Expression of E-Cadherin and H.pylori in Sudanese patients with gastric adenocarcinoma and gastrointestinal stromal tumors.

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المستخلص

المقدمة:

هنالك دور لبروتين الايكادرين و للملوية البوابية (جرثومة المعدة) فى سرطان المعدة و السرطان المعدى-المعوى الاسترومى و تطورهما. هدفت هذه الدراسة لايجاد رابط بين هذين السرطانين و الايكادرين و الملوية البوابية و اختبارهما كواصمات حيوية فى مجموعة من العينات السودانية المشخصة باى من سرطان المعدة أو السرطان المعدى-المعوى الاسترومى.

المواد و الطرق:

احتوت الدراسه على خمسون عينه محفوظة فى شمع البارافين و مأخوذة من عينات مشخصة مسبقا اما بسرطان المعدة أو السرطان المعدى-المعوى الاسترومى. و قد وفرت هذه العينات من معامل مختلفة بولاية الخرطوم. تراوح عمر المرضى مابين ١٥-٨٠ عام، متوسط عمر المرضى كان ٦٠ عاما. حساب القيمة الاحتمالية كان باستخدام مربع كاى (القيمة الاحتمالية تكون اقل من 0.05 للناتج ذوى الصلة). استعملت تكنولوجيا الاميونوهستوجيمسترى لختبار وجود بروتين الايكادرين و الملوية البوابية كواصمات حيوية.

النتائج:

مثل الذكور ٦٦% و الاناث ٣٤% من حاصل عدد عينات البحث الخمسون. ٧١% من العينات شخصت كحلات سرطان المعدة و ٢٩% من العينات شخصت بسرطان المعدى المعوى الاسترومى. عند ربط الايكادرين و الملوية البوابية كواصمات حيوية و علاقتهما بالسرطانات سابقة الذكر، فقد وجدنا ان هنالك رابط بين الملوية البوابية و الدرجات المتقدمة من سرطان المعدة بينما لم يكن هنالك وجود لبروتين تلك البكتيريا و سرطان المعدى-المعوى الاسترومى. من ناحية اخرى فقد ثبت توقف انتاج بروتين الايكادرين فى الدرجات المتقدمة لسرطان المعدة و سرطان المعدى-المعوى الاسترومى.

المناقشة:

تطور السرطان المعوى ذو النوع الغدى و السرطان المعدى-المعوى الاسترومى ينتج عن عدم وجود بروتين الايكادرين بينما بروتين الملوية البوابية ليس له صلة بتطور السرطان لكن ربما له صلة فى حدوثه. من ناحية اخرى فان هذه الدراسة لم تجد علاقة للملوية البوابيى بسرطان المعدى-المعوى الاسترومى. وعلية نأكد جدوى اختبار بروتين الايكادرين كواصم حيوى للكشف عن سرطان المعدة و سرطان المعدى-المعوى الاسترومى فى العينات السودانية.

Abstract.

Introduction: E-cadherin (epithelial-cadherin), is a trans-membrane glycoprotein and is down regulated in gastric cancer. *H. pylori* (Helicobacter pylori) infection is associated with down-regulation of E-cadherin at the early stage of gastric cancer development. Testing E-cadherin and *H.pylori* expression can provide potential clinical applications for diagnosis, prognosis, and therapeutic targets in gastric cancer. The role of E-cadherins and *H.pylori* in the progression of gastric cancer and gastrointestinal stromal carcinoma has never been studied in Sudanese samples before. This study aimed to

assess the effect of *H. pylori* infection and E-cadherin expression and their co-expression in gastric mucosa.

Materials and Methods: Fifty consecutive paraffin tissue blocks from patients diagnosed with gastric cancer (GCs) and gastrointestinal stromal carcinomas GISTs were enrolled to assess E-cadherin and *H.pylori* expression using immunohistochemistry biomarkers. The patients median age was 60 and mean age was 55.08. P-value was calculated using Chi- squire test (*P value <0.05* is significant).

