Editor's Note: Thank you for the opportunity to review this manuscript. I have made comments and asked questions in **BOLD**. I have also suggested some revisions, which are shown in **BOLD** script. Some sections/terms in the original text are underlined and are followed by comments and suggested modifications. Material to be deleted is indicated by STRIKETHROUGH script (i.e. ~~strikethrough~~).

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In addition to the comments and modifications made in the text, I have also included general comments below.

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General Comments:

Note that I found it preferable to use the abbreviation "BE" in titles/figures. This may need to be changed depending on the target journal's format requirements.

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ADDITIONAL COMMENTS (IF ANY…)

Risk factors associated with Barrett's epithelial dysplasia

Running title: Risk factors for Barrett's epithelial dysplasia

Key words: Barrett's esophagus, adenocarcinoma, *H. pylori*, P53, diastolic blood pressure

ABSTRACT

Background: **Risk factors for the progression of** Barrett's esophagus (BE)**,** ~~is~~ a pre-malignant condition, ~~however, risk factors progressing~~ to epithelial dysplasia and ~~subsequently to~~ adenocarcinoma remain unknown. **[NOTE: UNKNOWN ARE NOT WELL CHARACTERIZED?]** Since most ~~of~~ **cases of** Barrett's esophagus in Japan **are short segment type BE (SSBE) rather than** ~~is not~~ long segment type BE (LSBE)**,** ~~but short segment type BE (SSBE),~~ **the aim of** ~~our aim of~~ this study **was** ~~is~~ to elucidate risk factors **for progression** ~~progressing from~~ SSBE to dysplasia.

Methods: **Patients enrolled in the study (N**=6324**)** ~~patients from 2004 to 2008 were~~ underwent endoscopic examination **between 2004 and 2008**~~,~~ and **a diagnosis** ~~patients with~~ **of** BE **confirmed** ~~proven~~ **from** ~~by~~ biopsy specimen ~~were enrolled in this study~~. Physical **examination results** ~~record~~ and biochemical data were analyzed to identify risk factors **for progression** ~~progressing from BE to dysplasia~~. ~~In addition, we investigated the~~ **The** prevalence ~~rate~~ of *Helicobacter pylori* (*H. pylori*) infection and the expression of p53 by immuno-histological staining **were also investigated**.

Results: **A total of** 151 ~~Barrett's~~ **BE** patients were enrolled in ~~this~~ **the** study.

Histological examination classified patients into ~~three~~ **3** types, a specialized columnar epithelium (SCE) type (**n**=65 ~~patients~~), a junctional type (**n**=38 ~~patients~~) and a gastric fundic type (**n**=48 ~~patients~~). The incidence ~~rate~~ of dysplasia or adenocarcinoma from **BE of the** SCE type ~~of BE~~ was significantly higher than that of **the** other **2** ~~two~~ types (p<0.01). **Multivariate logistic analysis showed that** **o~~O~~**verexpression of p53 (OR=13.1, p=0.004), absence of *H. pylori* infection (OR=0.19, p=0.066), and **low** diastolic blood pressure (BP) (OR=0.87, p=0.021) were ~~identified as~~ independent risk factors **for** ~~associated with~~ ~~Barrett's epithelial~~ **epithelial** dysplasia **in BE patients** ~~by multivariate logistic analysis~~

Conclusions: Overexpression of p53, absence *H. pylori* infection**,** and low diastolic BP ~~were~~ **are** risk factors associated with progression of SSBE to dysplasia.

INTRODUCTION

Barrett's esophagus (BE) is defined as a condition in which normal squamous mucosa is replaced by columnar epithelium. This intestinal metaplasia of the distal esophagus is considered ~~as~~ **to be** a pre-malignant condition where metaplasia may progress to dysplasia and subsequently to adenocarcinoma [1, 2]. **BE** ~~Barrett's esophagus~~ is generally ~~accepted~~ **regarded** as a complication of chronic and severe gastroesophageal reflux disease (GERD). Elevation of the intra-abdominal pressure by obesity is a factor contributing to GERD**,** suggesting **that** obesity **is** ~~of~~ a risk factor for **BE** ~~Barrett's esophagus~~ [3]. Recently, a **constellation** ~~number~~ of lifestyle-related diseases, which together **comprise** ~~are recognized as~~ **the condition known as** metabolic syndrome (MS), ~~have~~ **has** received **increased** attention in Japan due to dietary changes **among the general population**, such as an increase in the consumption of fatty foods and alcoholic beverages. GERD and **BE** ~~Barrett's esophagus~~ **appear** ~~seem~~ to be ~~a~~ MS-related complication**s**, **given that** ~~because of~~ ~~relationship between~~ waist circumference, obesity**, and** ~~or~~ body mass index (BMI) **are associated with** ~~and~~ GERD [4-9].

