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Role of Helicobacter pylori on differential expression of angiogenic markers in gastric adenocarcinoma

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ABSTRACT

Animal studies showed that male gastric tissues respond more rapidly to Helicobacter pylori (H.pylori) infection but the possible mechanisms remained unclear. There is no data about gender specific activity of Androgen receptor (AR) as an independent unfavorable prognostic factor in gastric cancer and its interactions with H.pylori and angiogenesis in both genders. To compare the pathogenesis of H. pylori and to evaluate its role on tissue levels of Androgen Receptor (AR) and uPA as a major angiogenic factors in gastric adenocarcinoma, malignant and corresponding normal tissue specimens of 71 gastric adenocarcinoma were selected retrospectively. Modified Giemsa staining was used for identifying H.pylori infection and immunohistochemical methods were used to identify differential expressions of above markers in glandular, surface epithelial, tumoral, stromal, endothelial and lymphatic cells. Interestingly 83.3% of H.pylori positive males showed AR overexpression in their surface epithelial cells whereas the same interaction was not found in H.pylori positive females. Higher vascular invasion ($p=0.047$) and higher expression of uPA in stromal cells of male patients ($p=0.007$) clued us to different tumor progressive factors in males. Linear regression analysis showed H.pylori infection, surface epithelial AR and sex as three significant factors in tumoral uPA ($p=0.004$), stromal uPA ($p=0.012$) and lymphatic uPA ($p=0.016$) expression in males which play important roles on invasion and metastasis of gastric carcinoma. Present results suggest the strong role of H.Pylori on angiogenesis in males which could be considered as a new molecular mechanism of tumor progression by interacting with the receptor of male hormone and angiogenic pathways.

Key words: Gastric Adenocarcinoma, Androgen Receptor, Helicobacter Pylori, uPA, Angiogenesis.

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1-Introduction

Although the mortality rates of gastric cancer have declined during the last decades in most countries but gastric cancer remains a major public health issue as the fourth most common cancer and the second leading cause of cancer death worldwide (Crew KD and Neugut AI. 2006). Gastric cancer incidence rates are lower in women than men in both high and low-risk regions worldwide (Lindblad M et al.. 2004) but the reasons remained unclear. Helicobacter pylori infection is a well-recognized risk factor for gastric cancer development (Zullo A et al.. 2006). One animal study has showed that most of H. pylori-related gastric diseases were associated with male gender in mice (Fox JG et al..2003) moreover some evidences support the hypothesis that estrogen plays a protective role in gastric cancer risk of human (Lindblad M et al.. 2004, Freedman ND 2007). Although Androgen Receptor (AR) expression plays an independent unfavorable prognostic role in gastric cancer (Kominea A et al..2004) and androgen-producing enzymes in human gastric carcinomas are involved in the in situ production and possible regulation of androgenic activity in human gastric carcinoma (Nakamura Y et al..2006) but it is not clarified whether this androgenic activity is gender specific and which interactions may exist between AR and H.pylori in both genders.

H. pylori infection plays possible roles on expression pattern of uPA as a major angiogenic factor in gastric adenocarcinoma (Iwamoto J et al.. 2005) but the possible role of AR on expression patterns of mentioned angiogenic factor, tumor progression and clinical outcomes remained unclear in both genders. The purpose of this study was to clarify gender specific differences regarding H.pylori infection, AR and outlined angiogenic factor and their possible interactions for the first time in a clinical setting.

2-Methodology

2-1 Patients

This retrospective study enrolled patients (n=111) with gastric adenocarcinoma who underwent surgery in Imam Khomeini and shohadaye Tajrish University Hospitals during the years 2003 – 2006. Patients were excluded if they were habitual heavy drinkers or IV drug abusers, if they had evidence of other background diseases or history of chemotherapy and radiotherapy before their surgeries. After collecting demographic and pathological information, 71 cases that had necessary parrafinized tissue blocks from their resected tumors as well parrafinized blocks from their adjacent normal tissues were included for Giemsa and immunohistochemical studies.

2-2 Histological evaluation

Histopathological data containing tumor anatomical location, tumor pathological type, tumor size, histological differentiation (malignancy grade), pathological stage, lymphatic invasion, neural invasion, vascular invasion and secondary organ metastasis were collected and compared between males and females. Each surgical specimen was evaluated according to the Ackerman's guidelines of pathological studies on gastric adenocarcinoma (Rosai J 2004).

2-3 IHC Method

Tissues were deparaffinized and rehydrated as described before (Arbabi Bidgoli S et al. 2007). Following the blocking steps, the slides were incubated with primary antibodies at 1.200 optimized dilution for uPA (ab52327,Abcam) and 1:100 optimized dilution for Androgen receptor(clone AR 441, Dakocytomation) for 30 min. at room temperature. The results were visualized using the Streptavidine-biotin (LSAB2) immunoperoxidase detection kit and DAB chromogen (Dakocytomation- Denmark) based on the manufacturer's instruction with necessary modifications. Sections were also counterstained with Meyer's haematoxyline.

