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Original article

The effectiveness of Carraguard, a vaginal microbicide, in protecting women against high-risk human papillomavirus infection

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Background: A randomised, double-blind, placebo-controlled trial found the vaginal microbicide Carraguard unable to prevent HIV infection. A substudy assessed the association of genital high-risk human papillomavirus (HR-HPV) in women at study end with Carraguard use.
Methods: Participants received Carraguard gel or placebo plus condoms, and were instructed to use gel plus condoms during each act of vaginal intercourse. HR-HPV detection on cervical samples from 1,723 women was by Digene Hybrid Capture 2 analysis. Poisson regression analysis assessed the prevalence of genital HR-HPV for individuals receiving Carraguard relative to individuals receiving placebo.
Results: In the Carraguard arm (*n*=875) the end trial unadjusted HR-HPV prevalence was 23.5% (95% CI 20.8–26.3) and 23.0% (95% CI 20.2–25.8) in placebo arm (*n*=843). Significant risk factors for HR-HPV infection were younger age, being single, an abnormal pap smear, multiple sexual partners and promiscuous behaviour without the use of a condom. There were 348 compliant women (174 Carraguard, 174 placebo users), with relatively high adherence to gel use, who inserted 80% of their opened, returned applicators of test product with the proportion of applicator insertions to sex acts >30%. After adjusting for risk factors, these compliant Carraguard users were 0.62 as likely to be classified HR-HPV positive (95% CI 0.41–0.94) as compliant placebo users.
Conclusions: The prevalence of HR-HPV infection was lower in compliant Carraguard users than compliant placebo users. To our knowledge, this is the first report showing a negative association of HPV infection with a vaginal microbicide.

Introduction

Developing countries such as South Africa have a high burden of human papillomavirus (HPV)-associated cancers [1]. Cervical cancer screening and treatment programmes are inadequate in South Africa and HPV vaccines are unaffordable to most of the population. Vaccines, although highly successful, only fully protect from HPV-16 and HPV-18 [2] and there are at least 13 other HPV types causing cervical cancer [3]. In addition, HPV vaccines are focussed on young people, leaving older women unprotected. Furthermore, in South Africa, cultural norms and other factors preclude many women from insisting on the use of condoms. There is, thus, an urgent need to empower African women to protect themselves from sexually transmitted infections (STIs) including HPV. Potential vaginal microbicides had been tested in clinical trials for their effectiveness in protecting against sexually transmitted infections, especially HIV, with little success [4] until the recent tenofovir gel trial [5]. High adherers (*n*=336) to tenofovir gel use showed a 54% reduced HIV incidence in those using the tenofovir gel compared to those using the placebo gel (*P*=0.03) [5].

There had been no reports on the effects of microbicides on HPV transmission, until female sex worker users of a nonoxynol-9 (52.5mg, 3.5%), vaginal gel

(Advantage-S; Columbia Laboratories, Miami, FL, USA), showed increased HPV infection compared to placebo users [6]. The study concluded that the use of nonoxynol-9 did not prevent genital HPV infection and could increase the virus' ability to infect or persist [6]. The latter study was supported in a mouse model where nonoxynol-9 was shown to increase papillomavirus transmission [7]. In contrast, it has been shown that carrageenans inhibited papillomavirus infection *in vitro* [7,8]. Furthermore, it was recently reported that HPV-16 attaches to sperm, which, it is postulated, could increase the motility of HPV and penetration into cells. This cell binding and thus subsequent cellular penetration can be blocked by carrageenan [9].

Carrageenans are high molecular weight anionic polymers. The charges on these molecules bind to viral and cell envelopes and protein, and physically block infection and entry of pathogens into cells [10]. Studies have shown the effective blocking of the infectivity of papillomaviruses by sulphated polysaccharides such as heparin, cellulose sulphate and dextran sulphate [11]. HPV attachment to cells is thought to be by interaction of the virus and heparan sulphate on the surface of the cell. The sulphated polysaccharides are thought to block infection by mimicking heparan sulphate and competing with the virus for cell attachment [8]. Carrageenans are extracted from marine red algae and have been shown *in vitro* to inhibit the infectivity of genital HPV with almost a 1,000-fold greater potency than heparin (cell-free heparan sulphate) [8]. Carrageenan is commercially available as a thickener in cosmetics and food products and can be found in a wide range of products such as sexual lubricants, lubricated condoms and infant foods.

