget of India. A multipronged strategy with improvement in living standards for women, health education with emphasis on genital hygiene and safe sexual behaviour coupled with low intensity Pap smear screening can be considered for the control of HPV infection and cervical cancer in India.

7 SUMMARY

The present study has addressed the prevalence and determinants of HPV infection in a general population in Kerala, India and considered the implications for the control of HPV infection and cervical cancer.

In a suburban region of Trivandrum, the capital city of Kerala State in the Indian Union, a cohort of married women from the general population was formed. Nine thousand three hundred and twenty women were identified as eligible and were invited to clinics held in the field for interview and biological samples were collected after obtaining an informed consent. The overall compliance was 43.5% giving 4056 women for the study. Two thousand five hundred and thirty five women were interviewed for details on sexual behaviour and genital hygiene.

Testing for HPV was done by PCR as per standard protocol at the Institute of Cancer Research Laboratories in Sutton, UK. Prevalence and determinants were studied for all types of HPV.

The overall HPV prevalence was 6.1%. The prevalence was almost steady across the age groups. Women in the low socio-economic strata had a higher prevalence, 7.3% compared to 4% for those in the higher socio-economic strata. Women who were separated from their husband's had a prevalence of 8.4% compared to 5.9% for those who were living with their husbands.

Women whose husbands used condoms had a low prevalence of 2.9% compared to 6% for those who did not practise any method of contraception. However this was based only on 2 HPV positive women and may not be reliable.

Genital hygiene of husband and wife were combined into one variable and those who did not practice genital hygiene had a high prevalence (24% versus 7%). Women who reported promiscuity had a prevalence of 18.5% compared to 8.3% for those who did not. The prevalence rate was 10.2% for women whose husbands had promiscuity compared to 7.8% for those who had no report of promiscuity among husbands.

Poor socio-economic status, lack of genital hygiene of the couple and promiscuity of women emerged as the independent predictors of HPV infection in this population. The odds ratio for women in the lower social class was 2.16 (95 % CI 1.54–3.02) compared to those in the lower social class. The odds ratio was 3.76 (95% CI 2.56–5.51) for having HPV if they and their partners did not practise genital hygiene compared to those with good hygiene. Women who had a history of multiple sexual

partners had an odds ratio of 2.17 (95% CI 1.12–4.22) for HPV compared to those who reported no promiscuity.

If the factors low socio-economic status, poor genital hygiene and promiscuity of women are considered responsible for HPV infection in this population, then 40.6% of the infection can be prevented by improving the socio-economic status of the women, 14.2% can be prevented by improving genital hygienic practises of the couple and 2.3% can be prevented by women avoiding promiscuity.

The reported prevalence of HPV infection is mainly from western populations and they are not comparable to the socio-cultural milieu of the population in Kerala. Premarital sex is considered forbidden and multiple partnerships for women are negligible. Extramarital sexual relations of men seem to be in frequenting commercial sex workers.

Identification of genital hygiene and socio-economic status as independent predictors suggests that control of HPV infection can be achieved by improving the living standards of women. The cervical cancer incidence in Trivandrum Cancer Registry was 15 per 100,000 in 1991–92 and there are no organised cervical cytology screening programmes in the area. The subgroup of women who harbour HPV and who might ultimately develop cervical cancer are likely to be those in the low socio-economic strata with poor genital hygiene and multiple sexual partners.

This study has raised the problems of general population based epidemiological studies in low resource settings and identified the strategies to overcome them. The main limitation of this study was that all HPV types were considered together in the analysis. The subtyping of HPV was made available later and showed that 50% were high risk HPV types. Low socio-economic status and poor genital hygiene seem to be exerting their effects independently and this may also suggest a non-sexual route of HPV transmission. Identification of risk factors separately for the oncogenic and non-oncogenic types will be undertaken later as more data accrues and this will help to delineate the differences.

The prospects of HPV vaccination and HPV based screening are being considered as options for cervical cancer control. It might take many years for poor countries to establish organised population based screening programmes. Empowerment of women, improvement of living standards, and health education coupled with low intensity Pap smear and provision of equitable resources for treatment can control HPV infection and bring down the cervical cancer incidence in the poor countries of the world.

8 ACKNOWLEDGEMENTS

This study was carried out at the Regional Cancer Centre, Trivandrum and I express my gratitude to Dr. M Krishnan Nair F.R.C.R., Director of the centre for permitting me to undertake this study and for his guidance and support in my work and career.

I am indebted to my supervisor, Professor Matti Hakama D.Sc., who has provided me the opportunity to study in Tampere. He has been patient and considerate and found time during his busy schedule to steer me in the right direction. His kindness, sincerity and informal attitude have enriched my stay in Tampere and will remain as a source of inspiration in my life. I would like to express my deepest gratitude for his guidance, supervision, encouragement and for his help to make this thesis possible.

I thank my official referees, Professor Jorma Isola, M.D., Ph.D., from the Institute of Medical Technology, University of Tampere, Finland and Professor NS Murthy, Ph.D., from the Institute of Cytology and Preventive Oncology, Indian Council of Medical Research, New Delhi, India. Their valuable advice and comments helped me substantially to improve the quality of this work.

I am grateful to Professor Julian Peto and Dr. Judy Deacon from the section of Epidemiology, Institute of Cancer Research, Sutton, UK, who were the collaborators for this work in India and for carrying out the HPV testing in their laboratories.

The staff of the Tampere School of Public Health are gratefully acknowledged for their help and support. I thank Ms Virginia Mattila from the Language Centre and Ms Marita Hallila and Ms Catarina Ståhle-Nieminen from the school for their assistance in language correction, computerisation of the manuscript and in completing the formalities for submission and publication of the thesis.

My thanks are due to my colleagues in the Regional Cancer Centre, who have helped me considerably in conducting the study and in providing me the support and assistance. I thank all the women who participated in the study and helped us by providing the data and biological samples. My co-students from India, Mr. Jayadevan, Mr. Swaminathan, Mr. Budukh and Mr. Dhar deserve a special word of gratitude for their co-operation and company during my stay in Finland.

Thanks are also due to the Government of Finland and the Cancer Society of Finland for providing the financial support for my studies in the University of Tampere through the agreement between the Ministry of Education in India and Finland.

I wish to express my warmest gratitude to my wife, daughter, mother and brothers for their patience and loving support during my time away from the family and for all their care and concern.

9 ABBREVIATIONS

C.I = Confidence Interval

CIN = Cervical Intra Epithelial Neoplasia

CIS = Carcinoma in situ

DNA = Deoxyribonucleic Acid

FCB = First Child Birth

FIGO = International Federation of Gynaecology and Obstetrics

HPV = Human Papillomavirus

IARC = International Agency for Research on Cancer

M-M = Menarche-Marital interval

NBRR = Natural Background Radiation Registry

NCCP = National Cancer Control Programme

NCRP = National Cancer Registry Programme

PCR = Polymerase Chain Reaction

RCC = Regional Cancer Centre

SD = Standard Deviation

SES = Socio-economic status

STD = Sexually Transmitted Disease

WHO = World Health Organisation

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Appendices