Moreover, *Helicobacter pylori* (*H. pylori*) infection may play a key role in suppression of **BE** ~~Barrett's esophagus~~. Two main inhibiting roles for

development of Barrett's esophagus have been postulated in *H. pylori* infection; *H. pylori*-induced atrophic gastritis resulting in less gastric acid secretion and neutralization of the gastric acid by ammonia produced by *H. pylori* independent**ly** of gastric atrophy. Cag-A positive *H. pylori* infection is strongly associated with **a reduced** ~~reduction of~~ risk~~s~~ of esophageal adenocarcinoma, **and the** ~~whereas this~~ association **is** ~~was~~ independent of gastric atrophy**,** suggesting **the involvement of** a mechanism other than ~~a~~ **reduced** ~~less~~ acidic gastric reflux [3, 10]. ~~Although~~ **In Japan,** the prevalence ~~rate~~ of *H. pylori* is ~~descending~~ **declining** and ~~its eradication~~ **it can be** ~~is~~ easily **eradicated** ~~performed in Japan,~~ **; however,** it remains uncertain whether **the** incidence **of BE** ~~rate of Barrett's esophagus~~ will increase or **decreased** ~~not under condition with~~ **as a consequence of the** low prevalence ~~rate~~ of *H. pylori* infection [11, 12].

~~Barrett's esophagus~~ **BE** is **characterized by** ~~composed of~~ 3 types of columnar epithelium, namely cardiac type (junctional type), fundic type, and intestinal metaplasia type (specialized columnar epithelium type, SCE type). It has been shown that **there is an extremely high incidence of** adenocarcinoma in the distal esophagus ~~arises~~ **arising** from SCE in **patients with** ~~Barrett's esophagus with an extremely high incidence rate~~ **BE** [13].

~~Barrett's esophagus~~ **BE** is classified **as** ~~into~~ **either** long segment type (length≥3cm) ~~and~~ **or** short segment **type** (length≤3cm) ~~type~~. Barrett's esophagus in western countries means long segment Barrett's esophagus (LSBE), whereas short segments Barrett's esophagus (SSBE) is common in Japan. **[NOTE: WERE YOU REFERRING TO HOW IT IS DEFINED OR JUST WHICH TYPE IS MOST COMMON. I THINK YOU MAY HAVE MEANT: "In** W**estern countries, long segment Barrett's esophagus (LSBE) is most prevalent, while short segment Barrett's esophagus (SSBE) is most common in Japan."]** Hence, **in Japan,** dysplasia ~~or~~ **and** adenocarcinoma **arising** ~~derived~~ from LSBE is very uncommon, ~~whereas these arising~~ **but the incidence of cases arising** ~~from~~ SSBE are **steadily** ~~gradually~~ increasing ~~in Japan~~ [12].

A number of studies have shown that most ~~of~~ patients with ~~Barrett's esophagus~~ **BE** do not progress to cancer, **although some** ~~but~~ do ~~some cases~~ [14-16]. Thus, it is important to determine how **BE** ~~Barrett's esophagus develops~~ **progresses** to dysplasia and adenocarcinoma ~~or~~ **and to identify** ~~what~~ **the** type of **BE** patients ~~with Barrett's esophagus complicate~~ malignant transformation in SCE. **[GOAL IS UNCLEAR HERE. WAS IT TO DETERMINE *INCIDENCE* OF MALIGNANT TRANSFORMATION IN SCE CASES? *RISK FACTORS*?**

***MECHANISM*?]** It has been reported that central adiposity, metabolic syndrome, ~~or~~ **and** high body mass index (BMI) are associated with **BE** ~~Barrett's esophagus~~ and adenocarcinoma [4-10, 13]. In this paper, we present risk factors associated with ~~progressing~~ **progression** of **BE** Barrett's esophagus from non-dysplasia to high-grade dysplasia ~~including~~ **and** adenocarcinoma.