Based on preclinical evidence, there was a need to prove the concept in human clinical trials to determine whether carrageenan-based products can be used as a microbicide and protect against genital HPV. A carrageenan-based vaginal microbicide, Carraguard was developed by the Population Council (New York, NY, USA) and tested for its efficacy to prevent HIV infection in women in South Africa. Results showed no safety concerns with the use of Carraguard but no reduction in male-to-female sexual transmission of HIV was demonstrated [12]. At their final study visit, a subset of participants in the Carraguard Phase III trial, were enlisted to assess the prevalence of HR-HPV infection in Carraguard compared with placebo users. The findings from the study presented here showed an association of Carragurd use with HPV infection.

Methods

Study participants

This study constitutes a substudy of a Phase III randomised, placebo-controlled, double-blind trial of the microbicide Carraguard to determine whether Carraguard as a vaginal gel can prevent HIV infection in women [12]. The microbicide Carraguard (PC-515) was developed by the Population Council and is a non-contraceptive, odourless, colourless gel made from a specific type of carrageenan (product number PDR98-15; FMC, Philadelphia, PA, USA), which is derived from seaweed [12]. The placebo was methyl cellulose, a clear odourless, tasteless gel, with a proven safety profile [5]. The study was conducted over a 3-year period. Three centres were involved in the study; University of Cape Town, University of Limpopo Medunsa Campus, and the Medical Research Council (MRC) in Durban. The median age of women in the study was 31 years, (age range 16–72) years. Samples for HPV detection were not collected at screening visits or during the 3 years of the HIV study. This was a major limitation of the HPV substudy, not having at least a baseline cervical sample to compare with the samples taken at study end. Participants entered the HPV study in October 2006, 6 months before the study end on 31 March 2007, when there were 3,419 women in the HIV study. Samples for the HPV study were taken only from those women still eligible for a closeout visit pap smear (n=1,764). Amongst these there were 41 non-consents for various reasons leaving 1,723 women from whom samples were taken for HPV analysis. The demographics of these 1,723 women did not differ significantly from the women not included in the HPV study with the exception that the women in the study were on average 2 years older than those who did not enrol.

Women were asked to sign a separate informed consent form asking their permission for an extra specimen to be collected. The consent form explained the purpose of the study and women were given the option to choose not to have the extra specimen taken during their last pelvic examination. After the collection of the final pap smear, a second cervical specimen was taken from consenting women at their Carraguard Phase III trial closeout visit with a cytobrush. This was inserted into Digene transport medium (Gaithersburg, MD, USA) and transported to the laboratory and stored at -80°C. A total of 1,723 cervical samples for HPV detection were obtained: 874 received from Cape Town, 671 from Medunsa and 179 from the MRC, Durban.

Cervical HPV DNA analysis

Hybrid Capture 2 (HC2; Digene) was used to determine the combined prevalence of 13 cervical high risk HPV types: -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68. According the Digene HC2 user manual, 'high concentrations of anti-fungal cream, contraceptive jelly, or douche' could potentially inhibit the detection of HR-HPV at the lower limit of detection giving a false negative in these specimens containing HPV DNA levels that yield relative light units/cutoff (RLU/CO)

values near the assay cutoff. The sample taken for HC2 analysis was the third sample taken after removal of the cervical mucous plug and the pap smear sample. Thus, we postulate that the residual Carraguard concentration would be low and interference would be minimal, if at all. Furthermore, there were only 25 placebo-using participants with RLU/CO values close to cutoff and 6 in the final analysis whose positive HC2 result could have been potentially inhibited by the use of Carraguard.

