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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5413-revised.doc).

Title: Risk factors associated with Barrett's epithelial dysplasia

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Name of Journal: *World Journal of Gastroenterology*

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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
Reviewer1
Review report ESPS Manuscript Risk factors associated with Barrett's epithelial dysplasia by Mikiko Fujita, Yuri Nakamura, ,Saeko Kasashima, Ryoichi Misaka, and Hikaru Nagahara

The authors present a case series of patients with Barrett's esophagus. The presented analysis is focused at identifying risk factors of progression towards higher dysplasia grades and cancer. A total of 151 patients were enrolled, but the presented analyses focus on 65 patients with specialized columnar epithelium as these patients had most abundant P53 expression. The authors find that presence of H pylori and higher diastolic blood pressure is associated with lesser dysplasia, while P53 expression is associated with more dysplasia. The associations between H. pylori, P53 and dysplasia have been reported earlier, while the observed association between low diastolic blood pressure and dysplasia has not been previously reported. In contrast, earlier reports have associated different aspects of the metabolic syndrome to progression of dysplasia towards malignant transformation. The manuscript is easily read, with only minor typos and grammatical errors. However, there are several concerns to be addressed, and the manuscript is not recommended for publication in its present form. Please see below for details.
Major compulsory revisions
1. The concept of predicting disease progression without prospective data is problematic. Some follow-up data are presented in the discussion, but clearly the presented dataset can only discuss associations, and not prediction of prognosis (not to confuse with the statistical term "predictor").

We agreed this comment. The most of retrospective studies just show the association between factors and incidents, and the well-designed prospective studies can identify factors related to disease progression. We would like to identify the high risk group progressing to esophageal adenocarcinoma which will be common in Asian countries because of decrease of *H. pylori* –infected population and easy eradication of the agent. Thus, this study gave us the information about factors associated with change of Barrett epithelia from dysplasia negative to positive. Further study such as prospective studies is necessary to establish a high-risk group or identify risk factors related to Barrett adenocarcinoma. We changed the paragraph of " In conclusion……." in p 20 line 13.

2. The regression analysis is not presented optimally. The authors start by doing univariate analyses of several possible predictors. Often a somewhat higher critical P (for instance P<0.1) is used for picking candidate predictors for the multivariate analysis. With a critical P of 0.1, sex, H. pylori, P53, body weight, diastolic BP and hypertension should be entered in the multivariate analysis. When the multivariate regression is run, the model should be successively reduced to only include significant predictors (P<0.05). In conclusion: in the presented analysis negative H. pylori status is in fact NOT an independent predictor for dysplasia as the variable is not significant in the multivariate model. However, the loss of effect in the multiple regression model may depend on an interaction with one of the other model variables – this should also be explored. Finally, the model evaluation characteristics should be reported as well (model P and R2 equivalent).

We analyzed 26 factors including anthropometric, biochemical and pathological data shown in Table2. Firstly we performed univariate analysis and then multivariate analysis. In order to perform multivariate analysis about 10 samples are necessary to analyze one variable, hence 6~7 variables were used for one multivariate analysis because of the size of our study population (65 subjects). The first round of multivariate analysis was performed using *H.pylori* infection, p53, Body Weight, Diastolic BP, Hypertension and Gender, all of which p-values were less than 0.1. On the next round of analysis, TC, LDL-C, Diabetes, Systolic BP, Dyslipidemia and Fatty Liver, which p-values were less than 0.4, were employed. On the third step, we performed multivariate analysis using *H.pylori* infection, p53, diastolic BP and three variables selected from the second round analysis. Finally the variables with statistically significant variance, that is, p53, *H.pylori* infection and diastolic BP were only selected from the first round multivariate analysis.

The p-value of *H.Pylori* infection was 0.066, not less than 0.05, but its odds ratio was 0.187 that seemed to give a relatively strong effect on the association with Barrett epithelial dysplasia. Thus, we included it in the Table3. Multivariate analysis can reveal the interaction among variables, so if some variables, which p-value are not less than 0.05, are still retained at the higher position after analysis they may have an effect on the association between variables and the incident.

According to the reviewer's suggestion, we added this content into p13 line9.

Minor revisions

3. Abstract: The authors use the term "risk factor" for predictors with OR below 1 (H. pylori and diastolic blood pressure). This qualifies for being protective against dysplasia, and should not be termed a risk factor.

We presented that "negative *H. Pylori* infection and low diastolic pressure are risk factors", but this expression may give confusion, so we corrected as follows; Overexpression of p53 are risk factors associated with dysplasia of BE, however, *H. pylori* infection and diastolic BP inversely associated with dysplasia of BE might be protective to BE dysplasia.