METHODS

Study population

A total of 151 patients (105 **m**~~M~~ale, 46 **f**~~F~~emale) **with** histologically-diagnosed **BE** ~~as Barrett's esophagus~~ were enrolled ~~in the present study~~. They were chosen by endoscopic findings and confirmed their Barrett's esophagus by histology among 6324 patients **[NOTE: UNCLEAR. WERE THE 151 OUT OF 6324 THE PATIENTS IN WHICH BE HAD BEEN CONFIRMED BASED ON ENDOSCOPIC AND HISTOLOGICAL FINDINGS?]** who underwent endoscopic examination **between** ~~from~~ ~~Mar.~~ **March** 2004 to ~~Apr.~~ **April** 2008 at Xxxxx Hospital, Xxxxx Xxxxx Xxxxx University. Patients who had received antibiotics, proton pump inhibitors, steroids, or non-steroidal anti-inflammatory drugs ~~(NSAIDs)~~ were excluded from **the** ~~this~~

study. Patients were also excluded if they had peptic ulcer, underwent partial gastrectomy, consumed alcohol excessively, or had morbid diseases such as liver cirrhosis and uremia. Written informed consent was obtained from all patients.

Endoscopic examination

~~Barrett's esophagus~~ **BE** was diagnosed **based on** ~~by~~ endoscopic findings **of** ~~when~~ gastric-appearing mucosa or apparent columnar lined esophagus ~~was evident~~ proximal to the esophagogastric junction. The esophagogastric junction was defined as the pinch at the end of the tubular esophagus coinciding with the proximal margin of the gastric folds of the hiatal hernia. **SSBE was defined as an** ~~Length of Barrett's~~ epithelium **length** less than 3 cm ~~was referred to as short-segment Barrett's esophagus (SSBE)~~ and ~~longer than 3 cm as long-segment Barrett's esophagus (~~LSBE~~)~~ **as an epithelium length greater than 3 cm, as described previously** ~~according to the previous report~~ [12].

When abnormal columnar mucosa **characteristics**~~,~~ such as erosion**s**, red flare**s**, elevated region**s**, or mucosal break**s** ~~was~~ **were** observed between the proximal limit of the gastric folds and squamous epithelium, we **detected metaplastic change by** ~~performed~~ chromoendoscopy ~~to detect metaplastic change~~

~~by~~ **and** staining mucosa with crystal violet (Fig.1). ~~The~~ **For chromoendoscopy** ~~procedure of chromoendoscopy with crystal violet~~ ~~was as follows;~~ 200,000 units of pronase (Pronase MS; Kaken Pharmaceutical Co., Matsumoto, Japan) dissolved in 300 ml of warm water ~~were~~ **was** sprayed around the esophagogastric junction area with a spray-tube, and then a 0.03-% solution of crystal violet was applied on the same area. A few minutes later ~~after spraying the dyes~~, the sprayed area was washed thoroughly with water. When **the mucosa showed a** tubular or villous pit pattern, ~~a~~ **which is** typical ~~mucosal pattern (pit pattern)~~ of SCE in ~~Barrett's esophagus~~ **BE**~~,~~ was observed in the esophagogastric junction, we performed a targeted biopsy **in that** ~~from the~~ area [17]. ~~Barrett's esophagus~~ **BE** was confirmed by histological findings ~~of~~ **from** biopsy specimens in all patients.

Histology

All biopsy specimens were fixed in formalin, embedded in paraffin, sectioned, mounted on slides and then stained with hematoxylin and eosin ~~by means of~~ **using** standard techniques. Dysplasia was classified into **3** ~~three~~ grades~~,~~ -- mild, moderate and severe -- according to the guidelines of **t**~~T~~he Vienna classification **system** ~~of the~~ **for** gastrointestinal epithelial neoplasia [18]. To

perform ~~the~~ immunohistological staining of p53, an anti-human p53 antibody (DO-7 mouse monoclonal antibody, IR616, Dako, Denmark) was used according to the manufacturer's protocol. The expression level of p53 protein **was** ~~were~~ determined and graded ~~into three level,~~ based on the intensity of nuclear staining in columnar cells~~,~~ as follows**:**~~;~~ ~~(-),~~ no staining (-), ~~(+),~~ ~~the~~ positive nuclear staining in 5**%** to 10% **of** cells (+), and ~~(++),~~ ~~the~~ positive nuclear staining in more than 10% **of** cells **(++)**, **according to the** ~~along with the criteria~~ ~~by Rajesh N.~~ **of** Keswani et al. [19] (Fig.2). **All biopsy specimens were examined by a** ~~The single~~ **an** experienced gastrointestinal pathologist ~~examined all biopsy specimens~~.