Results: There was 33 (66%) samples from males and 17 (34%) from females. Thirty five (71%) were diagnosed with adenocarcinoma of gastrointestinal tract (GCs) and 15 (29%) were Gastro Intestinal Stromal Tumor (GISTs). Out of the 50 samples; 4 (8%) were diagnosed with well differential adenocarcinoma, 18 (36%) diagnosed with moderately differential adenocarcinoma, 13 (27%) diagnosed with poorly differentiated adenocarcinoma and 15 (29%) were diagnosed with gastrointestinal stromal tumor (GISTs). Expression of *H.pylori* were detected in 8 (16%) of GCs while in 42 (84%) of the samples *H.pylori* protein were not expressed. E-cadherin, was expressed in 18 (36%) while in 32 (64%) E-cadherin was unexpressed. Histological differentiation of GCs and GISTs showed significant statistical association (*P=0.02*) with E-cadherin under-expression. *H.pylori* has significant statistical association with histological differentiation of GCs (*P=0.01*) especially moderately differentiated and poorly differentiated adenocarcinoma, however, *H.pylori* expression was not detected in GISTs and well differentiated adenocarcinoma cases. Moreover *H.pylori* and E-cadherin co-expression showed insignificant association (*P= 0.391*) with neither GCs, nor with GISTs.

Discussion: Progression of gastric adenocarcinomas and GISTs is mainly dependant on under-expression of E-cadherin. *H.pylori* might play a role in GCs development, but it does not have a role in GISTs development. Our findings showed that E-cadherin can be used as biomarker for detection of GCs and GISTs in Sudanese patients.

Keywords: Gastointestinal adenocarcinoma, gastrointestinal stromal carcinoma, Gastric cancer in Sudan, E-cadherin expression, H. pylori.

1. Introduction:

Gastric cancer (GC) is the third most common cause of cancer-related death in the world (1). Depending on glandular architecture, cellular pleomorphism and mucosecretion., adenocarcinoma of the gastrointestinal tract may present three degrees of differentiation: well, moderate and poorly differentiated (2). GC is more associated with environmental factors such as infection with *H. pylori* (3, 4). Gastrointestinal stromal tumors (GISTs) on the other hand; are mesenchymal neoplasms of the gastrointestinal tract (5). GISTs are the most prevalent mesenchymal tumors, and are responsible for 2.2% of malignant gastric tumors without any gender preference (6). The association of *H.pylori* with GISTs is that H. pylori-induce epithelial response which can direct the homing of mesenchymal stem cells MSCs into the gastric mucosa (7, 8) and there MSCs play critical roles in gastric carcinogenesis and progression (9).

E-cadherin., a trans-membrane glycoprotein encoded by the *CDH1* gene, plays a significant role in adhesion and differentiation of epithelial cells (10). E-cadherin, expresses itself in the majority of the epithelial tissues on the cells' surface and has a very important role in establishing the

cellular polarity, maintaining of the epithelial integrity and cellular differentiation (11). Therefore, dysregulation of E-cadherin leads to alteration of cell polarity, increases cell survival, and promotes cell invasion and migration (12). These effects persuade cancer initiation and progression, including GC (13). It has been proposed that the loss of E-cadherin-mediated cell-cell adhesion is a precondition for tumor cell invasion and metastasis formation (14). Moreover, under expression of the E-cadherin is found in GC and correlates with metastatic ability (15).

Helicobacter pylori (*H. pylori*), which is a Gram-negative bacteria that selectively colonizes gastric epithelium and induces chronic inflammation (16, 17, 18, 19). This bacteria was classified by the World Health Organization as a class I carcinogen for GC, and as virtually all infected persons have superficial gastritis, it is likely that *H. pylori* plays a causative role early in the progression to adenocarcinoma (20). *H. pylori* infection is associated with down-regulation of E-cadherin, probably by generating cell signaling events that counteract the normal function of protein (21). The resulting increase in permeability mediated by the reduction in cell adhesion might allow *H. pylori* antigens to reach the gastric lamina propria and activate the mucosa immune system, with resultant tissue damage (22).

According to GLOBOCAN 2012; more than 70% of cases of stomach cancer occur in developing countries. This underlies the importance of studying the possible causation of GC in Sudan and similar countries and the molecular mechanism that lead to GC development. Therefore in this study we aimed to detect the expression of E-cadherin and *H.pylori* using immunohistochemical (IHC) technique and then correlate IHC findings with prognostic parameters for GC among the studied population.