Data analyses

The prevalence of HR-HPV infection was compared on an intention-to-treat basis. The data was modelled with modified Poisson regression [13]. All factors on which data is available, (for example, demographic information, reproductive history, sexual activity, alcohol and smoking) were assessed as potential confounders and included in the multivariate model if necessary. Crude prevalences were calculated in order to assess potential confounders for inclusion in the model. The final model's treatment prevalence ratio (PR), which reflects the risk of HR-HPV for Carraguard users relative to placebo users, was adjusted for all statistically significant confounders. Several scenarios were considered with regard to detecting 50% reduction in the prevalence of HPV infection. For the scenario used in the final analysis it was estimated that a sample size of 352 subjects (that is, 176 per treatment group) would provide 80% power to detect a 50% reduction in the prevalence of HPV with a two-sided test performed at the 0.05 level of significance. This calculation assumed that the population prevalence of HR-HPV was 24%. Samples sizes were estimated using the sample size calculator in STATA version 8.0 (STATA Corp., College Station, TX, USA).

Results

Of the 1,723 participants providing cervical samples, samples from 5 women were not received by the testing laboratory; there were thus 1,718 women available for the univariate analysis. There were 41 refusals from study end participants to provide samples for the HPV assessment, 4 from the MRC, Durban and 37 from the University of Cape Town. Reasons for refusals ranged from not interested (13), no reason (10), pregnancy (4), coloposcopy (4), too old (1), too young (2) and hysterectomy (7).

Of these 1,718 women, 875 women used Carraguard and 843 women used placebo gels. There was no difference in demographic data and sexual activity in Carraguard compared with placebo users (Table 1). Of the 1,718 women, 21 (1.2%) became HIV seropositive during the trial. Two of these women were excluded from the multivariate analysis because they were missing covariate values; one had no pap smear data and one no condom data at baseline in the Carraguard trial.

The prevalence of HR-HPV at the end of the trial differed per site: 20.9% (183/874; 95% CI 18.3–23.6) amongst Cape Town participants, 26.0% (173/665; 95% CI 22.7–29.3) amongst Medunsa participants and 24.6% (44/179; 95% CI 18.3–30.8) amongst MRC participants from Durban. Overall, the unadjusted HR-HPV prevalence was 23.3% (400/1718; 95% CI 21.3–25.2). In Carraguard users the prevalence was 23.5% (95% CI 20.8–26.3) and 23.0% (95% CI 20.2–25.8) in placebo users. Risk factors for HR-HPV infection were identified in the univariate analysis (Table 2). Significant risk factors found for HR-HPV infection were age younger than 35 years, PR 2.87 (95% CI 2.26–3.63) being from the MRC site, PR 1.25 (95% CI 1.04–1.50) having an abnormal pap smear, PR 2.86 (95% CI 2.39–3.43) being single (unmarried, separated or widowed) PR 2.60 (95% CI 2.07–3.25) promiscuous (multiple partners, without the use of condoms) behaviour (by themselves and/or their partner), PR 1.28 (95% CI 1.05–1.55) use of a condom at baseline, PR 1.65 (95% CI 1.38–1.97) and use of a condom in the study, PR 1.19 (95% CI 1.00–1.42). Factors, which the univariate models suggested were not significant covariates for predicting HR-HPV infection were; treatment group PR 1.03 (95% CI 0.87–1.22) having other sexually transmitted infections (chlamydia, gonorrhoea, HIV, syphilis, trichomoniasis; STI) PR 1.09 (95% CI 0.92–1.29) and degree of applicator use PR 0.93 (95% CI 0.74–1.16). However, these factors were included in the univariate model as they were of a priori interest. Of the 21 HIV-seropositive women in the study (10 Carraguard and 11 placebo, treatment categories) 2 from the Carraguard and 5 from the placebo groups had HR-HPV. Based on data available for this study, the univariate model suggests that HIV seroconversion was not a significant risk factor for HR-HPV infection PR 1.44 (95% CI 0.78–2.66). Having participated in the study for a period of more than 1 year was a significant factor predicting reduced likelihood of HR-HPV infection PR 0.70 (95% CI 0.58–0.84).