***H. pylori* infection**

The presence of gastric *H. pylori* was **determined** ~~proved~~ based ~~up~~on the results of Giemsa and/or Steiner's silver staining in ~~the~~ **a** minimum of 3 gastric surveillance biopsies (~~one~~ **1** obtained from **the** antral greater curvature, ~~one~~ **1** from **the** greater curvature of **the** mid to distal body, and **1** ~~one~~ from **the** lesser curvature in **the** proximal body). ***H. pylori* colonization was assessed by an** ~~The single~~ experienced pathologist~~s~~ blinded to **the** clinical data ~~determined *H. pylori* colonization~~. ~~The~~ **P**~~p~~atients who were not confirmed *H. pylori* infection by using

above histological analysis were further confirmed by **[NOTE: UNCLEAR. DID YOU MEAN THAT PATIENTS WHO TESTED NEGATIVE FOR *H. PYLORI* INFECTION BASED ON HISTOLOGICAL RESULTS ANALYSIS WERE RETESTED USING OTHER METHODS AND CLASSIFIED AS INFECTED IF THOSE RESULTS WERE POSITIVE]** measurement of *H. pylori* antibody test, 13C-urea breath test**,** or *H. pylori* antigen test in the stool.

Anthropometry and blood pressure

The dody w~~W~~eight**s of patients, while not wearing heavy outdoor clothing or shoes,** was measured to the nearest 0.1 kg using a digital scale~~, without heavy outdoor clothing or shoes~~. Height ~~was measured~~ **(**barefoot**) was measured** using a portable stadiometer. Waist circumference was measured to the nearest 0.1 cm using a plastic tape just above the umbilical portion ~~while~~ **with** standing in a relaxed **position** ~~manner~~ after gentle expiration. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured with a mercury sphygmomanometer on ~~the~~ each arm after at least 10 minutes of rest.

Definition of metabolic syndrome (MS) and biochemical analysis ~~***and biochemical analysis***~~

~~Metabolic syndrome (~~MS~~)~~ was diagnosed according to the criteria set out by the diagnostic criteria review committee of metabolic syndrome in Japan **NOTE: PLEASE RECHECK EXACT TITLE AS THIS SEEMS LIKE IT MIGHT BE A MISTAKE. DID YOU MEAN DIAGNOSTIC CRITERIA OF THE METABOLIC SYNDROME REVIEW COMMITTEE?]** [20]**:**~~;~~ central obesity (waist circumference ≥85 cm Japanese males, ≥90 cm Japanese females) plus any 2 of the following; raised triglycerides≥150 mg/dl or specific treatment for this lipid abnormality; reduced high density lipoprotein (HDL)-cholesterol < 40 mg/dl in males and females; raised blood pressure~~:~~ **(**systolic ≥130 mmHg or diastolic ≥85 mmHg**)**~~,~~ or treatment of hypertension; fasting glucose ≥110 mg/dl or previously diagnosed Type 2 diabetes mellitus.

After a 12-hour overnight fast, venous blood samples were taken for the measurement of plasma concentration**s** of ~~fasting blood~~ glucose, hemoglobin A1c (HbA1C), high sensitivity C-reactive protein (hs-CRP), total cholesterol, HDL-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, gamma glutamyltransferase (γ-GTP), **a**~~A~~spartate aminotransferase (AST), **and a**~~A~~lanine

aminotransferase (ALT).

Statistical analysis

Statistical analysis was performed using ~~computer software,~~ SPSS 17.0 computer software for Windows (SPSS Japan Inc.). Results **for** ~~of~~ continuous variables were expressed as means ± SD for each subject group. The statistical difference was determined by two-sided Student's *t*-test (for equal variance cases) or Welch's *t*-test (for not equal variance cases). Non-normally distributed variables were compared by the Mann-Whitney U test. Variables given as proportions were compared using the chi-square test. The relationships between risk factors and dysplasia including adenocarcinoma of **BE** ~~Barrett's esophagus~~ were examined by multivariate logistic regression analysis. A p-value <0.05 was ~~taken~~ **considered** to be statistically significant. Differences in mean laboratory data and anthropometric data across ~~three~~ **3** categories were evaluated using **1**~~one~~-way analysis of variance.

RESULT**S**

Endoscopic findings of Barrett's esophagus by crystal violet staining

Crystal violet staining was performed when we recognized **BE** ~~Barrett's esophagus~~ during routine endoscopic examination. The intestinal metaplastic lesion was stained with **a** violet color resulting in ~~an~~ easy recognition of **the** targeted biopsy (Fig.1a, b).

