*H. pylori* were related to osteoporosis but only in female: a cross-sectional study

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Abstract
Background Recently, more and more studies attach their attention to the extragastrointestinal effects of *Helicobacter pylori* (*H. pylori*). Osteoporosis is an asymptomatic disease which can eventually lead to fractures, have a significant impact on the life quality of the elderly. Sex as an influential factor plays a crucial rule in the development of osteoporosis. The aim of the study is to investigate the relationship between *H. pylori* and osteoporosis, and find the potential, influential biomarker in different gender, in Chinese population.

Method We conducted a cross-sectional study within the study population older than 50 years old, who have had regularly body examination in Beijing shijitan hospital health examination center in 2018 July to October. Participants for the patient profile, serum sample, *H. pylori* infection status, comorbidity, medicine use, lumbar dual-energy X-ray absorptiometry were collected. Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression among the normal, osteopenia, osteoporosis group with the *H. pylori* infection and the markers. And then we analyzed the correlation between the gender and the potential serum biomarkers.

Results There are significant differences between the *H. pylori* infection statue with the bone density in the female (P=0.044), but not in the male (P=0.381). And in female, *H. pylori* (OR=0.200, 95%CI 0.043-0.938, P=0.041) is related to osteoporosis, Body Mass Index (BMI) is close to have statistical significance (OR=7.706, 95%CI 0.936-63.497, P=0.058). Furthermore, pepsinogen1 (OR=0.246, 95%CI 0.091-0.662, P=0.006), Waist-to-Hip Ratio (OR=7.268 OR=2.291-23.196 P=0.001), triglyceride (OR=0.310, 95%CI 0.125-0.768, P=0.011), CA724 (OR=1.244, 95%CI 1.244-8.671 P=0.016), BMI (OR=0.071, 95%CI% 0.024-0.207 P≤0.001) have a significant difference within different gender, all excluding age as a confounder, all excluding age as a confounder.

Conclusion Low BMI, and *H. pylori* positivity were risk factors for osteoporosis but only for female not for male.

Background
*Helicobacter pylori* (*H. pylori*), a gram-negative, spiral-shaped microaerophilic bacterium has been
proved to be an important pathogen in gastrointestinal diseases\(^1\). Approximately 50% of the world population has been affected by the H. pylori, and about 800 million Chinese population are affected by this disease. It may cause chronic inflammation of gastric mucosa, which may lead to chronic atrophic gastritis, peptic ulcer diseases, and gastric cancer\(^2\), \(^3\). What's more, the latest reports have described the investigation of the extragastrointestinal effects of H. pylori, including metabolic syndrome\(^4\), fatty liver\(^5\), rheumatic and skin diseases\(^6\) and so on.

Osteoporosis is an asymptomatic disease characterized by a decreased density of normally mineralized bone that usually occurs in elderly person. In China, about 160 million people were threatened by osteoporosis. In the development of osteoporosis, there is often a long latent period before the appearance of the main clinical manifestation, pathologic fractures. Moreover, the most prevalent sequela of osteoporosis is compressing fracture of the vertebral bodies and the fracture of the ribs, proximal femurs, humeri, distal radiuses which would have a significant impact on the life quality of the elderly. Therefore, the prevention and early detection of osteoporosis is particularly important for the sensible population.

A majority of essay support the idea that at any given age, women have a higher risk of fracture than men\(^7\). However, men have the tend to have worse outcomes after fracture than women: they are twice as likely to die after a hip fracture than women\(^8\). What's more the gender-related factor is still remaining unclear. Therefore, to explore the difference of influencing factors between male and female osteoporosis will be helpful to explore the pathogenesis of osteoporosis and early prevention of occurrence and development of it.

