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ORIGINAL RESEARCH ARTICLE

The Bali STD/AIDS Study: human papillomavirus infection among female sex workers

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Summary: Female sex workers in low priced brothel areas in Denpasar, Bali, Indonesia participated in an intervention study designed to promote condom use and sexually transmitted disease (STD)/AIDS prevention. The intervention provided educational sessions for sex workers, STD treatment for sex workers, condom distribution, and media for clients. The brothel areas were divided into high and low areas for programme effort. The high effort areas received a more intensive behavioural intervention than the low effort areas. A clinic was available for STD treatment in both areas. Behavioural surveys and STD testing were used to evaluate the programmes. About 600 were evaluated for several STDs and completed personal interviews at enrolment and at six-month intervals during the 18-month study. About 50% of women were new to the study at each round. Human papillomavirus (HPV) testing of cervical swabbed specimens, using polymerase chain reaction methodology, was performed at the beginning of the study and 18 months later.

Human papillomavirus infection was initially high in these women (38.3%) and declined to 29.7% after 18 months ($P < 0.01$). The prevalence of HPV infection declined with age ($P < 0.01$). HPV infection was associated with a number of STD symptoms that were reported in personal interviews. These associations were stronger in the first time period. Infection with *Neisseria gonorrhoeae* was associated with HPV infection at baseline ($P = 0.03$). HPV infection declined in the study area with the more intensive educational programme ($P < 0.01$). The prevalence of HPV infection declined over time and was associated with study area and age of woman.

Keywords: Indonesia, prostitution, STDs, HPV

Introduction

Human papillomavirus (HPV) infection has been documented in 18–82% of women in a number of studies of female sex workers[1–10]. The prevalence of high risk types of HPV such as 16 and 18 has also been noted. In addition, HPV infection has been found to be associated with HIV infection[3,4,8]. HPV is also known to be strongly associated with the risk of cervical cancer[11].

From 1997–1999, an intervention study was conducted among female sex workers in Bali, Indonesia[12]. The objective of the project was to develop a long-term approach toward HIV prevention in these areas that combined behavioural approaches with sexually transmitted disease (STD) treatment. Two alternative intervention programmes, one with a more intensive education programme than the other, were tested in low price brothels in the Denpasar, Bali area. In addition to a number of group education and training sessions, women in the programme were also tested and treated for STDs every six months, and a drop-in clinic for evaluation and treatment was available in or near the complex areas.

Changes during the study period in bacterial STDs, AIDS and STD knowledge, and condom use were reported previously[12]. The objective of this paper is to describe the prevalence of HPV infection by type in this area, the association with STD symptoms and other STDs, and the change in prevalence over the study period.

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Methods

Context

The project was developed in seven large brothel complexes in and near Denpasar, Bali. These seven

complexes include all of the low price brothels in the study area. Clients of the sex workers are mainly Indonesian men. The women receive an average payment of about $3.00 US from each client and serve 3–4 clients per day. The brothel complexes are organized into a number of smaller units or 'clusters' that consist of a small area of land with a structure consisting of several rooms. An area in each cluster is usually also set aside to sell cold drinks and light refreshments. Each one of these clusters is owned by a pimp and supervised by him/herself or an assistant and may employ 4–12 women.

The intervention consisted of group sessions for sex workers, media regarding STDs and condom use for clients, and condom distribution. The intervention sites were divided into areas for high and low programme effort. The brothels were divided into these groups by assessing the size of the brothel area and the client mix (i.e. hotel workers, sailors) in each area. Areas were divided to create a balance of these two factors in each area. In the high effort areas, women received three educational sessions every six months. In the low effort areas, the women received only one session every six months. The brothels were visited by field workers 2–3 times per week to ensure condom and media supply. Media in the form of pamphlets and papers were also available to clients in the brothel areas.

