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Herpes Simplex Virus Type-2 (HSV-2) Assay Specificity and Male Circumcision to Reduce HSV-2 Acquisition

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Abstract

Three male circumcision (MC) trials which enrolled over 10,000 men of different ages, settings (urban vs. rural), countries (Uganda, Kenya and South Africa), and which utilized different surgical techniques were consistent in showing a 51–60% in reduction in HIV incidence [1–5], and all three trials reported that MC decreased high-risk human papillomavirus (HR-HPV) prevalence by 32–35% [6–8].

Keywords

Herpes Simplex Virus Type-2 (HSV-2); male circumcision; HIV; Uganda; Kenya; sexually transmitted infections

In addition, both the Ugandan and Kenyan trials showed that MC reduced genital ulcer disease by 47%–48% [1, 9]. Since the majority of genital ulcers in both men and women are due to herpes simplex virus type-2 (HSV-2) [10–12], it was hypothesized that MC also reduces HSV-2 acquisition. The Ugandan and South African trials evaluated this hypothesis and reported that MC reduced HSV-2 incidence by 25%–34% [8, 13]. Interestingly, however, Mehta *et al* did not find similar efficacy in the Kenyan trial in Kisumu [9].

All three trials utilized an enzyme-linked immunosorbent assay (ELISA) manufactured by Kalon Biological to detect incident HSV-2. However, interpretation of this assay to detect HSV-2 seroconversion can be problematic, since the index value to define a positive result may differ from the manufacturer's recommended cut off based on European and North American populations and HSV-2 viruses [14]. There are multiple reports of high false positive rates among African samples [14–18]. We and others found that a higher index cutoff value is required for optimal sensitivity and specificity [14, 16, 19]. When used according to manufacturer's instructions (index cutoff value 1.1), the Kalon ELISA had a sensitivity of 95.1% and a specificity of only 87.6% in Uganda compared to Western blot [14], whereas a Kalon index value cutoff of 1.5 resulted in a sensitivity of 91.7% and a

specificity of 92.4% [14]. Specificity increased to 97.6% with an index value of 2.5 and 98.4% with an index value of 3.5 [14]. In a validation study among men enrolled in the MC trial in Kisumu, the Kalon ELISA at the recommend manufacturer's cutoff had low sensitivity (92%) and specificity (79%) [20]. In their analysis, Mehta *et al* used the manufacturer's cutoff to evaluate the efficacy of MC to reduce HSV-2 incidence [9], a cutoff which is likely to have reduced specificity and may have biased their results towards the null.

Mehta *et al* noted that "no differences were found between circumcised and uncircumcised men at other cutoff values of 1.5, 2.0, 2.5 3.0 and 3.5 [9]." However, to determine whether improved specificity affected MC efficacy estimates in the Ugandan trial, we assessed HSV-2 seroconverters classified by higher Kalon ELISA index values which maximize specificity and reduce potential false positives. For HSV-2 seroconversion rate and person time calculations, it was assumed that HSV-2 infection occurred at the mid-time point between the last negative and first positive serological tests. Time from enrollment was accumulated to the 24 month follow-up visit or the last available sample visit, and HSV-2 seroconversions were estimated per 100 person-years. Incidence rate ratios (IRRs) and 95% confidence intervals (95%CI) were estimated using Poisson regression.

As HSV-2 assay specificity increased, MC efficacy to prevent HSV-2 infection increased, albeit modestly (Table). Thus, the previously reported 25–34% reduction in HSV-2 from the Ugandan and South African trials likely represent conservative estimates.

As Meta *et al* note in the discussion, the lack of an association between male circumcision and reduced incident HSV-2 in the Kenyan trial may be due to poor test performance. The results of the Ugandan and Kenyan trials showing reduced genital ulceration following MC and the results of the Ugandan and South African MC trials showing reduced HSV-2 acquisition appear consistent. The analysis in Table 1 suggests that higher assay specificity further strengthens the observed associations that MC reduces both HSV-2 incidence and genital ulcer disease.

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HSV-2 incidence among men in the intervention group (circumcised) and control group based on Kalon index value.

Table 1

Index cut-off value	Intervention group		Control group		Incidence rate ratio (95% CI)
	HSV-2 incident cases / person-years	HSV-2 incidence (cases per 100 pys)	HSV-2 incident cases / person-years	HSV-2 incidence (cases per 100 pys)	
1.5	116/2805.5	4.13	159/2814.25	5.65	0.73 (0.57–0.93)
2.5	59/2849.25	2.04	96/2902	3.31	0.62 (0.44–0.86)
3.5	31/2911.25	1.06	50/2937.25	1.70	0.62 (0.39–0.99)