There were 109/1,718 (6.4%) women who had at least one abnormal pap smear during the study, 43/109 (39.5%) women had an abnormal pap smear at screening but had a normal pap smear at month 12, 39/109 (35.8%) women had an normal pap smear at screening but had an abnormal pap smear at month 12, 11/109 (10%) women had abnormal pap smears at both screening and at month 12. The rest of the women had normal pap smears at screening and month 12 but had at least one abnormal pap smear subsequent to month 12. Women with an abnormal pap smear any time during the study were 2.5 times more likely to have HR-HPV than subjects who did not have an abnormal pap smear any

time during the study. There was no association found between treatment category and abnormal pap smear.

Table 3 presents the final multivariate model adjusted for all significant risk factors. Since the treatment compliance or adherence to gel use of 42% was a major problem in the parent study [12], a measure of compliance was added to the model. There were two candidates for the measure of treatment compliance. The 'insertion of >80% of opened, returned applicators' was determined by applicator staining, which detected whether the applicator had been inserted into the vagina [12]. The percentage of covered sex acts was calculated by dividing the average number of applicator insertions (confirmed by applicator staining) by the average

Table 1. Sociodemographic data and sexual behaviour for 1,718 women participants of the intention-to-treat population as reported at screening

Variable	*n*	Treatment group	
		Placebo (*n*=843)	Carraguard (*n*=875)
Site			
Medunsa	874	446 (51.0)	428 (49.0)
UCT	665	335 (50.4)	330 (49.6)
Medical Research Council	179	94 (52.5)	85 (47.5)
Median age, years	1,718	31 (23–40)	31 (23–40)
Ethnic group			
Black	1,709	839 (>99)	870 (>99)
Other	9	4 (<1)	5 (<1)
Marriage status			
Single, never married	980	475 (56)	505 (58)
Married/living as married	628	302 (36)	326 (37)
Divorced/separated/widowed	109	66 (8)	43 (5)
No response	1	0	1
Median number of sexual partners over the last 3 months	1,718	1 (1–1)	1 (1–1)
Unprotected sex over last 3 months			
Oral	1,718	27 (3)	19 (2)
Anal	1,718	7 (1)	6 (1)
Median vaginal sex over last 2 weeks	1,718	2 (3–6)	2 (3–6)
Sex for money			
Number of times	1,718	1 (<1)	5 (1)
Median ever		2 (2–2)	2 (2–2)
Forced sex			
With partner	1,718	21 (2)	34 (4)
With another partner	1,718	2 (<1)	2 (<1)
With someone else	1,718	1 (<1)	0 (0)
Steady partner now	1,718	838 (99)	868 (99)
Mean age of steady partner (range)	4	44 (31–57)	43 (43–43)
Steady partner circumcision	1,718	3 (<1)	1 (<1)
Male condom use at last sex act	1,717	452 (52)	420 (50)
Steady partner has other partners			
Yes	257	127 (15)	130 (15)
No	866	450 (51)	416 (49)
Don't know	593	297 (34)	296 (35)
Median number of partner's other partners	257	1 (1–1)	1 (1–1)
Abuse from steady partner over last 3 months			
Physical	1,716	21 (1)	28 (2)
Emotional	1,716	143 (8)	151 (9)
Economic	1,716	58 (3)	65 (4)
Other	1,716	3 (<1)	3 (<1)
Partner(s) other than steady	1,718	38 (4)	41 (5)
Abuse from other partner(s) over last 3 months			
Emotional	1,718	3 (<1)	7 (<1)
Economic	1,718	4 (<1)	1 (<1)

Data presented as median (IQR) or *n* (%) unless otherwise indicated. UCT, University of Cape Town.