The group sessions for women were designed to (1) increase AIDS/STD knowledge, (2) increase perceived susceptibility to these diseases, (3) improve skills related to condom use and partner negotiation, and (4) increase awareness of STD symptoms and the need for STD screening and treatment. A video was developed that included typical experiences of female sex workers including negotiating condom use with different types of clients, becoming infected with an STD, and social topics such as visiting with children and other family members in Java. The first session dealt mainly with informational issues about AIDS, STDs, and the symptoms of illness. The later sessions extended the discussion of STDs, condom use, and condom negotiation.

Women in the complexes were tested and treated for STDs every six months. A drop-in centre located near the complex was available for STD treatment during the study period. Women were encouraged to visit the clinic for treatment of genital symptoms.

The intervention was evaluated through a series of behavioural surveys of sex workers and clients and through STD testing of sex workers. The baseline survey was conducted in 1997–8 and three additional surveys were conducted at six-month intervals. Face-to-face interviews were conducted with female sex workers and clients in brothel areas. Interviewers received a training session of several days including obtaining informed consent, orientation to the field situation, probing, asking sensitive questions, and recording answers. The interviews consisted of questions on AIDS/STD knowledge, condom beliefs, self-efficacy for condom use, sexual behaviour, condom use, and STD symptoms and diagnoses. Interviews were conducted in private areas in the brothel complexes. All women working in the brothels were invited to participate and over 99% of these women participated in the survey interview.

After each interview was conducted, the woman was offered a physical examination for STD assessment. About 95% of the women consented to a vaginal examination. Women travelled to a nearby clinical site for the examination. Samples of cervical mucus were tested for *Neisseria gonorrhoeae* (Abbott LCx, probe system, Abbott Laboratories, Abbott Park, Illinois, USA), *Chlamydia trachomatis* (Abbott LCx, probe system, Abbott Laboratories, Abbott Park, Illinois, USA), HSV (polymerase chain reaction [PCR], herpes simplex virus [HSV]-1 and HSV-2, primers obtained from Midland Certified Reagent Company, Midland, TX), and *Trichomonas vaginalis* (InPouch TV, BioMed Diagnostics, San Jose California, USA). Serum was tested for syphilis (*Treponema pallidum* haemagglutination assay [TPHA] (positive), rapid plasma reagin [RPR] (positive, titre $\geqslant 8$) and HIV (anonymous) (enzyme-linked immunosorbent assay [ELISA]/Western blot). Candida was identified by culture in-house cultures (Sabouraud agar, obtained from Liofitchem diagnostici, Roseto Degli Abruzzi, Italy). Candida cultures were read at two days. *Candida* species were identified using 10–40× microscopy of representative colonies.

Women were considered positive for syphilis if the TPHA was positive and the RPR was positive with a titre of at least eight. Laboratory tests for syphilis, trichomonas, and candida were processed at Kerti Praja Foundation in Denpasar, Bali. The testing for gonorrhoea, chlamydia, and HSV was conducted at the University of Michigan Hospital Clinical Microbiology Laboratories. The HIV testing was conducted at the laboratories of the Naval Medical Research unit in Jakarta, Indonesia. Treatment was provided according to a syndromic diagnosis at the time of examination. The World Health Organization protocol for syndromic treatment was followed at the first round and was then modified to increase sensitivity to STDs in this population. When lab results were obtained from laboratories out of the area at a later date, untreated women were contacted and medication was provided for non-viral STDs.

The specimens for molecular testing to be performed at the University of Michigan were inoculated into transport tubes supplied by Abbott Laboratories for use with the LCx test, were initially kept at room temperature and were transported to a -70°C freezer within five hours of collection. Shipment to Michigan occurred quarterly with specimens remaining frozen on dry ice during transport.

Quality assurance was maintained in the Bali laboratory by using trained laboratory technicians for all testing and testing known positives when available to assure the quality of the reagents. Quality control at the University of Michigan laboratories included routine inclusion of negative and positive controls.

Testing for HPV was performed using a PCR test by L Gregoire at Wayne State University. Samples for DNA analysis were sent frozen and remained frozen until the time of processing. Phenol and chloroform extraction was performed on the cellular extract. The DNA was then precipitated from the aqueous phase with the addition of 3M Na acetate and 100% ethanol. The resulting DNA pellet was then further purified using InstaGene Matrix (BioRad) according to the manufacturer's protocol.

