Reviewer's code: 02575643

COMMENTS TO AUTHORS

THE SAME COMMENTS MADE TO THE EDITORS

RESPONSE:

We thank the reviewer for his/her effort and favorable comments.

Reviewer's code: 00058573

COMMENTS TO AUTHORS

Good study. Few queries 1. Why were subjects with liver diseases and cardiovascular diseases excluded from the study? Please provide explanation 2. It would have been better if the CONTROLS were taken from normal population and not from high risk zone. 3. ESCC is being used in abstract without giving its full version.

RESPONSES:

We thank the reviewer for his/her effort and favorable comments.

1 Some risks maybe happen when patients with liver diseases and cardiovascular diseases accept esophageal endoscopy examination, and we carried out the endoscopic screening test in villages where the equipment conditions for medical emergency were not satisfying, so in this study we excluded these subjects.

2 Iodine staining endoscopic screening is suitable for high-incidence area of esophageal cancer, but it is difficult to obtain enough samples in low-incidence area. And in this study, all esophageal diseases and normal controls were diagnosed by histopathology, so the possibility of misdiagnosis was very small. In addition, all subjects lived in similar

environment, so the data obtained from them is comparable.

3 To keep insistence with other content, we changed "ESCC" to "invasive cancer" in the abstract.

Reviewer's code: 03475059

COMMENTS TO AUTHORS

Summary "Predicting malignant direction of esophageal squamous cell lesions by combined biomarkers in endoscopic screening program" revealed combined assessment of p53, CEA and CA19-9 could predict the malignant potential of esophageal lesions. The concept of the study is clinically relevant, but there are several questions. Major comments 1. The specificity of all lesions was 88%. Is it correct? Since the sensitivity was different among these lesions, the specificity should be different. 2. Why was school year or per capita income related to esophageal lesions? 3. Positive rates of p53 and CEA were significantly related to age. Is there still relationship between these biomarkers and esophageal lesions when age is adjusted? 4. There are several biomarkers relating to squamous cell carcinoma such as CYFRA. Why did you choose three biomarkers? 5. Stained biomarker in the biopsy specimens may be different from detected biomarker in the serum. The concentration of these biomarkers may be too low to detect in the serum, but it is better to show it. This data can indicate one of limitations in this study. 6. As you noted, the positive rates of biomarkers in normal specimens were too high. If iodine staining was used, it should be easy to take biopsy from normal esophageal mucosa. What kind of endoscopy system was used? In addition, are doctors who performed endoscopy well-trained endoscopists? Minor comments 1. There are a □ several times in page 6. This should be corrected. 2. "P<0.01" should be "p<0.01". "p" should be a small character.

RESPONSES:

We thank the reviewer for his/her effort and favorable comments.

1 We calculated the sensitivity, specificity, and +PV by comparing the four disease groups with normal control group, but the specificity was only related with the positive(n=9) and negative (n=71) result in the normal control group, so the specificity of all lesions was same. But we still thank the reviewer for his/her favorable suggestion, and we made the following modifications:

(1)Replaced "In the ROC curves of the combination of the three biomarkers, the sensitivity and specificity were 58.2% and 88.8% for invasive cancer, 25.5% and 88.8% for HGD, 11.2% and 88.8% for LGD, and 6.5% and 88.8% for BCH, respectively" with "In the ROC curves of the combination of the three biomarkers, the specificity was 88.8% for the normal controls, and the sensitivity was 58.2% for invasive cancer, 25.5% for HGD, 11.2% for LGD, and 6.5% for BCH" in the "results" of the "ABSTRACT".

(2)Replaced "In the ROC curves of the combination of the three biomarkers, the sensitivity and specificity were 58.2% and 88.8% for the ESCC group, 25.5% and 88.8% for the HGD group, 11.2% and 88.8% for the LGD group, and 6.5% and 88.8% for the BCH group, respectively" with "In the ROC curves of the combination of the three biomarkers, the specificity was 88.8% for the normal control group, the sensitivity was 58.2% for the ESCC group, 25.5% for the HGD group, 11.2% for the LGD group, and 6.5% for the BCH group " in the last paragraph of the "RESULTS".

(3)Deleted the columns of "specificity" and its 95%*CI* in the Table 6 and added the following content in the footnotes: the specificity was 88.8% for the normal control group, with a 95%*CI* of 79.7%-94.7%.

2 We found the school year or per capita income was related to esophageal lesions, such

as compared with the normal control group, the ESCC group accepted less education and lower income. The result was consistent with those in many other high-incidence areas of esophageal cancer. Maybe those who accepted less education knew less about the prevention of esophageal lesions, and those who had lower income were influenced by more risk factors, such as eating more salty food.

3 Positive rates of p53 and CEA were significantly related to age. There is still relationship between these biomarkers and esophageal lesions when age is adjusted. We gave the *ORs*(95%*CIs*) of p53, CA19-9 and CEA protein positive expressions with BCH, LGD, HGD and ESCC after adjusted for age and other significant influencing factors in Table 4.

4 In recent years, some reports showed that the over survival (OS) of the patients with high CYFRA 21-1 levels was worse than that of those with low CYFRA21-1 levels (P = 0.001). In multivariate analysis, a low level of CYFRA21-1 was the most significant independent predictor of good OS (P = 0.007). It is not clear whether the CYFRA21-1 level changed in the early stage of the progression of ESSC in our study period. Meantime, we detected many biomarkers, including CYP1A, CA19-9, MPO, Ki-67, CEA, p53, ALDH2(Aldehyde dehydrogenase), Cyp2E1, SCCA2 and MTHFR. From above biomarkers, we found the three ones (p53, CA19-9 and CEA) were related to the ESSC.

5 Stained biomarker in the biopsy specimens may be different from detected biomarker in the serum, and the concentration of these biomarkers may be too low to detect in the serum. We mentioned this limitation in the "DISCUSSION":

Another study reported that CEA mRNA was expressed in the blood, even though tumor markers CEA and CA19-9 were within the normal range in patients with

relapse[45]. From above studies we calculate that in the serum of patients with early stages of carcinoma the level of CEA or CA19-9 protein expression may be too low to be detected. Opposed to this, CEA or CA19-9 protein expression in the biopsies of the initial steps of esophageal carcinogenesis can be easily detected by immunohistochemistry. In the present study the main discovery was that the positive expressions of CEA and CA19-9 proteins increased along the axis of BCH, dyplasia and carcinoma of esophagus. The results are useful to deeply comprehend the mechanism of the evolving process of esophageal cancer from the molecular level of protein expression.

6 It is true that the positive rates of the three biomarkers in normal specimens were high in this study. In the endoscopic screening examination, for subjects with a non-staining area of the mucosa, the biopsy specimens were obtained and the histopathology diagnosis was carried out by two pathologists. So the specimens obtained from the control group in this study may be different with the biopsies obtained from normal subjects who had normal iodine staining mucosa. The high positive frequency of the biomarkers in the control group may be due to this.

We used Olympus1T140 electronic gastroscopy, Japan.

Doctor Zhou Rui-xue got the technical training of endoscopic examination for one year in the Department of Digestion，Cancer Institute & Hospital, Chinese Academy of Medical Science. Professor Wang Guo-qing from the Department of Digestion，Cancer Institute & Hospital, Chinese Academy of Medical Science gave on-the-spot guidance of gastroscopy.

RESPONSES TO OTHER COMMENTS:

We thank the reviewer for his/her effort and favorable comments.

1 We did not find the □ in the paper, and we calculated that maybe it is related to the browser.

2 We read the newly published paper of WJG and found that the *P* is capital and italic. We have revised it and other statistical symbols according the format of WJG.