There are some well-established risk factors for the emerge of osteoporosis, for example age, sex, body mass index, alcohol assumption smoking\(^9\). And recent articles have attached their attention on the H. pylori and the osteoporosis. Partially due to the chronic inflammation induced by the H. pylori infection, which may increase the production of tumor necrosis factor-\(\alpha\), interleukin-1, and interleukin-6, and increasing the osteoclast activation and inhibiting osteoblast survival\(^10\). The association for osteoporosis and H. pylori has been studied by many Japanese scientists and remain controversial. In
addition, there are still some deficiencies in existing research. For example, the number of sample size is insufficient, lack of Chinese data, lack of investigation of different gender and lack of sufficient serum markers. The aim of the study is to investigate the relationship between H. pylori and osteoporosis and the potential influential biomarkers in different gender in Chinese population.

Methods

Study Population

Briefly, men and women, older than 50 years old not included 50 years old, would be recruited who have had regularly body examination in Beijing shijitan hospital health examination center in 2018 July to October. In order to be eligible, we excluded the patients using the drugs and having the comorbidities which may cause secondary osteosis. The drugs included glucocorticoids, thyroid/parathyroid drugs, psychotropic drugs, anticonvulsants, selective estrogen receptor modulators (SERMs), vitamin D, calcium, and bisphosphonate. The patient who has had gastrectomy, inflammatory bowel disease, malignant diseases, chronic kidney disease, diabetes mellitus, hypo/hyperthyroidism, hypo/hyperparathyroid disorder, acromegaly and rheumatoid arthritis (including collagen disease). The female patients were not in pregnancy or lactation. Furthermore, the patients who have had diagnosed of H. pylori infection before or have potentially active against H. pylori drugs in 1 month would be recruited as well.

Data collection

Among all the eligible individuals, 243 eligible patients gave our permission and provided their basic information, including demographics (age, gender, race), smoking status, and medicine use. All the ethics approvals have been given by the ethics committee of Beijing shijitan hospital affiliated to capital medical university and have been performed in accordance with the Declaration of Helsinki. All the serum samples were taken on an empty stomach and the data of blood routine, glucometabolic, liver function, renal function, lipid metabolism, ion, tumor markers, pepsinogen(PG), pro-gastrin-releasing peptide (proGRP) has been collected. The patient’s weight, height, waist circumference, hip circumference had been measured as well. And the Diastolic and systolic blood pressure have been measured in the morning. Body Mass Index (BMI) was calculated by taking a person's weight, in
kilograms, divided by their height, in meters squared. Waist-to-Hip Ratio (WHR) is measured as waist circumference divided by hip circumference. And the H. pylori infection statue was measured by c13 breathing test at the same day with an empty stomach. proGRP, PG1, PG2 and tumor marker were measured using enzyme-linked immunosorbent assay methods.

Diagnosis of Osteoporosis.
The bone mineral density (BMD) of the lumbar vertebrae 2–4 (L2-4) was measured by DXA using a Discovery A (HOLOGIC, Bedford, Massachusetts). The results will provide BMD (g/cm2) and young adult mean bone mineral density. The diagnosis of osteoporosis was performed in accordance with the World Health Organization diagnostic criteria by the World Health Organization (WHO) Collaborating Center for Metabolic Bone Diseases. A value for BMD within one standard deviation (SD) of the average BMD of normal adults will be regarded as normal. Osteopenia is defined as a value for BMD that lies between 1 and 2.5 standard deviation compared with the below the young adult mean value. BMD deduced by more than 2.5 SD below the young adult mean value will be classified as osteoporosis11.

Statistical analyses
We used SPSS statistical software version 22.0 for data analyses. Continuous variables were reported as means ± standard deviation, whereas categorical variables were presented as percentages. Study subjects were first classified into three groups using the classification standard according to the BMD: normal, osteopenia, osteoporosis. The Kolmogorov-Smirnov test was used to verify whether the data fitted normal distribution. Summary and groping data for baseline characteristics (the laboratory examination) were compared using t-test for continuous variables and Fisher's exact test for categorical variable. Moreover, we divide patients into men and women based on gender. And we used Fisher's exact test to verified whether men and women have gender difference in the relationship of H. pylori and osteoporosis.

Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression among the three subgroups. In order to further analyzed the relationship between the H. pylori infection status and osteoporosis, we created a model using total cholesterol(TC),
triglyceride (TG), uric acid (UA), BMI, WHR, Low-density lipoprotein cholesterol (LDL-C), glycosylated hemoglobin (Ghb), H. pylori infection statue, and the BMD groups, which analyzed the different gender separately. All the study population's High-density lipoprotein cholesterol (HDL-C) are all in the normal value, so we did not include it in the model.

Meanwhile, we analyzed the marker between the male and female. We separate the patient according to the gender and further analyzed the patient's basic data using the t-test for the for continuous variables and Fisher's exact test for categorical variable.

What's more, we further analyzed the relationship between the gender and the marker to find the gender difference in the relationship between the H. pylori infection and osteoporosis. The markers including PG2, PG1, PG2/PG1, proGRP, TC, LDL-C, UA, TG, glucose (GLU), CA724, CEA, BMI, WHR, PG1, PG2, PG1/PG2, ProPG entered in the model using two percentile. Other serum biomarker entered into the model as factors using their normal value as the grouping criterion. All the model excluded age as confounder. P-value < 0.05 was considered to be statistically significant.

**Result**

Data were analyzed from 243 patients aged from 50 to 67 years old who have body examination in Beijing shijitan hospital health examination center. The baseline characteristics of the participants were shown in tablet 1. The mean age is 54.53±0.238 and 167 (68.7%) were male, 76 were female. Among the study population, 47 study population have osteoporosis, 83 study population have osteopenia and 113 study population have normal BMD. The mean age of the osteoporosis osteopenia and normal group is 54.14±3.393, 54.98±3.838 and 54.70±4.185. Unfortunately, there is no statistical differences in age among the study population of osteoporosis, osteopenia and normal BMD groups. There are 38.2% of the male have normal BMD, 32.9% of the male are osteopenia, 28.9% of the male are osteoporosis. And there are 50.3% of the female have normal BMD, 34.7% of the male are osteopenia, 15.0% of the male are osteoporosis. And there is statistical difference between these three groups in gender percentage (P=0.034).

Among all the patients, 84 study population have H. pylori infection and 159 study population without H. pylori infection. The mean age of the with H. pylori infection and without H. pylori infection is
54.36±0.436 and 54.63±0.283 and 71.4%, 67.3% of patients were male, separately. Table 1 shows CA724 (t=5.758, P=0.022), PGII (t=13.016, P≤0.001), PGI/PGII (t=3.875, P≤0.001) has a statistical difference among patients with H. pylori infection and without H. pylori infection, and there is no statistic differences between the H. pylori infection statue and the bone density.

In table 2, we separate the study population according to the gender. And we found there is significant differences between the H. pylori infection statue with the bone density in the female (P=0.044), but not in the male (P=0.381).

We analyzed the relationship the H. pylori infection and the osteoporosis in female in table 3. And we find that H. pylori (OR=0.200, 95%CI 0.043-0.938, P=0.041) is related to osteoporosis. And BMI is close to have statistical significance (OR=7.706, 95%CI 0.936-63.497, P=0.058). And with the decrease of BMD, BMI decrease as well.

Meanwhile, we analyzed the marker between the male and female which have been shown in table 4. And we find that SP (P≤0.001), BP (P≤0.001), BMI (P≤0.001), WHR (P≤0.001), Hb (P≤0.001), platelet (P≤0.001), UA (P≤0.001), TC (P=(C (P≤0.001), GLU (P=0.003), CEA (P≤0.001), PGI (P=0.020), PGI/PGII (P=0.047), Ghb (P=0.029), Iron (P≤0.0011) has a statistical difference among male and female.

And the relationship between the sex and the marker has been shown in table 5. And we found that PG1 (OR=0.246, 95%CI 0.091-0.662, P=0.006), WHR (OR=7.268 OR=2.291-23.196 P=0.001), TG (OR=0.310, 95%CI 0.125-0.768, P=0.011), CA724 (OR=1.244, 95%CI 1.244-8.671 P=0.016), BMI (OR=0.071, 95CI% 0.024-0.207 P≤0.001) have a significant difference within different gender, all excluding age as a confounder.