The resulting DNA solutions were submitted to PCR using primers specific for moderately repetitive human genomic sequences to evaluate the integrity of DNA. Samples yielding the expected 350 bp amplified fragments were suitable for amplification and samples were tested for the presence of HPV sequences.

Detection of HPV DNA sequences was performed as previously described[13]. Briefly, amplification reactions were performed in a volume of 100 μL in 67 mM Tris–HCl (pH 8.8), 2.0 mM MgC12, 37 mM KCl with the deoxyribonucleotides at a final concentration of 200 uM each and primers at 1 μM and 2.5 U Taq DNA polymerase. Thirty cycles were performed where a cycle consists of a denaturation at 94°C for one minute, annealing at 46°C, a first extension at 55°C for 30 seconds and a second extension at 72°C for 30 seconds. Positive and negative controls were included with all PCR series and were analysed in parallel with the clinical specimens.

The amplified fragments were dotted on biotrans nylon membranes (ICN) and hybridized using oligoprobes specific for HPV 16, 18, 31, 33, 35, 45, 52, HPV 6 and 11 were detected but not discriminated. Amplified fragments were also electrophoresed through 2% agarose gels and transferred to nylon membranes (ICN). The resulting membranes were submitted to a non-stringent (Tm-40) Southern blot hybridization using as a probe a mixture of HPV 16, 18, 31 and 52 in an attempt to identify HPV sequences different from the one above.

The protocol for the study was approved by the Health Sciences Institutional Review Board of the University of Michigan and the IRB of Kerti Praja Foundation.

Measures

STD prevalence was measured by whether or not the woman had the infection using the method of diagnosis described above. It was coded 1 = infection present, 0 = infection not present.

Age was measured as the woman's age in single years.

Round refers to the round number of the evaluation survey and examinations.

New participant included all women new to the study, regardless of round of data collection. It was coded one if it was the first round in which the woman participated, 0 otherwise.

High programme area was coded one if the woman was working in the area with the more intensive educational programme, 0 otherwise.

Study cluster is the unit in which the woman works which consists of a pimp and 4–10 other women.

Statistical methods

Initially, differences between the high and low intervention groups and time periods were evaluated using analysis of variance. Following this, logistic models were estimated using the SAS (Statistical Analysis System version 6.2) procedure Proc MIXED. This procedure uses restricted maximum likelihood estimation (REML) for parameter estimation and allows us to adjust for covariance between individuals due to repeated measures and study cluster. For categorical dependent variables, the associated GLIMMIX procedure was used to estimate the logistic models. An observation is included in the data file for each time that a woman participated in an interview or STD examination. The model included identification number and cluster as random variables and age, programme area, round number, and whether or not the woman was new to that round as independent variables. The interaction variable 'round*programme' area (the interaction of round and programme area) was also included as an independent variable. The coefficient of the variables 'round*programme' area is of importance to the evaluation of the education for commercial sex workers since it indicates if the rate of change in HPV infection was different for the two programme areas. With the interaction term in the model, the dichotomous variable programme area indicates whether or not the programme areas differed initially on the dependent variable. Hence, the models will evaluate whether or not the rate of change was different in the two programme areas controlling for initial differences in the dependent variable, and age and mobility of sex workers.

Results

Demographics

Table 1 shows the demographic characteristics of the study participants. The average age of women was in the mid twenties and the average number of years of schooling about five years. Although the majority of women were once married, most (80%)

Table 1. Demographic characteristics of study participants

	Round 1 (baseline)	Round 4
Mean age (years)	25.3	26.1
Mean education (years)	4.6	5.5
% married	6.0	5.7
% never married	12.8	15.4
% divorced/separated/widowed	81.2	78.9
Clients per day	2.9	3.8
New enrollees	100%	46%
Price for sex (Indonesian rupiah)	11,019	18,993
Price for sex (US$)	2.70	2.56
Mean income/day	7.84	9.71
Dates of study	11/97–2/98	6/99–9/99
No.	631	618

Age, education, and clients per day were significantly different between the two rounds at $P<0.01$
Differences in significance between the income measures were not calculated due to the rapidly changing economic conditions

of the women were widowed, divorced, or separated at the time of interview. Although reports tend to be understated, the women reported that they had been working in Bali for a mean of 13 months and a median of six months.

