

September 11, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13170-edited.doc).

Title: Detection of HPV DNA in esophageal carcinoma, in Greece

Author: Georgios Georgantis, Theodoros Syrakos, Theodoros Agorastos, Spiridon Miliaras, Asterios Gagalis, Georgios Tsoulfas, Konstantinos Spanos, Georgios Marakis

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 13170

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

1) Reviewer comment: The authors stated in the Methods that "The study was conducted at the University Hospital and the patients came from the area of Northern Greece." This region may not reflect the incidence of ESCC nor HPV infection in the country as a whole as stated in the title of the paper. Despite this, the authors concluded that "Our data has shown that Greece is a low risk region for HPV-related cancer of the esophagus." This conclusion is not supported by the regional sample of patients that the study investigated.

Response: We would like to thank the reviewer for the thoughtful comment. Although the point is well taken, we would like to point out that a) the University Hospital acts as a referral center not only for Northern Greece but also for other parts of the country (with 20% of our patients coming from outside Northern Greece), and b) Greece is a small and rather homogeneous country, something which would make these generalizations more plausible. However, we have added in the discussion the following sentence: "Another limitation is the fact that, even though the University Hospital is a referral center for the whole of Northern Greece and also Greece is a relatively homogenous country, it does represent only part of the country and so a sampling issue could be raised. "

2) Reviewer comment: The authors should explain why they choose the primers they used in the study and provide supporting citation(s).

Response: In the methods the paragraph "DNA isolation and identification" was changed to "DNA extraction and PCR" with appropriately changed content. Also, a paragraph (highlighted) discussing this issue was added in the Discussion and 5 relevant references were added.

3) Reviewer comment: They should also provide the manufacturer's name & country in all reagents, kits, etc.

Response: These changes were made in the text as requested.

4) Reviewer comment: The authors mentioned that the biopsies were performed according to a standard protocol. The detail of this protocol should be provided in order to allow evaluation of the validity of the sampling process.

Response: The following was added to the methods section, in order to describe the biopsy protocol: "The esophagogastroduodenoscopies and the esophageal biopsies were performed by specialized gastroenterologists, who had performed at least 500 esophageal biopsies each. Areas that were considered suitable for biopsy were those that had erythema or a nodular appearance or friable tissue or ulcerations, scars or neopithelialization. The endoscope provided a high definition picture and

could also be used for therapeutic purposes, if the need arose. Special biopsy forceps (called jumboforceps) were used for the esophageal biopsies, which can take a significant amount of tissue both in terms of volume, as well as depth from areas with noted pathology. From each region, four random samples surrounding the lesion were taken in a radius of 1-2 cm."

5) Reviewer comment: The info for reference 17 is not enough to allow retrieval of this reference. The authors should provide the reference URL.

Response: As this was not a pubmed paper, a new reference is provided.

6) Reviewer comment: English language and style should be more clear and readable.

Response: Further English language and style changes were made as requested. One of the authors, Georgios Tsoulfas, is a native speaker of English and undertook the corrections.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

A handwritten signature in blue ink, appearing to read 'G. Tsoulfas', with a stylized flourish at the end.

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