Discussion
Osteoporosis is a an important health and societal burden in elder people, not only women, but also may. In men, osteoporosis is underrecognized and undertreated. Only a few man screen for osteoporosis, even after fracture. The treatment rate is much lower than female. Meanwhile more men than women die every year due to hip fracture. Hence, we also included men as study population in order to find out the risk factors for osteoporosis.
The research about the influence of sex on osteoporosis remains controversial, but it is undeniable that most of the studies believe that there are differences in the pathogenesis of osteoporosis between men and women, the reasons are as follows. First, Differences in clinical outcomes of osteoporosis in men and women may be rooted in the biologic properties of bone. Barrett-Connor E holds the idea that there are sex-specific differences in the number of osteoprogenitor cells, and are different in hormone response, and hormone regulation\textsuperscript{15, 16}. Second, men had a greater bone size, trabecular BMD and bone area at the radius and tibia than women, even after adjusting for weight and height, which may lead to decrease of osteoporosis and fracture\textsuperscript{17}. Thirdly, men undergo a slowly decrease of BMD with the increase of age, while women experience a profound period of rapid bone resorption, especially after entering into menopause\textsuperscript{18}. Last but not least, essays support the idea that men are more likely to suffered from the secondary disease, for example, rheumatoid arthritis, alcoholism, excessive smoking, and gonadal deficiencies and others\textsuperscript{19}, which may lead to sustainable bone loss. Unfortunately, the relationship between osteoporosis and H. pylori infection is still controversial. Some studies hold the view that there is no difference between men and women in the relationship between H. pylori and osteoporosis\textsuperscript{20, 21}, while others only think that H. pylori is related to osteoporosis only in women\textsuperscript{22}, while others think that there is no correlation between them in female\textsuperscript{23}. In our study, we analyzed the relationship between the H. pylori infection and the osteoporosis. And we found that H. pylori infection is related to osteoporosis in female but not in male. We think this may be due to the difference in the etiology of osteoporosis between men and women. However, we did not find any further study on it, which need more investigation.

After analyzing the difference between the male and female, we found that there is significant differences in BMI, WHR, CA724, and PG1 in the study population. And it provides further follow up research on gender differences between H. pylori and the osteoporosis.

Most of the study hold the view that obesity is related to osteoporosis, however the effect of obesity remains unclear. In one hand, obesity has traditionally been considered positive to bone because of
the beneficial effect of mechanical loading\textsuperscript{24}. In the other hand, people hold the view that BMI may do harm to BMD. Osteoblasts and adipocytes are both steam from marrow mesenchymal stromal cells. And the osteoblasts and adipocytes are in a competitive relationship, which the increase of adipocytes will inhabit the osteoblasts\textsuperscript{25}. In our study, the P-value is closely (\(P = 0.058\)) to have significant difference, and the previous study hold the view that low BMI was independently significantly associated with decreased BMD which is same as our result\textsuperscript{26}. Therefore we still hold the view that BMI is related to the osteoporosis. Moreover, we believe that higher BMD found in obese people may partly own to the increased mechanical loading and strain, in addition, it’s a complicated problem that cannot be generalized.

Furthermore, we find that there is a relationship between the P.G. and H. pylori infection. P.G. can be used as a surrogate marker for the evaluate of gastric mucosal status. In patients with H. pylori infection, the PG II levels were higher and the PG I/II ratios were lower than those without H. pylori infection, which is same as our result\textsuperscript{27}.