The number of clients per day and the price for each sex act varied over the study period. A very severe economic crisis was experienced by Indonesia during the study. The amount of money that a woman earned per client was reduced sharply in real terms due to the decline in the value of the rupiah. The number of clients the women served each day also increased ($P<0.01$). The mean income per day of the women recovered in the later rounds.

The study area was characterized by a significant turnover of sex workers each round. The study defined all women as new the first round that they participated. The proportion of women who were new was 46% in Round 4.

Prevalence of HPV infection

Table 2 shows the prevalence of HPV infection stratified by age and geographic area of the study. In the first round, 38.3 of women tested positive for HPV infection. The prevalence declined to 29.7% by the end of the study ($P<0.01$). HPV infection varied substantially by the age of the woman. The highest rates were observed by women under 18 (69.2% Round 1, 48.3% Round 4). Rates declined with each older age group with just over 20% for women 31 or more testing positive.

At the beginning of the study, the HPV prevalence was similar in both areas (36.5% high area *vs* 39.1% low area, $P>0.05$), but by Round 4 the prevalence rates were significantly different (16.6% high area *vs* 37.3% low area, $P<0.01$). The rates declined significantly in the high programme area, but not the low programme area. These changes were further examined below in the multivariate analysis.

The laboratory was able to determine the type of HPV virus for about half of the sample while others displayed interference with the test, or were indeterminate. The most common types of HPV detected were those of unknown type (53% at Round 1 and 65.8% at Round 4), followed by the high risk types of 16 and 18 (17.2% Round 1 and 20.2% Round 4). These were followed in frequency by the medium risk type, with the low-risk types of 6 and 11 being less commonly detected. The reduction in HPV prevalence between Round 1 and Round 4 was noted in the lower risk types of HPV, with no reduction seen in those types most commonly associated with cervical cancer (type 16 and 18) or in the unknown types of HPV.

Association with self reported symptoms

In the personal interview conducted before the STD examination, women were asked about their experience with a number of STD symptoms. They were asked if they had ever experienced these symptoms, whether they experienced them in the last six months, and whether they had them during the past month. Results are presented in Table 3 only for the questions on experience in the last six months, since results were usually the same across the three measures.

In Round 1, in which the HPV prevalence was 38.3%, for each genital symptom the odds were greater than one of having the symptom if the woman was HPV positive with almost all of the

Table 2. Prevalence of human papillomavirus (HPV) infection by type

	Round 1		Round 4	
Positive for HPV	38.3%	209	29.7%	158
Negative for HPV	61.7%	334	70.3%	374
Total	100%	541	100%	532
Age (years)				
<18	69.2%	26	48.3%	29
18–24	44.9%	216	33.5%	155
25–30	34.7%	199	29.5%	217
31+	21.6%	97	21.1%	95
Area				
High intensity	36.5%	197	16.6%	169
Low intensity	39.1%	343	37.3%	327
Type of HPV				
6, 11	9.1%	19	1.3%	2
16, 18	17.2%	36	20.2%	32
31, 33, 35	14.3%	30	8.9%	14
45, 52	6.2%	13	3.8%	6
Unknown type	53.1%	111	65.8%	104
Total	100%	209	100%	158

Differences by age group were significant at $P<0.01$ in both rounds
The high programme area was significantly different from the low programme area at Round 4 ($P<0.01$) but not at Round 1. HPV infection differed significantly between Round 1 and Round 4 in the high programme area ($P<0.01$) but not the low programme area

Table 3. Odds ratio of human papillomavirus infection by symptoms reported in personal interviews

