Author's response to reviews

Title: Soluble Fas/Fas Ligand might serve as a diagnostic tool for gastric adenocarcinoma

Authors:

Samaneh Boroumand-Noughabi (s_n_bromand@yahoo.com)
Hamid Reza Sima (simahr@mums.ac.ir)
Kamran Ghaffarzadehgan (kghafarzadehgan@hotmail.com)
Mostafa Jafarzadeh (mostafa.jafarzadeh@gmail.com)
Hamid Reza Raziee (razieehr@mums.ac.ir)
Hanieh Hosseinnezhad (hosseinnezhad.hanieh@gmail.com)
Omeed Moaven (omeedmoaven@gmail.com)
Mohammad Taghi Rajabi-Mashhadi (rajabimt@mums.ac.ir)
Amir Abbas Azarian (azarianaa1@mums.ac.ir)
Mojtaba Mashhadinejad (m.mashhadinejada@gmail.com)
Jalil Tavakkol-Afshari (tavakolaj@mums.ac.ir)

Version: 2 Date: 19 March 2010

Author's response to reviews: see over
Dear Editor,

All the authors of this manuscript and I, appreciate your reviewer’s careful reviews of our manuscript. We have prepared a revised manuscript addressing the reviewers’ questions and comments. In addition, we have also addressed every one of the reviewer’s comments below separately. I believe that considering our answers to the reviewer’s comments and all the revisions made in our manuscript, the new revised manuscript should be acceptable for publication. I appreciate your time and consideration of this matter.

Reviewer: Yue Fu

Reviewer's Comment:
1. This study is designed to verify sFas/sFasL as biomarker for early diagnosis of gastric cancer. And as far as we know, dysregulation of Fas/Fas Ligand has been proposed to play a role in the development of many kind of cancer including gastric carcinoma. Researchers have found that serum sFas levels been higher in patients with gastric carcinoma (Akiko Tamakoshi, Int. J. Cancer, 2008; Liang QL, Ai Zheng, 2002), and serum sFasL level may be useful in evaluating preoperative diagnosis and serve as a prognostic biomarker for stomach cancer (Tsutsumi S, Cancer, 2000; Lim SC, Oncol Rep, 2002). So, according to upon researches, it is pity that this manuscript is not presented sufficient innovation to meet publication criteria. (Major Compulsory Revisions)

Authors’ response: Although few studies have been previously published on this subject, following facts are noteworthy. This study has some distinguishing features compared to others. None of them have included patients in preneoplastic stages. We included healthy controls and patients in different preneoplastic stages as well so that we could deduce the applicability of this biomarker as an “early diagnostic tool”. On the other hand, despite the following reports, we have concomitantly studied sFas and sFasL. Moreover, clinicopathological significance of these markers is still controversial and has yet remained to be clarified as different reports have been published. It looks that further studies are required to better deduce the underlying fact.
As mentioned in the comment of the reviewer, following studies have been reported. A brief summery shows the differences between the current study and the published reports and highlights some controversial facts as well which emphasizes on the significance and novel aspects of the current study which introduce it suitable for publication in this journal:

**Akiko Tamakoshi**, Int. J. Cancer, 2008: They reported mean serum level of sFas in gastric cancer patients is more than controls while, as evidenced by the figure 1 in the paper, the difference was not statistically significant. Preneoplastic stages were not included in the controls and the level of sFas ligand in serum was not assessed in that study.

**Yatsuya H**, J. Epidemiol, 2005: In a nested case-control analysis they reported that sFas level in female stomach cancer cases was significantly higher than that of controls. As for controls, only healthy volunteers were included and preneoplastic stages were not included in the controls and the level of sFas ligand in serum was not assessed in that study.

**Liang QL**, Ai Zheng, 2002: They showed a statistically significant increase in serum level of sFas between gastric cancer patients and healthy controls.

Regarding serum sFasL level in gastric cancer following studies are published in the literature:

**Tsutsumi S**, Cancer, 2000: they didn’t find statistically significant difference in the serum level of sFasL between gastric cancer patients and healthy controls. No patients in preneoplastic stages were included.

**Lim SC**, Oncol Rep, 2002: the researchers showed that serum level of sFasL in gastric cancer patients is more than controls, while they did not find any relation between sFasL level and prognostic factors (grade, stage, histologic type etc).