In our study, we find that H. pylori infection is associated with the decrease of bone density. First, H.pylori infection may cause systemic inflammation and increase the production of tumor necrosis factor-\(\alpha\), interleukin-1, and interleukin-6\textsuperscript{28}. And these cytokines directly involved in the formation of the deduction of BMD. Second, osteoporosis may be related to the decrease of vitamin B12 level\textsuperscript{29}. Meanwhile, H.pylori infection may lead to the deficiency of B12. Serin et al's article has selected 145 patients without atrophy, erosions or ulcers. And they find that the histopathological scores for both antral and corpus H. pylori density and inflammation were significantly inversely associated with serum vitamin B12 levels\textsuperscript{30}. Last but not least, most patients chronically infected with H. pylori manifest a pangastritis with reduced acid secretion due to bacterial virulence factors, inflammatory cytokines, and various degrees of gastric atrophy\textsuperscript{31}. Calcium is ionized in acidic conditions and absorbed in the small bowel. Therefore, in either hypochlorhydria or achlorhydric stomachs, calcium absorption is impaired\textsuperscript{32}. What's more patient with long term use of acid suppressants, for example,
proton pump inhibitor may lead to osteoporosis or the decrease of BMD as well. Limited experimental evidence indicates that PPI may influence the calcium absorption leading to compensatory physiologic responses including secondary hyperparathyroidism, which may cause the increase in the rate of osteoclastic bone resorption\textsuperscript{33}. Although the effect of Helicobacter pylori infection to the decrease of bone density is supported by most of the researchers, the effect of early eradication therapy is still not enough. Replogle ML hold the view that the early eradication therapy may eliminate chronic inflammation from H. pylori \textsuperscript{34}. Some article has also reported an improvement in B12 after complete eradication\textsuperscript{30, 35}. And this result still requires further research.

Expect the virtue we have achieved; our study had several limitations. First, we have not been able to get the time of H.P. infection, so different infection time may have an impact on the results. Second, the sample size of our data is not large enough, and the study populations only include the patients from Beijing shijitan hospital and might have confounding factors because of the difference in the distribution of hospital patients. Third, the patients were all Chinese and the findings might not be generalizable to other ethnic population. In addition, we only found some differences between men and women but failed to further explore them.

In summary, our study finds that H. pylori infection and BMI is related to osteoporosis, but only in female. And we find that BMI, WHR, CA724, and PG1 have a significant difference in male and female, which provide direction for the further investigation of the difference in gender between the relationship of H. pylori infection and osteoporosis.

**Abbreviations**
lumbar dual-energy X-ray absorptiometry (DXA), odds ratios (ORs), confidence intervals (CIs), Helicobacter pylori (H. pylori), selective estrogen receptor modulators (SERMs), pro-gastrin-releasing peptide (proGRP), Body Mass Index (BMI), Waist-to-Hip Ratio (WHR), bone mineral density (BMD), World Health Organization (WHO), standard deviation (SD), total cholesterol(TC), triglyceride(TG), uric acid(UA), Low-density lipoprotein cholesterol (LDL-C), glycosylated hemoglobin(Ghb), High-density lipoprotein cholesterol (HDL-C), glucose(GLU), pepsinogen(PG), calcium(CA); diastolic blood pressure(DP); Hemoglobin(Hb); systolic blood pressure(SP);
Declarations

**Ethics approval and consent to participate**

All the ethics approval has been given by the ethics committee of Beijing shijitan hospital affiliated to capital medical university and have been performed in accordance with the Declaration of Helsinki. All involved patients have given their informed verbal consent before included in the study.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets analysed during the current study are not publicly available because it includes the study population personal information which is illegal to open but are available from the corresponding author on reasonable request.

**Competing interests:**

The authors declare that they have no competing interests in this section

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**Authors’ contributions:**

LH has made substantial contributions to the designed of the work; ZLC has made contributions to collection of the data.; DFX has analyzed and interpreted of the data; WJW has drafted the manuscript and revised it. All authors have read and approved the manuscript