number of reported sex acts. Since applicator insertion was verifiable while the denominator used to calculate covered sex acts was not verifiable, applicator insertion was selected as the basis for the measure of compliance. There were 426 women (215 Carraguard users and 211 placebo users), who inserted 80% of their opened, returned applicators. Unfortunately, despite having claimed insertion of 80% of applicators, many of these women, who were compliant in the sense that they were reasonably truthful, did not cover a substantial number of their sex acts. Sample size calculations indicated that a sample of 352 women would provide 80% power to detect a 50% difference between the treatment groups in the number of HPV positive women at the end of the study. Based on sample size calculations it was known that approximately 175 subjects were needed, in each of the treatment groups in order to have sufficient power to obtain statistically significant results. In order to obtain this many 'truthful' subjects for analysis we were forced to select 30% covered sex acts as the lower bound for compliance. In essence, women who were only 30% compliant with respect to covered sex acts were not as compliant as we would have preferred, but in order to have sufficient statistical power

Table 2. Unadjusted factors impacting on HR-HPV prevalence amongst participants in the Carraguard microbicide trial

			Univariate associations with HR-HPV in women	
Variable	*n*	%	PR	95% CI
Age				
≤35 years	1,054	61.4	2.87	2.26–3.63
>35 years	664	38.6		
Participant Site				
Medunsa versus UCT	665 (Medunsa)	38.7 (Medunsa)	1.19	0.89–1.68
874 (UCT)	50.9 (UCT)			
MRC versus UCT	179 (MRC)	10.4 (MRC)	1.25	1.04–1.50
874 (UCT)	50.9 (UCT)			
Cervical cytology				
Abnormal pap smear	113	6.6	2.86	2.39–3.43
Normal pap	1,604	93.4		
Treatment group				
Carraguard	875	50.9	1.03	0.87–1.22
Placebo	843	49.1		
Marital Status				
Single	1,048	61.0	2.60	2.07–3.25
Married or behaved as married	670	39.0		
Condom use baseline				
Yes	872	50.8	1.65	1.38–1.97
No	845	49.2		
Condom use in study				
Yes	864	50.3	1.19	1.00–1.42
No	854	49.7		
Sexual activity				
Promiscuous behaviour, own and partner	356	20.7	1.28	1.05–1.55
Non-promiscuous behaviour	1,362	79.3		
Sexually transmitted infections[a]				
Present	855		1.09	0.92–1.29
None	863			
Duration of study participation				
More than one year	1,329	77.4	0.70	0.58–0.84
One year or less	389	22.6		
Applicator use (Carraguard or placebo)				
Inserted >80% returned applicators and >30% covered sex acts	426	24.8	0.93	0.74–1.16
Inserted ≤80% of returned applicators or ≤30% of sex acts covered	1,292	75.2		

HR-HPV, high-risk human papillomavirus; MRC, Medical Research Council; PR, prevalence ratio; UCT, University of Cape Town. [a]Sexually transmitted infections included: chlamydia, gonorrhea, HIV, syphilis and trichomoniasis.

Table 3. Multivariate analysis of associations with HR-HPV in study participants

Covariate/interaction	Prevalence ratio	95% CI	Interaction *P*-value
Site			
MRC/UCT	0.96	0.66–1.02	
Medunsa/UCT	0.96	0.72–1.28	
STI	1.11	0.94–1.31	
Average coital frequency	1.17	0.99–1.40	
Longer time in study	0.80	**0.67–0.95**	
Abnormal pap smear	2.54	**2.10–3.07**	
Used condoms at baseline	1.44	**1.17–1.77**	
Treatment by compliance			0.01
Carraguard/placebo (compliant)	0.62	**0.41–0.94**	
Carraguard/placebo (non-compliant)	1.07	0.89–1.28	
Age by relationship			0.01
Age ≤35/age ≥35 (single)	2.87	**2.06–3.99**	
Age ≤35/age ≥35 (non-single)	2.07	**1.06–2.41**	
Promiscuity by condom use			0.00
Promiscuous/not promiscuous (used)	0.97	0.75–1.24	
Promiscuous/not promiscuous (no use)	1.70	**1.30–2.20**	

Each covariate was adjusted for all of the other covariates appearing in the model. Bold CI values are significant. HR-HPV, high-risk human papillomavirus; MRC, Medical Research Council; STI, sexually transmitted infection; UCT, University of Cape Town.