**Yoshikawa T**, J Surg Res 2008: in this study a decrease in serum sFasL level in Gastric cancer patients than controls was found. However they have only compared 17 controls to 20 patients and the control group was consisted of only healthy volunteers and not preneoplastic stages.
**Reviewer's Comment:**

2. The conclusion that Serum level of sFas may serve as a non-invasive tool for "early" diagnosis of gastric cancer is not supported by the data well. Although the authors observed an increasing gradient for mean serum level of sFas, the difference of serum sFas level between three non-tumoral subgroups (especially precancerous lesions vs. chronic active gastritis and precancerous lesions vs. normal) had not been statistically analyzed. The claim was not convincing.

(Major Compulsory Revisions)

**Authors’ response:** The fact that we observed significant difference between cancer and noncancerous patients (either preneoplastic or chronic active gastritis) will prove the specificity of this marker for early detection of cancer. In other words the significant difference in between these two groups is sufficient for deduction and either we find significant differences between precancerous lesions vs. chronic active gastritis and precancerous lesions vs. normal or not, will not affect our observation i.e. significantly higher level of sFas in gastric cancer and thus introducing the possible applicability as an early diagnostic tool. For an early diagnostic tool, in order to be applied for the purpose, there should be a significance difference between cancer group and noncancerous patients which is observed. A large-scale study is further required to propose a precise cut-off and validate this biomarker as an early diagnostic tool.

**Reviewer's Comment:**

3. Data showed in tables and figures is not well presented.

In table 2, 3 and 4, as well as figure 1, I cannot find the exact number of the sample in each subgroup.

In table 2 and 4, the unit of the serum level of sFas/sFasL is not presented.

**Authors’ response:** The required data are added.

**Reviewer's Comment:** In figure1, the data only show the exact value of sFas in four groups, however statistical significance has not been presented in the figure.

(Major Compulsory Revisions)
Authors’ response: The figure shows mean serum level of sFas ± standard error of means and Mann-Whitney U test. P values are added.

Reviewer's Comment: 4. Since the data do not prove the conclusion in the title it may be changed to remove the words “sFas ligand”.

Authors’ response: The title is changed to: “Soluble Fas might serve as a diagnostic tool for gastric adenocarcinoma”

Reviewer's Comment:
5. Interestingly, the authors found serum level of sFasL in patients with non-cardiac type of gastric cancer was significantly higher than that in patients with cardiac type of gastric cancer. This deserves further research.

Authors’ response: The authors agree with the reviewer that this finding deserves further research in a large-scale study as a validation set, however, it requires a separate study which is beyond this manuscript.

Reviewer's Comment:
6. Table 2: “meam” should be changed to “mean”.

Authors’ response: The change was performed.

Reviewer's Comment:
7. Table 1, actually, is not necessary.
The data showed in the table is not relative to the purpose of the investigation.

Authors’ response: The table is removed based on the comment of the reviewer.
Reviewer: Kostas SYRIGOS

Reviewer's report:
The authors evaluate the clinical significance of soluble Fas as a diagnostic marker of early gastric carcinoma. They demonstrated that patients with adenocarcinoma have increased serum sFas, compared to healthy individuals and patients with non-malignant situations. The study is well designed and the paper well written. Nevertheless the authors should address the following points:

-Reviewer's comment: Similar studies in other malignancies have been negative

Authors’ response: Different results have been reported in different malignancies including both positive and negative reports. Following brief reports represent this broad spectrum.

El-Sarha AI et al, Pathol Oncol Res. 2009: They showed that serum sFas level in breast cancer patients is significantly more than healthy controls and has a negative correlation with survival.

Pignataro L et al, J Surg Oncol. 2003: The researchers did not find a significant difference in serum level of sFas between Laryngial carcinoma and healthy control groups.

Ugurel S et al, Clin Cancer Res. 2001: In this study an increase in sFas level between melanoma patients and healthy controls was found. Serum sFas level was related to poor prognosis.

Akhmedkhanov A et al, BMC Cancer. 2003: They found that serum sFas levels were similar in women subsequently diagnosed with ovarian cancer and in controls and conclude that serum sFas may not be a suitable marker for identification of women at increased risk of ovarian cancer.

Kondera-Anasz Z et al, Apoptosis. 2005: In this study significant increases of the mean value of sFas and sFasL were found in the serum of women with uterine tumor compared to the control group. The mean levels of these parameters increased in consecutive stages of the clinical extent of the uterine cancer (I-III).
**Furuya Y et al., Endocr J. 2003:** The researchers showed that serum sFas levels in patients with metastatic prostate cancer were significantly higher than that of control patients with benign prostate hyperplasia. Patients with low levels of serum sFas had a higher survival rate and compared with patients with high levels of serum sFas. They concluded that sFas levels might be associated with poor prognosis in metastatic prostate cancer.