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Tables
Table 1 Baseline Characteristics of the patients according to the H. pylori infection statue

|                  | Total          | H. pylori-     | H. pylori+     | P-value |
|------------------|----------------|----------------|----------------|---------|
| age (years)      | 54.53±0.238    | 54.63±0.283    | 54.36±0.436    | 0.589   |
| sex              |                |                |                |         |
| Female           | 31.3%          | 32.7%          | 28.6%          | 0.305   |
| Male             | 68.7%          | 67.3%          | 71.4%          |         |
| BMD              |                |                |                |         |
| H. pylori-       | 46.5%          | 45.9%          | 47.6%          | 0.892   |
| H. pylori+       | 34.2%          | 35.2%          | 32.1%          |         |
| P-value          |                |                |                |         |
| age (years)      | 54.36±0.436    | 54.63±0.283    | 54.36±0.436    | 0.589   |
| sex              |                |                |                |         |
| Female           | 31.3%          | 32.7%          | 28.6%          | 0.305   |
| Male             | 68.7%          | 67.3%          | 71.4%          |         |
| BMD              |                |                |                |         |
| H. pylori-       | 46.5%          | 45.9%          | 47.6%          | 0.892   |
| H. pylori+       | 34.2%          | 35.2%          | 32.1%          |         |
| P-value          |                |                |                |         |
| SP(mmHg)         | 123.68±1.121   | 122.52±1.350   | 125.85±1.983   | 0.158   |
| BP(mmHg)         | 75.62±0.826    | 75.17±1.030    | 76.47±1.385    | 0.457   |
| BMI(kg/m²)       | 24.71±0.209    | 24.64±0.250    | 24.84±0.379    | 0.655   |
| WHR              | 0.901±0.004    | 0.901±0.006    | 0.900±0.007    | 0.948   |
| Hb(g/L)          | 148.81±0.877   | 148.81±1.078   | 148.82±1.519   | 0.993   |
| Platelet(*10⁹/L)|                |                |                |         |
| Ca(mmol/L)       | 2.314±0.006    | 2.313±0.007    | 2.315±0.011    | 0.905   |
| UA(μmol/L)       | 363.26±5.200   | 363.01±6.644   | 363.71±8.308   | 0.949   |
| TC(μmol/L)       | 4.984±0.056    | 4.963±0.066    | 5.024±0.102    | 0.608   |
| TG(μmol/L)       | 1.606±0.058    | 1.595±0.074    | 1.627±0.096    | 0.796   |
| HDL-C(μmol/L)    | 1.303±0.019    | 1.303±0.023    | 1.303±0.034    | 0.994   |
| LDL-C(μmol/L)    | 2.674±0.041    | 2.663±0.048    | 2.695±0.076    | 0.713   |
| GLU(μmol/L)      | 5.484±0.088    | 5.360±0.095    | 5.717±0.179    | 0.080   |
| CEA(ng/ml)       | 2.544±0.098    | 2.422±0.112    | 2.775±0.188    | 0.087   |
| PGI(ug/L)        | 53.886±1.808   | 51.760±2.463   | 57.911±2.328   | 0.106   |
| PGII(ug/L)       | 9.144±0.330    | 7.886±0.343    | 11.527±0.624   | 0.000   |
| PGI/PGII         | 6.415±0.125    | 6.843±0.140    | 5.605±0.221    | 0.000   |
| proGRP(pg/ml)    | 31.796±0.883   | 31.765±9.444   | 31.852±1.708   | 0.963   |
| CA724(U/ml)      | 3.523±0.408    | 4.103±0.603    | 2.378±0.333    | 0.022   |
| Ghb(%)           | 5.836±0.093    | 5.767±0.080    | 1.530±0.214    | 0.404   |
| Iron(μmol/L)     | 19.24±0.476    | 19.210±0.588   | 19.30±0.816    | 0.926   |

WHR, waist-to-hip ratio; TC, total cholesterol; Ghb, glycosylated hemoglobin; TG, triglyceride; UA, uric acid; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; Ca, calcium; Cre, creatinine; DP, diastolic blood pressure; Hb, hemoglobin; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; UA, Uric acid; BMD, bone mineral density; OR, odds ratio; proGRP, pro-gastrin-releasing peptide; WHR, Waist-to-Hip Ratio; SP, systolic blood pressure; PG, pepsinogen; GLU, glucose; Hb, Hemoglobin

Bold indicates statistically significant values.