~~***High complication rate of D***~~***dysplasia in SCE~~ ~~***specialized columnar epithelium***~~ ~~**(SCE)** ***type BE of Barrett's esophagus***~~

The average age of **the** 151 **BE** ~~Barrett's~~ patients was 62.9 years (±10.6 **years**) and **the ratio of** male**s (n=105)** to female**s (n=46)** ~~ratio~~ was 2.3:1 ~~(105 to 46)~~. **The d**~~D~~demographic characteristics of ~~Barrett's~~ **the** patients according to pathological classification are shown in Table 1. ~~151~~ ~~Barrett's~~ **BE** patients ~~could be~~ **were** classified into **3** ~~three~~ categories**:**~~,~~ ~~specialized columnar epithelium (~~SCE~~)~~ type (**n**=65 ~~patients~~), junctional type (**n**=38 ~~patients~~)**,** and gastric fundic type (**n**=48 ~~patients~~), and the **incidence** ~~ratio~~ of complicating **[UNNECESSARY?]** dysplasia **in these 3 groups was** ~~were~~ 30.8% (20/65), 7.9% (3/38) and 4.2% (2/48), respectively. The ratio of dysplasia in **patients with** SCE type **BE** was

significantly higher than **in patients with** ~~those in~~ junctional**-** and gastric fundic-type **BE** ~~with a statistically significant difference~~ (p=0.02 and p=0.002, respectively).

Variables associated with dysplasia in SCE type BE ~~***of Barrett's esophagus***~~

We focused on **the** SCE type **of BE** ~~Barrett's esophagus~~ because of **the** high ~~complication~~ **rate** of dysplastic change **associated with this condition,** as shown in Table1. We compared variables between **SCE-type BE** patients with and without dysplasia ~~in SCE type of Barrett's esophagus~~ (Table 2). *H. pylori* infection, p53 over expression (Fig. 2), body weight, and diastolic BP were identified as risk factors strongly associated with dysplastic change ~~of Barrett's esophagus~~. In contrast, ~~body mass index (~~BMI~~)~~, waist circumference, MS complication**s,** and variables ~~linking~~ **related** to glucose or lipid metabolism were not associated with dysplasia. ~~In next step, we~~ **We then** conducted multivariate logistic analys**e**~~i~~s ~~upon~~ **of those** variables **that's showed significant** ~~presenting relatively low~~ p-value**s** in **the** univariate analysis shown in Table 2~~,~~ **;** namely, gender, *H. pylori* infection, body weight, p53 overexpression**,** and **low** diastolic BP. Among these **these variables**, p53 overexpression, *H. pylori* infection**,** and **low** diastolic BP were

independent risk factors associated with dysplasia complicated in **patients with** BE of the SCE type ~~Barrett's epithelium~~ (Table.3).

Risk factors associated with progression of SCE from non-dysplastic epithelium to low-grade and high-grade dysplasia

We speculated **that** risk factors associated with progression of SCE to dysplasia might have a linearly increasing or decreasing tendency from non-dysplasia to low and further high-grade dysplasia. **[NOTE: THIS IS A BIT CONFUSING. DID YOU BASICALLY MEAN THAT YOU ASSESSED THE LINEARITY OF THE RELATIONSHIP BETWEEN RISK FACTORS AND PROGRESSION?]** We classified dysplasia into ~~two~~ **3** groups~~,~~ **-- no dysplasia (n=45),** low-grade **dysplasia (n=14),** and high-grade **dysplasia (n=6)** ~~group~~ including adenocarcinoma~~, -- and the compared variables among three groups, 45 patients with non-dysplasia, 14 with low-grade dysplasia and 6 with high-grade dysplasia including adenocarcinoma~~ (Table~~.~~ 4). ~~By~~ **Based on** analysis of variance, **6** ~~six~~ variables **were significantly associated with progression of SCE from non-dysplasia to high-grade dysplasia:**~~,~~ length of BE, *H. pylori* infection ~~rate~~, p53 overexpression, body weight, GERD**,** and **low** diastolic BP ~~revealed~~

~~association with progression of SCE from non-dysplasia to high-grade dysplasia with a statistically significant difference~~. ~~Furthermore,~~ **O**~~o~~nly ~~three~~ **3** of **these 6** ~~six~~ variables~~,~~ ~~length of BE, *H. pylori* infection rate and p53 over expression~~ showed **a** linear correlation with progression ~~of SCE from non-dysplasia~~ to high-grade dysplasia**; i.e., length of BE, H. pylori infection, and p53 overexpression** ~~including adenocarcinoma~~ (Table 4).