4. Introduction: p 4: the abbreviation MS must be defined.

We gave the abbreviation for MS, Metabolic syndrome, in p4 line 9.

5. p 5 line 10 typo: *decrease

We corrected, "decreased" to "decrease".

6. p 5 second paragraph: the statement "…extremely high incidence…" does not seem to be backed up by references 29 and 30.

We replaced Ref 29 and 30 to new one.

7. Introduction: last paragraph, second sentence must be restructured.

We corrected the paragraph to the following;
"Thus, it is important to determine how BE progresses to dysplasia and adenocarcinoma and to identify the type of BE patients who may have a possibility of malignant transformation in SCE."

8. Study population: were the patients enrolled in a consecutive series, and if so, over how long time (which years) did the enrolment take place.

As reviewer's comment, the patients analyzed in this study were enrolled in a consecutive series. We added the paragraph "enrolled in a consecutive series from April 2004 and March 2008" in p 6 line 10.

9. H. pylori infection: the authors used an array of different methods for detecting H. pylori - how were the results interpreted if different methods yielded ambiguous results? The algorithm should be stated.

If there was no evidence of *H. pylori* infection in pathological specimen, we confirmed *H. Pylori* negative by combination of serum HP specific antibody test with 13C-urea breath test or *H. pylori* antigen test in the stool. We added this statement in p 9 line14.

10. In table 4 there seems to be a non-linear relationship between diastolic blood pressure and dysplasia. Do we have an interaction here? This should be explored.

The diastolic BP gave us the statistically significant difference between dysplasia and non-dysplasia. We interpreted the non-linear relationship of diastolic BP among three groups shown in Table 4 as follows: If the variables were associated with malignant potential of Barrett epithelia it may show the linear relationship among three groups, normal, low grade and high grade dysplasia. The diastolic BP, however, may be associated with the step such as columnar epithelial metaplasia relatively early step of transformation. We added this statement in p19 line 14 in discussion section.

11. As none of the blood samples yielded any interesting results, they could either be omitted completely from the manuscript, or reduced to a couple of sentences in materialts/methods and results. Table 2 could be considerably smaller.

We agreed the reviewer's comment, so we reduced contents about blood samples on p11 line 1. We keep the paragraphs about definition of MS.

12. Table 4 does not add to the conclusions of the regression analysis and could

Table 4 gave us the results of analysis of variance among three categories, so it is not the results from regression analysis as reviewer's comment. Hence, we corrected the paragraph of p19 line15 to that; When we analyzed the relationship variables among three categories such as patients with no dysplasia, low- or high- grade dysplasia by analysis of variance, we detected no difference in diastolic blood pressure (Table 4).

Reviewer 2

The authors showed the risk factors associated with Barrett's epithelial dysplasia. They enrolled 151 patients with BE in a single arm hospital. This study was well-organized and well investigated. They clearly showed that p53 expression, absence of Hp infection and low diastolic BP are risk factors associated with dysplasia of BE. To improve the quality of this paper, the authors should revise it according to the following suggestions;

1) To investigate the role of BP, the authors should analyze the use of
anti-hypertensive drugs, especially Ca antagonist among patients.

We analyzed anti-hypertensive drugs in patients. 23 out of 45 (51.1%) in dysplasia (+) subjects and 9 out of 20 (45%) in subjects with no dysplasia took anti-hypertensive drugs. In addition Ca antagonist user were 14/45 (31.1%) in dysplasia (+) and 6/20 (30%) in no dysplasia subjects. Hence, there was no statistically significant difference in anti-hypertensive user, especially Ca antagonist in two groups. We mentioned these results in p19 line10.

2) To investigate the role of gastric acidity or gastric atrophy, the authors should analyze the grade of endoscopic atrophy classified by Kimura-Takemoto.

We analyzed the degree of atrophy of the stomach. The ratio of atrophy (+):(-)=8:12 in dysplasia, 30:15 in normal, respectively (p=0.58). Furthermore we classified the degree of atrophy according to Kimura-Takemoto' classification; O-III, O-II, O-I C-III, C-II, C-I and the number of patients in each categories were 0,0,1,4,2,1 in dysplasia and 8,1,0,6,10,5 in normal, respectively (p=0.15). We presented these results in discussion section, p18 line 13 and also added the Kimura-Takemoto's paper in references.

3 References and typesetting were corrected

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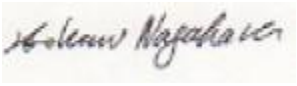

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