2-4 H.pylori identification

Routine formalin fixed and paraffin wax embedded pairs of tumoral and non tumoral surgical samples from 71 adult patients were obtained. As the patients were not in access during the time of study and the accessible data in hospital registry was not reliable regarding their presence of H.pylori infection therefore we were unable to evaluate the H.pylori status by other methods e.g. culture, biopsy urease test (CLO), serology, and C-urea breath test (UBT) .It is confirmed that the modified Giemsa staining is a reliable, cheap, easy to perform, and convenient histological means of identifying H.pylori in gastric samples (12) therefore all standard histological sections were prepared and stained with modified Giemsa staining method (Rotimi O 2000, Wabinga HR 2002).

2-5 Scoring Method

In non tumoral slides glandular epithelial cells and in tumoral slides tumoral, stromal, Lymphatic, endothelial and surface epithelial cells were assessed for expression of markers and each antigen scored separately. uPA with membranous expression and AR with nuclear staining were considered for scoring system. All cells were scored as 3+ if they had strong staining (>50%), 2+ if they had moderate staining (25-50%), 1+ if they had mild staining (5-25%) and 0 if staining was <5% or no staining.

2-6 Statistical analyses

To compare means of two groups student t test, to assess

the association between expression of markers and clinicopathological data nonparametric chi -square test, to estimate the linear relationship between a dependent variable and one or more independent variables (covariates) linear regression analysis was conducted. Relative risks and Odds ratios were calculated by Cochran's and Mantel Haenszel statistics using SPSS 10 (Rober FW 2000). Probability values of <0.05 and Odds ratios>1 were considered significant.

3-Results

3-1 Patient's characteristics

The clinicopathological characteristics of 111 gastric adenocarcinoma patients (75 males and 36 females) were compared between both genders. Following differences were recorded:

1- Females showed higher histological grades (*p=0.002, OR=4.13 CI 95% 1.448-11.799*). Sex was evaluated as a relative risk factor of higher malignancy grades that means 61.8% of females showed higher histological grades (poor and undifferentiated tumors) whereas only 29.6% of males were detected in the same situation.

2-Gender specific difference was detected in vascular invasion (*p=0.047*).Vascular invasion of tumoral cells was recorded in 80.4%t of males whereas the same pattern was detected in 58.3% of females only.

3-2 Giemsa staining

Giemsa staining on 71 cases showed H.pylori infection in non tumoral tissue slides of 10/47(21.3%) of males whereas this frequency was recorded in 2/24 (8.3%) of females (*p=0.196*).Surprisingly H.pylori infection was rarely detectable in tumoral cells of both genders .We found the same pattern in other gastric adenocarcinoma patients who were previously admitted in the same hospitals and were not included in this study. No statistical association was detected between H.pylori infection and clinicopathological features of patients .Due to the lack of H.pylori infection in tumoral tissue slides of most patients we considered H.pylori infection in adjacent normal tissue slides for the rest of study.

3-3 Differential expression of AR in both genders

Nuclear expression of AR was not detectable in stromal, lypmphatic and vascular cells of gastric tissue specimens. Differential scores of AR were recorded on the basis of AR expression in tumoral (TAR), Surface Epithelial (SEAR) and Glandular Epithelial (GEAR) cells.

Out of 71 cases ,17(24%) patients showed mild and moderate TAR expression (2cases 2+ and 15 cases 1+) while only 5(7%) of cases showed mild GEAR staining .Out of three AR expressive cells, the highest level belonged SEAR(52%).

Both genders were negative to Lymphatic, Vascular and stromal AR expression and no statistical significant difference was recorded between males and females regarding GE AR, SE AR and TAR expression.

3-4 Association between H.pylori infection and Androgen Receptor expression

Out of H.pylori positive cases 71.4% showed SEAR while SEAR was detectable in only 45% of H.pylori negative patients (*OR=3.056, CI 95% 0.475-19.657*). Linear regression analysis showed H.pylori infection as a significant predictor of SEAR expression (p=0.001). When we excluded females who didn't show the same pattern, males showed the same significant association (*p=0.003, OR=5, CI 95%0.442-56.62*). That means 83.3% of H.pylori positive males showed SEAR expression whereas all (100%) H.pylori positive females were negative to SE AR (Fig. 1).