~~The expression level~~ Correlation between ~~of~~ p53 expression ~~correlated with~~ and progression of SCE from non-dysplasia to low- and high-grade dysplasia

Given the ~~results of~~ strong association **observed between** ~~of~~ p53 overexpression ~~with~~ **and** dysplasia **seen** in **the** multivariate logistic analysis (Table 3), we analyzed the ~~expression~~ level of p53 **expression** and its association with progression of non-dysplastic SCE to low- and high-grade dysplasia including adenocarcinoma. The expression ~~level~~ of p53 was categorized ~~into three groups,~~ **as** ~~(-),~~ no ~~p53~~ expression **(-)**, ~~(+),~~ moderate ~~p53~~ expression~~,~~ **characterized by** ~~the~~ positive nuclear staining in 5**%** to 10% **of** cells **(+)**, ~~(++),~~ **and** high ~~p53~~ expression, **characterized by** ~~the~~ positive nuclear staining in more than 10% **of** cells (Fig. 2). As shown in Table 5, only 10% of **patients in the** non-dysplastic

SCE **group** expressed p53 at **a** low level, whereas **expression was high** ~~did all SCE~~ **in the group** with high-grade dysplasia ~~at high level of p53 expression~~ ($p<0.01$).

DISCUSSION

A number of reports**, based on endoscopic, biochemical, and anthropometric data,** have identified ~~gastroesophageal reflux disease (~~GERD~~)~~, absence of *H. pylori* infection, MS, waist circumference**,** and body weight as risk factors associated with the presence of ~~Barrett's esophagus~~ **BE** ~~by analyzing endoscopic findings, biochemical and anthropometric variables~~ [4-9]. **One of the most notable** ~~The most interesting~~ findings ~~came~~ from ~~the~~ epidemiological reports **has been** ~~that~~ a strong inverse association between *H. pylori* infection and **dysplasia of** Barrett'**s epithelium** ~~dysplasia~~ [10-12].

Esophageal adenocarcinoma derived from **BE** ~~Barrett's~~ esophagus is not common in Japan **as** compared **with** ~~to~~ **W**~~w~~estern countries, whereas gastric carcinoma is more prevalent in Japan ~~than in western countries~~. This inverse relationship may reflect the high ~~and low~~ prevalence ~~rate~~ of *H. pylori* infection in Japan and **the low prevalence in W**~~w~~estern countries~~, respectively~~.

Another **notable epidemiological** ~~big~~ difference between **these regions** ~~Japan and western countries in Barrett's esophagus~~ is **the** ~~a~~ length of **BE** ~~Barrett's esophagus,~~ **; i.e,** ~~that is,~~ SSBE is common in Japan but LSBE **is more prevalent** prevailing in **W**~~w~~estern countries. **The underlying reasons for this difference are not currently known [NOTE: IS THIS WHAT YOU MEANT?]** ~~There has been no critical input to interpret this difference~~.

Herein, w~~W~~e ~~present here~~ **have identified** risk factors associated with progression of **BE** ~~Barrett's esophagus~~ from non-dysplasia to high-grade dysplasia including adenocarcinoma. In our **cohort,** ~~case~~ 94% of **BE cases** ~~Barrett's esophagus~~ **were the** ~~was~~ SSBE **type** (Table 2). ~~The~~ **O**~~o~~verexpression of p53 was most important risk factor for progression to dysplasia and adenocarcinoma (Table 3), and **the level of p53** ~~its~~ expression ~~level were~~ **was** strongly related to the grade of dysplasia (Table 5). A number of studies have shown that p53 overexpression is increased ~~along~~ **in parallel** with progression of histological changes from metaplasia to high-grade dysplasia and adenocarcinoma [21-23]. **In specimens obtained from surgical resection, expression of** ~~It has been shown that~~ p53 ~~expression was~~ **has been** observed in the region of adenocarcinoma as well as **in** adjacent dysplastic epithelia ~~by using specimen obtained from surgical resection~~

[24]. In addition, in many cases, p53 mutations are found at the identical residue in both adenocarcinoma and adjacent dysplastic epithelia **[NOTE: DID YOU MEAN THAT A MUTATION RESULTING INA CHANGE IN A SPECIFIC AMINO ACID RESIDUE WAS THE SAME IN BOTH TISSUES IN THE SAME PATIENTS?]** [25]. These results suggest~~ed~~ that ~~the~~ p53 mutation, **which is** relatively uncommon in non-dysplastic **BE** ~~Barrett's esophagus~~, **is** ~~was~~ an important step in the progression to~~ward~~ adenocarcinoma [26]. Galipeau et al. showed that inactivation of p53 by mutation is strongly associated with progression to aneuploidy**,** possibly through the loss of p53-mediated apoptosis and cell cycle arrest [27]. The accumulation of these aneuploid cell populations has been shown to increase the risk of developing adenocarcinoma [28, 29].

