

## Format for ANSWERING REVIEWERS



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7253-review.doc).

**Title: An increased expression of TNF- $\alpha$  is associated with advanced colorectal cancer stages**

**Author:**

Omar A. Al Obeed, Khayal A. Alkhayal, Ahmed A. Zubaidi, Mansoor-Ali Vaali-Mohammed, Abdulmalik Al Sheikh, Robin Boushey, James H. Mckerrow and Maha-Hamadien Abdulla

**Name of Journal:** World Journal of Gastroenterology

**ESPS Manuscript NO:** 7253

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

**Reviewer #1 comments:**

The manuscript was well prepared, yet the following items should be considered for reversion.

Majors :

1) In order to prove that “high levels of TNF- $\alpha$  expression could be an independent diagnostic indicator of colorectal cancer”, it should be better to add 30 cases of early CRC or colorectal adenomas with dysplasia.

I agree with the Reviewer, but unfortunately most of the patients in our study were admitted to the hospital in late Stage III. In the future, we plan to recruit patients for colorectal cancer screening. This will enable us to conduct the study on a far greater number of patients, and include early CRC patients.

We have established a Colorectal Cancer BIOBANK, and have begun collecting fresh tumor and adjacent tissue in order to conduct research on a greater number of patients, as well as obtain high-quality RNA for measurement of mRNA levels in colorectal patients.

2) How about the other tumor biomarkers such as serum or tissue CEA and CA 19-9? What is the co-relationship between TNF- $\alpha$  and CEA?

In this population, there is no correlation between CEA and TNF-alpha. We will consider evaluating the co-relationship between TNF-alpha and CEA in the future studies with a greater number of study participants.

Minors: 1) As Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a pro-inflammatory cytokine, there might be some difference in the expression of TNF- $\alpha$  between sporadic and colitis associated cancer, therefore, patient family history, past history with or without colitis should be included in table 1 or in section Materials and Methods (Clinical Samples). Most of the cases (90%) were sporadic.

2) There were not any data to prove that “targeting TNF- $\alpha$  might be a promising prognostic tool in clinical staging of CRC”, as the follow-up or survival data were not seen in the text.

We acknowledge that our study needs further evaluation of the relationship of TNF-alpha presence as a prognosticator of colorectal cancer stages and survival. It was very difficult to obtain this information in our group of only 30 patients because no follow-up or survival data was available.

It will be our major aim in the future to show that TNF is associated with advanced tumor stages by conducting more research on a larger population.

**Reviewer 2 comments:**

The paper concerns the assessment of the expression of TNF- $\alpha$  in colorectal cancer patients. Authors stated that high levels of TNF- $\alpha$  expression could be an independent diagnostic indicator of colorectal cancer, and targeting TNF- $\alpha$  might be a promising prognostic tool in clinical staging of CRC. Major comments:

(1) I recommend shortening the manuscript significantly (especially Introduction and Materials and Methods) as well as the number of cited literature.

I agree with the Reviewer. The Introduction and Materials and Methods sections have been shortened. Please see attached revised version with deletions.

(2) In the Abstract it is stated that “Multivariate analysis confirmed the presence of elevated TNF- $\alpha$  gene expression in cancer cells, which strongly correlated with tumor progression”. In the body of the paper I did not find any data concerning multivariate analysis. “Tumor progression” in more advanced stage or tumor progression after the treatment? The latter data are not presented.

Multivariate analysis has been deleted from the Abstract.

We were indicating that there was tumor progression in more advanced stages, not tumor progression after treatment.

(3) In the Discussion authors stated “We showed that increased TNF- $\alpha$  gene transcription and protein expression levels in late stages of tumor progression are associated with an increased tumor recurrence rate...”. Again no data concerning tumor recurrence rate were found.

The sentence has been corrected in the revised version.

“We showed the presence of elevated TNF- $\alpha$  gene expression in cancer cells, which strongly correlated with advanced stages of tumor”. See revised manuscript

(4) Figure 2 and Table 1 should be omitted and the data should be placed in the Results section.

Table 1 has been deleted (see revised version). With all due respect to the Reviewer, we think it is important to show TNF-alpha immunohistochemistry staining (Figure 2).

(5) When the groups are small it is better to use absolute numbers instead of percents.

The percentages have been changed to absolute number. Please see the revised version of the manuscript.

3 References and typesetting were corrected

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

Sincerely yours,



Maha- Hamadien Abdulla, Ph.D.

Assistant Professor, Director of colorectal research center

Department of Surgery, King Khalid University Hospital, King Saud University,

P.O.Box 7805(37), Riyadh 11472, Saudi Arabia. [mabdulla@ksu.edu.sa](mailto:mabdulla@ksu.edu.sa)

Telephone: +966-1- 467-1575

Fax: + 9661-469-2728