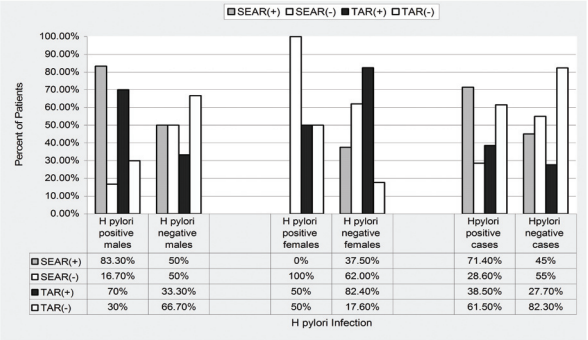

Figure 1. *This Figure shows that 83.3% of H.pylori positive males were AR (+) in their surface epithelial cells whereas all (100%) H.pylori positive females were negative to surface epithelial AR expression.*

H.pylori infection was not recognized as a significant predictor of TAR in both genders. As we didn't find any clinical significance for TAR and GEAR therefore we considered SEAR as the main Androgen Receptor for the rest of this study.

3-5 Role of H.pylori, gender and Androgen receptor on uPA expression

uPA was detected in tumoral, surface epithelial and lymphatic cells of intestinal, diffuse and mucinous gastric adenocarcinoma.Significant difference between stromal uPA expression of males and females (*30% vs.53.7%, p=0.007*) was recorded.

H.pylori infection, SEAR and sex were detected as three significant factors of tumoral uPA (*p=0.045*) and stromal uPA (*p=0.03*) expression due to linear regression analysis.

When the same study was conducted in two genders separately, significant effects of H.pylori, SE AR and

vascular invasion on Tumoral uPA (*p=0.004*), Stromal uPA (*p=0.012*) and lymphatic uPA (*p=0.016*) was recorded in males. uPA expression in females was not correlated with above factors.

4- Discussion

Although most of H. pylori-related gastric diseases are associated with male gender but the role of gender as a risk factor of H. pylori infection is still controversial moreover the pathogenesis of gastric cancer with higher prevalence in males cannot be explained by H. pylori only (Gunther JD et al. 2005) therefore it might be necessary to recognize other unknown factors in both genders. We have compared the clinicopathological features of H.pylori positive and H.pylori negative males and females and revealed only significant gender-related differences in their histological grades and vascular invasion at the first step. By conducting the study we recorded H. pylori infection and Surface Epithelial AR(SEAR) as two independent cofactors of tumoral and stromal expression of uPA in male patients only. Although no significant difference was found between tissue levels of AR in males and females which is in accordance with the unique previous study (Kominea A et al..2004) but we showed the important role of H. pylori on male gastric cancer development by inducing SEAR and angiogenic factors for the first time in our clinical setting.

We have proposed Androgen Receptor as one of male specific unknown factors which was overexpressed in surface epithelial cells of 53% of patients. Surprisingly 83.3% of H.pylori positive males showed AR overexpression in their surface epithelial cells whereas it was not found in H.pylori positive females. Although it is not so easy to interpret the mechanism of H.pylori infection on inducing AR overexpression but it is suggested Cortisol has the ability for binding to AR, if androgens are absent or deficient (Maruyama S and Sato S 1985) and one recent study has also demonstrated that patients with gastric H. pylori colonization have significantly lower cortisol levels when compared with H. pylori negative cases which may cause AR overexpression in male surface Epithelial cells. Although they found a negative correlation between H. pylori colonization and urine cortisol output in normal population (Koşan B et al.. 2008) but more studies are indicated to find out the exact underlying mechanisms of AR overexpression in H.pylori positive gastric cancer males. It sounds also necessary to find the association between cortisol, serological androgen levels and surface epithelial expression of AR in H.pylori positive and H.pylori negative males and females .The most likely scenario is that gastric cancer in males may be the result of a sequence of changes, some of which may have been initiated by H. pylori and its direct role on SEAR expression. We found one of these effects on uPA expression in present study.

In fact higher vascular invasion (*p=0.047*) and higher expression of uPA in stromal cells of male patients (*p=0.007*) clued us to different tumor progressive factors in males which may originated from AR expression and H.Pylori infection. Linear regression analysis showed H.pylori infection, SEAR and sex as three significant factors with significant roles on Tumoral uPA (*p=0.004*), Stromal uPA (*p=0.012*) and lymphatic uPA (*p=0.016*) expression males which play important roles in the invasion and metastasis of gastric carcinoma and serve as effective markers of the biopathological behavior of gastric tumor (Deng W et al. 2007).

It was previously hypothesized that the toxic effects of H.pylori on angiogenesis occurs in early preclinical disease phase or in long-lasting aggressive infections, but only when high H.pylori IgG levels are persistent (Mangia A et al..2006) .This study suggests the toxic role of H.pylori on angiogenesis by its effects on AR expression in male cases without considering the IgG levels. Present results suggest the strong role of H.Pylori on angiogenesis in male which could be considered as a new molecular mechanism of tumor progression by interacting with the receptor of male hormone and angiogenic pathways.

As the existence of sex-specific angiogenesis, H.pylori infection and AR have seldom been addressed in previous works therefore present findings would give a valuable insight into gastric cancer progression and ultimately be of use in gender specific treatment strategies of gastric adenocarcinoma.

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