The possible causal role of p53 ~~possibly participates causally~~ in tumorigenesis as well as tumor progression in ~~Barrett's esophagus~~ **BE has been postulated** ~~because~~ **based on histological evidence showing that** p53 mutations are more frequent in advanced stage**s** in histology. **[NOTE: IS THIS OK? IN CASES IDENTIFIED AS ADVANCED BASED ON HISTOLOGICAL EVIDENCE?]** Thus, it is important to address the hypothesis that p53 overexpression could predict progression of non-dysplastic Barrett's esophagus to

adenocarcinomas. **[NOTE: SLIGHTLY VAGUE/AWKWARD. DID YOU WANT TO STATE THIS AS A HYPOTHESIS HERE, OR DID YOU SPECIFICALLY WANT TO MENTION THAT IT IS IMPORTANT TO *ADDRESS* THE HYPOTHESIS?]** Younes et al~~.~~ studied p53 accumulation via immunohistochemistry in **54 patients [CORRECT?]** with Barrett's metaplasia, dysplasia, ~~and~~ **or** adenocarcinoma**;** ~~and found that~~ p53 accumulation increased **in parallel** ~~along~~ with histological progression~~,~~ from metaplasia to adenocarcinoma ~~in 54 patients~~ [30]. Follow-up biopsies were available in 23 out of 54 patients who had dysplasia in at least ~~one~~ **1** biopsy specimen. Only 1 of 21 (4.8%) patients with all p53-negative biopsies **[MULTIPLE BIOPSIES IN EACH PATIENT WERE ALL NEGATIVE?]** had histological progression. In contrast, 2 of 3 (67%) patients with p53-positive biopsies progressed to high-grade dysplasia or intramucosal carcinoma (~~one~~ **1** patient **was** lost to follow up). ~~Thus, their~~ **These** retrospective data suggest~~ed~~ that p53 accumulation increase~~sd~~ the risk of progression from low-~~grade~~ to high-grade dysplasia. ~~Their~~ **These** data **are** ~~is~~ **also** consistent with our results **showing** that the ~~expression~~ level of p53 **expression** was correlated with the grade of dysplasia (Table 5), suggesting that mutated p53 expressed at an early stage and may stimulate **[NOTE: "…, which is expressed at**

an early stage, may stimulate…" OR "is expressed at an early stage and may stimulate"?] the tumor progression in **the** metaplasia-dysplasia-adenocarcinoma sequence of **BE** ~~Barrett's esophagus~~.

We found a strong inverse association between *H. pylori* infection and the progression of dysplasia **in BE** ~~of Barrett's esophagus~~ (Table 2)**;**~~,~~ ~~thus~~the lowest prevalence ~~rate~~ of *H. pylori* was observed in **the** high-grade dysplasia group (Table 4). Many studies **have reported** found that ~~persons~~ **the absence of** ~~without~~ *H. pylori* colonization **is associated with a greater likelihood** ~~were more likely to~~ **of** develop**ing** esophageal dysplasia and adenocarcinoma [11, 31-33]. Hence, *H. pylori* infection appears to have a protective effect against the development of ~~Barrett's~~ dysplasia and adenocarcinoma **in BE** [34]. The mechanism through which the absence of *H. pylori* colonization is associated with ~~Barrett's~~ dysplasia **in BE** is unknown, but there are several ~~potential~~ possibilities. First, *H. pylori* infection, in particular the more virulent Cag A-positive strain**,** may suppress acid production leading to gastric atrophy; **[NOTE: UNCLEAR. DOES IT LEAD TO OR PREVENT GASTRIC ATROPHY?]** this may lower the risk of **BE** ~~Barrett's esophagus~~ and esophageal adenocarcinoma [33, 35]. With regard~~s~~ to the possible outcome of *H. pylori* eradication in **BE** ~~Barrett's patients~~, only a few ~~study~~ **studies**

have reported that the ~~short-segment Barrett's esophagus~~ **SSBE** developed at 24 months after *H. pylori* eradication **[NOTE: UNCLEAR. IS THIS EVIDENCE IS SUPPORT OF ERADICATION? DID IT DELAY THE ONSET OF SSBE?]** [36, 37]. In Japan, the prevalence of *H. pylori* infection has been decreasing **[RECENTLY? WHAT WAS APPROXIMATE TIME SPAN? DECADE(S)?]** and **the use of** ~~eradication of~~ *H. pylori* **eradication therapy** has ~~been~~ flourished ~~during recent years~~. For this reason, **the incidence of BE** ~~Barrett's esophagus~~ and adenocarcinoma **is likely to** ~~will~~ increase, and it is **therefore** important to determine risk factors ~~taking the potential~~ for malignant change**s** associated with the development ~~for~~ **of** dysplasia and adenocarcinoma **in BE** ~~of Barrett's esophagus~~ after *H. pylori* eradication.