Table 4. The prevalence of HPV in women using Carraguard or placebo microbicide preparations according to level of adherence to gel use

Adherence status	Fraction	Prevalence	Lower 95% CI	Upper 95% CI
Carraguard adherent				
Yes	30/174	0.17	0.12	0.23
No <80%	176/701	0.25	0.22	0.28
No 50–80%	44/171	0.26	0.19	0.32
No <50%	12/51	0.24	0.12	0.35
No ≤30%	120/479	0.25	0.21	0.29
Placebo adherent				
Yes	46/174	0.26	0.20	0.33
No <80%	148/669	0.22	0.19	0.25
No 50–80%	48/229	0.21	0.16	0.26
No <50%	11/60	0.18	0.09	0.28
No ≤30%	89/380	0.23	0.19	0.28

Adherent, >80% returned applicators with >30% covered sex acts; non-adherent 50–80%, returned applicators with >30% covered sex acts; non-adherent <50%, returned applicators with >30% covered sex acts; non–adherent ≤30%, no more than 30% covered sex acts. HPV, human papillomavirus.

to detect a difference we had to accept this compromise. Restricting the definition of compliant (high adherence to gel use) subjects to 'women who inserted >80% of their returned, opened applicators and covered >30% of their sex acts' provided a sample of 348 women (174 Carraguard users and 174 placebo users). The median percent covered sex acts for this group was 58% as opposed to 50% for the 426 women who inserted >80% of their returned, opened applicators. For these reasons, 'women who inserted >80% of their returned, opened applicators and covered >30% of their sex acts' was selected as the measure of compliance.

The prevalence of HPV was determined in women using Carraguard or placebo according to their level of compliance or adherence to gel use (Table 4). For women with high adherence (that is, inserted >80% of their opened returned applicators and had >30% covered sex acts) using Carraguard, the prevalence of HPV was 17.2% (30/174) and for those using placebo 26.4% (46/174). For women with low adherence (that is, inserted <80% of their opened returned applicators and had ≤30% covered sex acts) using Carraguard the HPV prevalence was 25.1% (176/701) and using placebo 22.1% (148/669). These were unadjusted values and differences were not significant (Table 4).

Poisson regression analysis as described above showed that for women who were compliant and had high gel adherence (that is, inserted >80% of their

opened returned applicators and had >30% covered sex acts), the prevalence of HR-HPV for Carraguard users relative to placebo users was PR 0.62 (95% CI 0.41–0.94; Table 3). Women with an abnormal pap smear were 2.5 times more likely to harbour genital HR-HPV than women with a normal pap smear (95% CI 2.10–3.07). Of those women who were single, younger women (that is, women at most 35 years old) were 2.8 times more likely than older women to have genital HR-HPV (95% CI 2.06–3.99) and if not single were 2.1 times more likely to have genital HR-HPV (95% CI 1.06–2.41). Those women who demonstrated promiscuous sexual behaviour (or their partner was promiscuous) and who did not use a condom were 1.7 times more likely to have genital HR-HPV than non-promiscuous women who did not use a condom (95% CI 1.30–2.20). Women who participated for >1 year in the study were less likely to have HR-HPV PR 0.80 (95% CI 0.67–0.95). The site where the women were recruited and average coital frequency did not impact significantly on genital HR-HPV presence and nor did the method of treatment (Carraguard versus placebo) in non-compliant users (Table 3).

Discussion

As far as we are aware, this study constitutes the first demonstration of the possible reduction of genital HPV infection by a vaginal microbicide. The presence of HR-HPV determined only at the final visit, as described in the present study, can merely assess difference in HR-HPV prevalence between Carraguard and placebo users. This difference will include new HR-HPV acquisition, HR-HPV persistence, or potential reactivation.