**Reviewer’s comment:** Controls are of younger age compared to patients (50 vs 65). This might hamper the statistical significance.

**Authors’ response:** Although there were differences between the age of cases and controls, there are evidences that support the fact that the level of sFas is not affected with age or sex and this difference does not affect the results of our studies:


**Reviewer’s comment:** Patients should be evaluated for co-morbitidies that influence the serum levels of sFas, such as renal and cardiac failure.

**Authors’ response:** patients with these co-morbid conditions have been excluded and none of the enrolled patients and controls had any other serious disease at the time of obtaining the specimens
Reviewer: Wataru Ichikawa

Reviewer's comment:
#Major 1
The authors indicated that the serum Fas is "statistically" higher in gastric cancer group as compared with non-tumoral group. However, in the cancerous group, the serum Fas decreased according to lymph node involvement. It seems to be strange. The authors should discuss this observation.

Authors’ response: The following part is added to address the concerns of the reviewer.
We observed a lower serum level of sFas in patients with lymph node involvement. When tumor involves lymph nodes, antitumour immunity will be provoked [33] which may result in production of more Fas bearing immune cells and subsequently sFas may be consumed more following binding and neutralizing these Fas receptors.

Reviewer's Comment:
#Major 2
Although the descriptive characteristics of gastric adenocarcinoma and non-tumoral groups are quite different with the exception of opium addiction (Table 2), the authors compared the serum Fas/Fas ligand among two groups. This small cohort including 112 samples could not resolve the problem of co-existing factors to affect the serum Fas/Fas ligand. If the authors would clearly indicate the positive associations of soluble Fas/Fas ligand and gastric carcinogenesis, more number of samples should be collected to perform the multi-regression analysis.

Authors’ response: Although the descriptive characteristics of gastric adenocarcinoma and non-tumoral groups are different, this difference reflects the reality since these parameters are the risk factors of gastric cancer. In order to test the biomarker your patients and controls must be representatives of the population and these differences are expected to be observed. On the other hand, analysis of controls with positive risk factors without including those who do not have the risk factors still reproduce same result with statistical differences as well. Although increasing
the samples size will definitely increase the statistical power leading to a stronger conclusion, we think that the current sample size is large enough for a preliminary introduction of this biomarker as other reviewers have not been addressed this problem either. With no doubt, further studies with a larger sample size will be helpful for approving this finding.

**Reviewer's Comment:**

#Minor 1 Table 2 is missed.

**Authors’ response:** No, it was attached as an additional file.

**Reviewer's Comment:**

#Minor 2

The information for patient characteristics should be described more in detail. For example, numbers of patients should be indicated for each characteristics (Table 1, 3, 4, and Figure).

**Authors’ response:** The required details are added.

- **Reviewer:** Stamatios Theocharis

**Reviewer's comment:** The manuscript of Boroumand-Noughabi et al, clearly describes alterations of soluble FAS/FASL between gastric cancer and gastritis samples. Some important alterations with clinicopathological parameters have been achieved. The work is well organized and the methodology is appropriate. Nevertheless, the article remains of minor clinical importance as there is no correlation with tissue FAS and FASL expression. It is important the authors to provide immunostainings of FAS and FASL expression in the same tissue samples. After that the paper should be accepted for publication. Typos throughout the manuscript must be corrected.

**Major Compulsory Revisions**
Authors’ response: Although performing IHC analysis of Fas and FasL will add to the scientific value of the manuscript and better underlying pathological correlations may be provided and the power of conclusion may be increased, the authors believe that the principal message of this study, applying this non-invasive biomarker as an early diagnostic tool, is informative enough, even without providing IHC analysis. Therefore, although we agree that IHC analysis is a useful addition to this manuscript, due to our limitations it is not possible to be added at a short time while the current design and method is enough conclusive.

Typos throughout the manuscript were corrected.

Sincerely,

Hamid Reza Sima, MD
Director, Gastroenterology and Hepatology fellowship
Assistant professor of Medicine,
Division of GI and Liver Diseases,
Mashhad University of Medical Sciences,
Mashhad, Iran
Tel: (511) 844-6156
Fax: (511) 859-8818
Email: simahr@mums.ac.ir