In **our** multivariate logistic analysis, diastolic blood pressure was an independent risk factor associated with progression of **BE** ~~Barrett's esophagus~~ from non-dysplas**tic**~~ia~~ to dysplastic epithelium (Table3). Although this is ~~a~~ **the** first report of **a relationship between** diastolic blood pressure ~~involving Barrett's esophageal~~ **and** dysplasia **in BE**, ~~it is difficult to interpret~~ the underlying mechanisms **are unclear**. **We detected** ~~There is~~ no difference in ~~a~~ diastolic blood pressure between **groups of patients with** low**-** ~~and~~ **or** high-grade dysplasia

(Table 4)**,** suggesting **that** direct or indirect effects of diastolic blood pressure **may occur** at an early stage of dysplastic change **in BE** ~~of Barrett's esophagus~~.

In ~~a~~ **our** univariate analysis**,** body weight was extracted as a risk factor for dysplasia **in BE** ~~of Barrett's esophagus~~, but ~~not was~~ BMI and waist circumference **were not**. In a Swedish study of 189 cases of newly diagnosed esophageal adenocarcinoma, a strong positive association was found between BMI and esophageal adenocarcinoma when controlling for GERD symptoms [4]. A study from the Veterans Association in the United States found ~~those~~ **subjects** with a BMI>30 had a 4**-**fold greater risk for **BE** ~~Barrett's esophagus~~ ~~when~~ **as** compared **with** ~~to~~ controls with a BMI<25 [5]. More recently, several studies **have** revealed that waist circumference, but not BMI, has~~d~~ **a** modest independent association~~s~~ with the **incidence** ~~risk of~~ **BE** ~~Barrett's esophagus~~, dysplasia and adenocarcinoma. ~~The other~~ **Other** studies **have reported** ~~suggested~~ **that a** higher waist-to-hip ratio **is** associated with **BE** ~~Barrett's esophagus~~ when **data are** adjusted for GERD symptoms and BMI [5-7, 9]. In our **multivariate logistic analysis** ~~case~~**,** anthrometric variables were not extracted as risk factors ~~in multivariate logistic analysis~~.

In conclusion we **demonstrated that** ~~presented~~ p53 overexpression, absence of *H. pylori* infection**,** and low diastolic blood pressure ~~as an~~ **are** independent risk factor ~~associated with~~ **for** progression of ~~Barrett's esophagus~~ **BE** from non-dysplasia to dysplasia. Future studies are needed to elucidate the ~~underlying~~ mechanisms **underlying the association** of these risk ~~factors involving~~ **with the sequence of progression of** dysplasia to adenocarcinoma **in BE** ~~sequence of Barrett's esophagus~~.

Figure Legends

Figure 1**.** ~~Barrett's esophagus~~ **BE** stained by crystal violet. (a) Regular observation of **BE** ~~Barrett's esophagus~~. (b) Staining with crystal violet in the same region.

Figure 2**.** Immunostaining of p53. The upper **panel shows** ~~picture is~~ hematoxylin and eosin staining and the lower **panel shows** immunostaining of p53 using the identical sample. (a) (-), no p53 expression. (b) (+), moderate p53 expression~~,~~ **characterized by** ~~the~~ positive nuclear staining in 5**%** to 10% **of** cells. (c) (++), high p53 expression~~, the~~ **characterized by** positive nuclear staining in more than 10% cells.

Table 1**.** ~~The~~ **C**~~c~~haracterization of **the 3** ~~three~~ type**s of BE** ~~ of Barrett's esophagus~~.

Table 2**.** Univariate analysis of variables associated with Barrett's epithelial dysplasia.

Table 3**.** Multivariate analysis of variables associated with Barrett's epithelial dysplasia.

Table 4**.** Analysis of variance **for the** ~~among~~ ~~three~~ **3** categories of **BE:** ~~Barrett's esophagus~~, non-dysplasia, low-grade dysplasia**,** and high-grade dysplasia.

Table 5**.** Relationship **between** ~~of~~ the ~~expression~~ level of p53 **expression** and the grade of dysplasia.

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