Table 2 the relationship between the H. pylori infection and the BMD in different gender

|                  | H. pylori infection (-) | H. pylori infection (+) | P-value |
|------------------|-------------------------|-------------------------|---------|
| Female           |                         |                         |         |
| Normal BMD       | 20                      | 9                       | 0.044   |
| osteopenia       | 21                      | 4                       |         |
| osteoporosis     | 11                      | 11                      |         |
male

|                | H. pylori infection (+) | H. pylori infection (-) | P-value |
|----------------|-------------------------|-------------------------|---------|
| Normal BMD     | 53                      | 31                      | 0.381   |
| osteopenia     | 35                      | 23                      |         |
| osteoporosis   | 19                      | 6                       |         |

**Bold indicates statistically significant values.**

Table 3 Multivariable analysis for different markers and BMD

|                | OR           | 95% CI        | P-value |
|----------------|--------------|---------------|---------|
| TC (mmol/L)    |              |               |         |
| Q1 (3.65-5.20) | 1.694        | 0.354, 8.093  | 0.509   |
| Q2 (5.21-7.66) | 0            | 0             |         |
| BMI (kg/m²)    |              |               |         |
| Q1 (17.9-23.9) | 7.706        | 0.936, 63.497 | 0.058   |
| Q2 (24.0-31.9) | 0            | 0             |         |
| UA (umol/L)    |              |               |         |
| Q1 (187-357)   | 0.571        | 0.092, 3.557  | 0.548   |
| Q2 (358-571)   | 0            | 0             |         |
| TG (mmol/L)    |              |               |         |
| Q1 (0-1.70)    | 0.232        | 0.033, 1.631  | 0.142   |
| Q2 (1.71-4.47) | 0            | 0             |         |
| LDL-C (mmol/L) |              |               |         |
| Q1 (0-3.12)    | 0.749        | 0.126, 4.446  | 0.750   |
| Q2 (3.13-4.20) | 0            | 0             |         |
| Ghb (%)        |              |               |         |
| Q1 (0-6.0)     | 0.754        | 0.422, 1.346  | 0.339   |
| Q2 (6.1-7.3)   | 0            | 0             |         |
| C13            |              |               |         |
| Q1 (without H. pylor) | 0.200   | 0.043, 0.938  | 0.041   |
| Q2 (with H. pylor) | 0            | 0             |         |
| WHR            |              |               |         |
| Q1 (0.79-0.84) | 3.330        | 0.616, 17.993 | 0.162   |
| Q2 (0.85-0.97) | 0            | 0             |         |