	Round 1				Round 4			
Symptoms in last 6 months	Percent reporting symptom	Odds ratio	95% Confidence interval	*P* value	Percent reporting symptom	Odds ratio	95% Confidence interval	*P* value
Increased vaginal discharge	30.4	1.96	(1.35, 2.85)	<0.01	36.7	1.13	(0.76, 1.68)	0.31
Coloured vaginal discharge	13.9	1.93	(1.18, 3.16)	<0.01	13.5	1.68	(0.99, 2.86)	0.04
Foamy or thin discharge	16.1	1.84	(1.16, 2.92)	<0.01	20.6	1.07	(0.67, 1.71)	0.43
Fishy vaginal odour	15.0	1.83	(1.13, 2.94)	<0.01	24.8	1.27	(0.82, 1.96)	0.16
Vaginal bleeding between periods	8.5	1.87	(1.02, 3.43)	0.03	8.5	1.47	(0.76, 2.83)	0.16
Genital itching	27.6	1.53	(1.05, 2.26)	0.02	31.0	1.21	(0.81, 1.83)	0.20
Genital sores	23.3	1.89	(1.27, 2.84)	0.01	32.3	1.51	(1.01, 2.25)	0.03
Painful genital ulcers	17.6	1.67	(1.07, 2.61)	0.02	24.0	1.08	(0.69, 1.70)	0.40
Painful bumps on genitals	8.5	2.27	(1.23, 4.18)	<0.01	8.3	1.22	(0.62, 2.39)	0.34
Sore inguinal nodes	10.6	1.53	(0.88, 2.65)	0.09	8.7	2.40	(1.27, 4.51)	<0.01
Pain on urination	23.7	1.75	(1.17, 2.61)	<0.01	26.2	1.38	(0.90, 2.11)	0.08
Frequent urination	27.2	1.42	(0.96, 2.08)	0.05	30.2	1.18	(0.78, 1.78)	0.25
Pain in lower abdomen	48.1	1.41	(0.99, 1.99)	0.03	54.2	1.02	(0.70, 1.51)	0.49
Pain inside with intercourse	20.0	1.76	(1.15, 2.69)	<0.01	35.3	1.23	(0.83, 1.85)	0.17
Pain at vulva with intercourse	8.0	2.06	(1.12, 3.78)	0.01	12.9	1.35	(0.78, 2.34)	0.18
No.		540				496		

symptoms significantly associated with HPV infection. Increased, foamy or thin, coloured and fishy smelling discharge were associated with increased odds of HPV infection. Genital sores, itching, abdominal pain and pain during intercourse were also associated with HPV infection. However, in the Round 4 data, although the odds ratios remained greater than one, most were not statistically significant. This may be related to a reduced power to detect significance due to the lower prevalence of HPV infection at Round 4.

Association with other STDs

There were very few significant associations between the presence of HPV infection of the cervix and the presence of condyloma and other STDs (Table 4). The diagnosis of condyloma in the examination occurred only for a small number of cases and was significantly associated with HPV infection only at Round 4. *N. gonorrhoeae* was associated with HPV infection at Round 1, but not Round 4. The other STDs, including chlamydia, syphilis, and trichomonas were not associated with HPV infection of the cervix. HIV testing, with unlinked samples, indicated a low prevalence in this study (1/600, Round 1, 2/544 Round 4).

Multivariate analysis

Comparisons between the study areas with differing educational intensity may be confounded by differences in the age of the women and duration of their stay in the study complexes. To assess differences between areas, controlling for differences in age and entry of new women and initial levels of HPV, a logistic regression mixed model was assessed. The dependent variable for the logistic model was the presence of HPV infection. Independent variables were the age of the woman, programme area, the study round, and whether or not the woman was new to the study that round. An interaction term, 'round*programme area', was added to the model to test whether or not the two

Table 4. Association of human papillomavirus infection with diagnosis of condyloma and other sexually transmitted diseases

