



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	International STD Research & Reviews
Manuscript Number:	2013_ISRR_7983
Title of the Manuscript:	ONCOGENIC HUMAN PAPILLOMAVIRUS DETECTION IN PENILE LICHEN SCLEROSUS: AN UPDATE

PART 2:

FINAL EVALUATOR’S comments on revised paper (if any)	Authors’ response to final evaluator’s comments
Study design and description: the authors have confirmed that the paper includes two subgroups (46+46 patients+controls already published and the new 46+46 patients+controls), specifying in the discussion that the samples have been collected through a 19-year period and reporting the HPV data separately for the two subgroups. However, this is not clearly stated in the abstract, that should be modified accordingly (in “duration of study” and “methodology” sections). Methodology: the DNA extraction method and the evaluation of sample adequacy are now reported (were all the samples amplified by the housekeeping gene primers?); also the methods for HPV search and typing are now reported, but are not clear. Nested PCR for HPV implies the use of MY09/MY11 primers in the first step followed by the use of GP5+/GP6+ primers in the second step, while cycle sequencing is performed on the amplified product (after the first or second PCR) for genotyping. Paragraph 2.3 should be modified. The other two points raised in my first revision have been adequately addressed.	In <i>Place and Duration of Study</i> section of the Abstract, the sentence: “Dermatology Clinic, University of Catania, Italy, between January 2007 and July 2008” has been modified as follows: “The study has been performed in the Dermatology Clinic of the University of Catania, Italy, spanning a 19-year period.” At the beginning of the <i>Methodology</i> section of the Abstract, the sentence: “We previously demonstrated a high (17.4%) HPV detection rate in a study on 46 patients with genital LS. In this retrospective analysis we extended the analysis to a larger number of patients in order to strengthen these former data.” has been added as suggested. In Paragraph “2.2 – DNA extraction” (Lines 74-78) the sentence: “Sample adequacy was established by spectrophotometric analysis, and DNA quantity and integrity was monitored through amplification of part of the β-globin gene in replicate tubes.” has been changed as follows: “Sample adequacy, DNA quantity and integrity were established by spectrophotometric analysis. Each sample was monitored through amplification of the β-globin housekeeping gene.” Paragraph “2.3 – HPV detection and genotyping” (Lines 79-90) has been modified as follows: “Ten ul of DNA solution from Biopsy specimens and brushings were assayed by a highly sensitive two-steps nested polymerase chain reaction (PCR) technique based on general MY11/MY09 PCR primers and GP5+/GP6+ consensus primers, followed by cycle sequencing. In the first step, 30 cycles of DNA amplification were performed in a reaction mixture of 50 μl containing 10 mM Tris-Cl pH 8.3, 50 mM KCl, 2.5mM MgCl2, 200 μM of each dNTP, 2.5 units 1U of Taq DNA polymerase and 0.05 mM of MY11/MY09, with denaturation at 94° for 1 min, annealing at 55° for 1.5 min, and 72° for 1 min followed by extension at 72°C for 5 min. In the second reaction, 2ul of PCR product were amplified in a reaction mixture of 50 μl containing 10 mM Tris-Cl pH 8.3, 50 mM KCl, 2.5mM MgCl2, 200 μM of each dNTP, 2.5 units 1U of Taq DNA polymerase and 0.05 mM of GP5+/GP6+ denaturation at 94° for 30 sec annealing at 47° for 30 sec, and 72° for 30 sec followed by extension at 72°C for 5 min. Agarose gel of 10 ul of nested PCR product reveal visible bands in the HPV. The reaction were purified, and cycle sequencing was performed as previously described to determine the type of HPV using an automated genetic analyzer [11-12]. The homology of the consensus sequence was then compared with those of known HPV types available through the GenBank database. Statistical evaluation was performed using conditional logistic regression analysis.”