**Abbreviations as in Table 1; OR, odds ratio;**

**Bold indicates statistically significant values.**

Table 4 Baseline Characteristics of the patients in different gender
|                  | Total          | Female         | Male            | P-value |
|------------------|----------------|----------------|-----------------|---------|
| age (years)      | 54.53±0.238    | 54.08±0.487    | 54.74±0.266     | 0.197   |
| SP (mmHg)        | 123.68±1.121   | 116.61±2.058   | 126.87±1.258    | 0.000   |
| BP (mmHg)        | 75.62±0.826    | 68.03±1.461    | 79.06±0.875     | 0.000   |
| BMI (kg/m²)      | 24.71±0.209    | 23.32±0.374    | 25.36±0.236     | 0.000   |
| WHR              | 0.901±0.004    | 0.878±0.006    | 0.911±0.006     | 0.000   |
| Hb (g/L)         | 148.81±0.877   | 135.66±1.295   | 154.80±0.773    | 0.000   |
| Platelet (x10⁹/L)| 238.82±3.521   | 258.93±7.336   | 229.67±3.691    | 0.000   |
| Ca (mmol/L)      | 2.314±0.006    | 2.319±0.012    | 2.311±0.007     | 0.513   |
| UA (umol/L)      | 312.20±8.427   | 312.20±8.427   | 386.49±5.689    | 0.000   |
| TC (mmol/L)      | 4.984±0.056    | 5.240±0.102    | 4.868±0.065     | 0.002   |
| TG (mmol/L)      | 1.606±0.058    | 1.378±0.085    | 1.706±0.074     | 0.011   |
| HDL-C (mmol/L)   | 1.303±0.019    | 1.451±0.032    | 1.235±0.021     | 0.000   |
| LDL-C (mmol/L)   | 2.674±0.041    | 2.759±0.075    | 2.636±0.049     | 0.163   |
| GLU (mmol/L)     | 5.484±0.088    | 5.159±0.102    | 5.631±0.118     | 0.003   |
| CEA (ng/ml)      | 2.544±0.098    | 2.051±0.132    | 2.768±0.126     | 0.000   |
| PGII (ug/L)      | 53.886±1.808   | 47.672±2.305   | 56.714±2.385    | 0.020   |
| ProGRP (pg/ml)   | 9.144±0.0330   | 8.841±0.608    | 9.283±0.393     | 0.536   |
| PGII/PGII        | 6.415±0.125    | 6.048±0.214    | 6.582±0.152     | 0.047   |
| proGRP (pg/ml)   | 31.796±0.883   | 31.752±1.431   | 31.818±1.117    | 0.972   |
| CA 724 (U/ml)    | 3.523±0.408    | 3.676±0.472    | 3.449±0.562     | 0.796   |
| Ghl (%)          | 5.836±0.093    | 5.617±0.062    | 5.942±0.133     | 0.029   |
| Iron (umol/L)    | 19.24±0.476    | 16.98±0.810    | 20.33±0.556     | 0.001   |

Abbreviations as in Table 1; OR, odds ratio;

Bold indicates statistically significant values.

Table 5 Multivariable analysis for different markers and gender
| Variable          | Q1(0-34.03) | Q2(34.04-92.80) | OR    | 95% CI       | P-value |
|-------------------|------------|----------------|-------|--------------|---------|
| ProGRP (pg/ml)    |            |                | 2.773 | 1.203, 6.385 | 0.017   |
|                   |            |                | 0     | 0            |         |
| PGII (ug/L)       |            |                | 1.468 | 0.508, 4.242 | 0.478   |
|                   |            |                | 0     | 0            |         |
| PG I/PG II        |            |                | 1.071 | 0.425, 2.702 | 0.884   |
|                   |            |                | 0     | 0            |         |
| PG (ug/L)         |            |                | 0.246 | 0.091, 0.662 | 0.006   |
|                   |            |                | 0     | 0            |         |
| WHR               |            |                | 7.286 | 2.291, 23.196| 0.001   |
|                   |            |                | 0     | 0            |         |
| TC (mmol/L)       |            |                | 2.020 | 0.793, 5.140 | 0.141   |
|                   |            |                | 0     | 0            |         |
| LDL-C (mmol/L)    |            |                | 2.474 | 0.804, 7.622 | 0.114   |
|                   |            |                | 0     | 0            |         |
| UA (umol/L)       |            |                | 0.837 | 0.340, 2.063 | 0.699   |
|                   |            |                | 0.186 | 0.021, 1.619 | 0.128   |
|                   |            |                | 0     | 0            |         |
| TG (mmol/L)       |            |                | 0.310 | 0.125, 0.768 | 0.011   |
|                   |            |                | 0     | 0            |         |
| GLU (mmol/L)      |            |                | 0.527 | 0.166, 1.675 | 0.278   |
|                   |            |                | 0     | 0            |         |
| CA724 (U/ml)      |            |                | 1.244 | 1.244, 8.671 | 0.016   |
|                   |            |                | 0     | 0            |         |
| CEA (ng/ml)       |            |                | 0.186 | 0.021, 1.619 | 0.128   |
|                   |            |                | 0     | 0            |         |
| BMI (kg/m^2)      |            |                | 0.071 | 0.024, 0.207 | 0.000   |

Abbreviations as in Table 1; OR, odds ratio;
Bold indicates statistically significant values.

*Male and female have different normal value in UA and WHR. UA 149-416umol/L in male, 89-357umol/L in female, WHR <9.0 in male, <8.5 in female.