The results of this study have been supported by *in vitro* evidence of the blocking by carrageenan of HR-HPV entry into cells [7,8]. Carraguard possibly acted as a physical barrier to HR-HPV within the genital area and competed with HR-HPV for attachment to epithelial cells as reported in *in vitro* studies. It does appear that Carraguard may be the active ingredient which prevents HR-HPV infection. We were not able to confirm whether the methyl cellulose-containing placebo did have a protective effect against HPV infection as is postulated for HIV [12]. There was however no reduction in HR-HPV prevalence in high adherence placebo users who inserted >80% applicators with >30% covered sex acts compared with low adherers to gel use who inserted <80% applicators with <30% covered sex acts.

Carraguard did not demonstrate protection from HIV infection [12]. HIV transmission is more complicated than HPV transmission. HPV only infects epithelial cells and transmission is by virus particles, whereas HIV can be transmitted via virus or infected cells. The HIV target cell can be dendritic cells or T-cells and once the virus or virus-infected cell moves from the surfaces protected by the microbicide then infection can also take place away from the vaginal surface [14]. The recent evidence of the prevention of HIV infection by the vaginal gel containing tenofovir has been a most welcome breakthrough in the search for an antiretroviral microbicide [5].

Promoting adherence to the recommended use of vaginal microbicides is a challenge in microbicide trials as adherence to the protocol is generally lower than required. In the present study only 348/1,718 inserted >80% of their returned gel applicators and with a calculated >30% coverage of sex acts. Therefore it is important to include information on adherence when analysing the data. The reduced prevalence of HR-HPV in Carraguard users compared with placebo users was demonstrated for those with higher adherence to gel use (P=0.01). Similar results were seen for HIV reduction in compliant, high adherer users of tenofovir gel (P=0.03) [5]. In this trial, that gave significant protection from HIV infection, 336/889 participants reported gel adherence >80% and 40% of participants were recorded as low adherers (<50% of sex acts covered) [5]. In the Carraguard trial, we believe that there was under reporting of average number of sex acts by participants and over reporting of applicator use despite the staining of the applicators to determine whether these had been inserted into the vagina. There was no way to determine where the applicator gel had been used. Improved adherence to the use of Carraguard by more women and by each woman before more sexual acts might have greatly increased protection of HPV infection by Carraguard users compared with placebo users. Greater adherence to applicator use amongst more women would have also increased the power of the comparison. To control for poor adherence in the Tenofovir trial, a comprehensive adherence support programme assisted participants with their applicator use and discouraged applicator sharing and misuse [5]. This was most beneficial and increased the overall percentage of high adherers to gel use compared to the Carraguard trial. Importantly, most women from the tenofovir trial declared their commitment to the use of a vaginal microbicide should it prevent HIV infection, indicating high adherence to the use of a commercial product should this become available [5].

To conclude, it could be most beneficial to investigate the protection by Carraguard of HPV genital infection in a trial assuring high adherence to applicator use combined with a longitudinal assessment of HPV infection over a number of time points. A major limitation of the present study was that there was no baseline or intermediate measurement of genital HR-HPV infection.

Prevalence of HR-HPV was only measured at study end. No safety concerns have been recorded for the use of Carraguard. We are of the belief that Carraguard may have potential for use as an effective vaginal microbicide against HR-HPV infection.

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ALW was the principal investigator (PI), directed the HPV study and edited the manuscript; DM, carried out analysis of data and wrote the manuscript; DG carried out statistical analyses; BRA conducted HPV DNA testing; GR was the PI in Durban, collected samples and commented on the manuscript; MH developed the study design and carried out data collection; LA was PI at the University of Cape Town, developed the study design and carried out data collection; NC carried out data collection; FG carried out data collection; KA was PI in Medunsa and carried out data collection.

Disclosure statement

The authors declare no competing interests.

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