	Round 1				Round 4			
	Prevalence	Odds ratio	95% Confidence interval	*P*	Prevalence	Odds ratio	95% Confidence interval	*P*
Condyloma	4.3%	1.16	(0.81, 1.66)	0.23	2.4%	4.83	(1.43, 16.299)	<0.01
Neisseria gonorrhoeae	61.0%	1.45	(1.00, 2.09)	0.03	55.8%	1.15	(0.78, 1.70)	0.28
Chlamydia trachomatis	42.1%	1.16	(0.81, 1.66)	0.23	43.5%	0.98	(0.67, 1.45)	0.51
Syphilis RPR=8	10.3%	0.77	(0.43, 1.39)	0.24	4.4%	0.86	(0.33, 2.25)	0.48
Trichomonas	10.9%	1.12	(0.65, 1.95)	0.39	4.0%	0.99	(0.37, 2.63)	0.60
No.	525				477			

RPR=rapid plasma reagin

Table 5. Logistic mixed models of the effects of age, mobility and programme variables on the prevalence of HPV infection

Variable	Coefficient	Standard error	Odds ratio	*t*	*P*
Logistic regression					
Dependent variable: HPV					
Intercept	1.64	0.67	5.14	2.45	0.01
Round	−0.07	0.09	0.93	−0.73	0.47
High programme area	0.57	0.35	1.77	1.61	0.11
Round*high programme area	−0.54	0.12	0.58	−4.34	<0.01
Age	−0.09	0.02	0.91	−4.76	<0.01
First enrolment No.	0.14	0.29	1.16	0.50	0.62

HPV = human papillomavirus

programme areas were changing at different rates. Initial models also included number of clients per day, condom use, and the woman's education, but these variables were dropped due to their lack of significance in the models.

Results of this analysis are shown in Table 5. The variable study round and programme area were not significantly related to HPV infection. However, the coefficient of the variable round*programme area was negative and significant indicating the HPV infection rate decreased more in the high intensity programme area over time than in the low programme intensity area ($P < 0.01$). Age of woman was also significantly related to HPV infection. The prevalence of infection declined with age ($P < 0.01$). The variable reflecting which round the woman was first enrolled was not significantly related to HPV infection.

Discussion

Consistent with other studies of HPV infection among sex workers, the women in this study had a substantial prevalence of HPV infection. The rates observed here are lower than those reported in several other studies[2,5,7]. The sample from this study is different from most others in that about 95% of women working in the low price brothels were included in the study. Many other studies include clinic populations that may be less representative of the population of sex workers. Some of the other studies also included many women who were HIV positive. Unlinked samples indicated a very low prevalence of HIV infection in this study (1/600, Round 1, 2/544 Round 4).

An association of decreasing HPV infection with age was noted in this study as in other studies[5,6,14]. The rates were particularly high for the women under 18 (69.2% Round 1, 48.3% Round 4). This association with age held in the multivariate analysis when several other potentially confounding variables were controlled. The HPV data were also tested for association with condom use, number of clients, and the amount of time involved in sex work, but the associations were not significant. This may be due to the high level of exposure that all women in the study have given the large number of clients and the inconsistency of condom use.

HPV infection was associated with a number of symptoms of STDs that were reported in the personal interviews. These associations have been noted previously, although the mechanism remains unclear[14,15]. These associations were significant for the first round, but not for the fourth. This may be due to a reduced power to detect significance because of the lower prevalence of HPV infection in Round 4. The size of the odds ratios were low to moderate in both rounds. The 95% confidence intervals overlap for each symptom between the two rounds.

Generally, HPV infection was not associated with the other STDs that were assessed in the study. The one exception to this was the association with *N. gonorrhoeae* in Round 1. Also, the diagnosis of clinically apparent condyloma, which might prompt medical evaluation and treatment, was very rare.

The decline in HPV infection between Round 1 and Round 4 was associated with the difference in programme area. HPV infection declined more substantially in the area with the more intensive intervention. This decline was not related to the number of new women who entered the area and occurred despite the high levels of the other STDs. A Zairian (Democratic Republic of Congo) study noted a decrease in another viral STD, HIV when STD treatment had been amplified while the level of chlamydia infection remained substantial[15].

In summary, in this group of women with a high exposure to sexually transmitted infections, the prevalence of HPV was noted in about one-third of the sample, but declined during the course of the study. The prevalence was higher among younger women and among those with gonorrhoea infection. The prevalence declined more in the area that was given a more intensive behavioural programme.

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