2. Materials and Methods:

2.1. Samples:

A total of 50 paraffin block samples of gastric cancer (GC) were selected from the available paraffin blocks of collaborating pathology labs in Khartoum state. The samples constitute 33 male and 17 female patients. Their age ranged from 15 to 80 years old (table 1). 35 patients were diagnosed with different grades of gastric adenocarcinomas and 15 were diagnosed with gastrointestinal stromal tumors (GISTs) (table 1).

Table (1): Distribution of cases on the different pathological diagnosis, age and sex:

Number of patients	Sex distribution	Age range / years	Histopathological diagnosis
4 cases	4 males	42-80	WDAC
18 cases	15 males and 3 females	27- 77	MDAC
13 cases	8 Males 5 females	31- 71	PDAC
15 cases	6 males and 9 females	15-75	GISTs

**WDAC = well differential adenocarcinoma, MDAC = moderately differential adenocarcinoma, PDAC = poorly differentiated adenocarcinoma, GISTs = gastrointestinal stromal tumor*

2.2 Immunohistochemistry (IHC):

Paraffin waxed sections of 3µm thickness were cut and placed on slides and then mounted onto Stalinized slides (Fisher brand). Following de-paraffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval for *H.pylori* and E-cadherin using PT link Dako An Agilent Technologies Company (allows the entire pre-treatment process of deparaffinization, rehydration and epitope retrieval to be combined into a well-documented, 3-in-1 specimen preparation procedure). Briefly, slides were placed in slide tank containing sodium citrate buffer (pH 9.0), then boiled at high Temp for 20 minutes, then sections were cooled at RT. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 minutes, then Slides were incubated with 100-200 µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then were rinsed in Phosphate buffer saline. The primary antibody for *H.pylori* and E-cadherin, was ready to use. After washing with PBS for 3 min, binding of antibodies were detected by incubating for 20 minutes with dextrin labeled polymer (Thermo Shandon Limited). Finally, the sections were washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides were counterstained with Mayer's haematoxylin for one minute and washed in water and blued in 0.05% ammoniated water for 16 seconds, then washed in tap water ,dehydrated through ascending grades of ethanol (50%, 70%, 90%, 100%) two minutes for each then cleared in two change of xylene two minutes for each, and mounted in Di-N-Butyle Phthalate in xylene (DPX) mounting media.

Results obtained were detected by researcher and confirmed by experienced histopathologist

2.3. Statistical analysis:

Patients' data were analyzed using statistical package for social science (SPSS) computer program. Frequencies, means, chi-square tests and independent t- test were used. P-value was calculated using Chi- squire test (*P value <0.05* is significant).The project is a retrospective study, ethical approval were obtained from the hospital which provided the samples.

3.1. Results:

The 50 sample were divided into 35 (71%) were adenocarcinoma and 15 (29%) were GISTs. Median age was 60 and the mean age was 55.08. The 50 samples were divided into 33(66%) were male and 17 (34%) were female. Out of 50 samples 4 patients (8%) with well differential adenocarcinoma (WDAC), 18 patients (36%) were diagnosed with moderately differential adenocarcinoma (MDAC), 13 patients (27%) were diagnosed with poorly differentiated adenocarcinoma (PDAC) and 15 patients (29%) were diagnosed with gastrointestinal stromal tumor (GISTs) (table 2).

Gastric cancer revealed positive expression of *H.pylori* in 8 (16%) samples and negative expression of *H.pylori* in 42 (84%) samples. E-cadherin, on the other hand, was expressed in 18(36%) while in 32 (64%) E-cadherin was unexpressed (figure 1, 2, 3). Histological differentiation of GC and GISTs showed significant statistical

association (*P=0.02*) with E-cadherin under-expression (table 3). *H.pylori* has significant statistical association with histological differentiation of GC (*P=0.01*) and was not detected in GISTs cases (table 4). However, *H.pylori* and E-cadherin expression showed insignificant association (*P= 0.391*) (table 5).

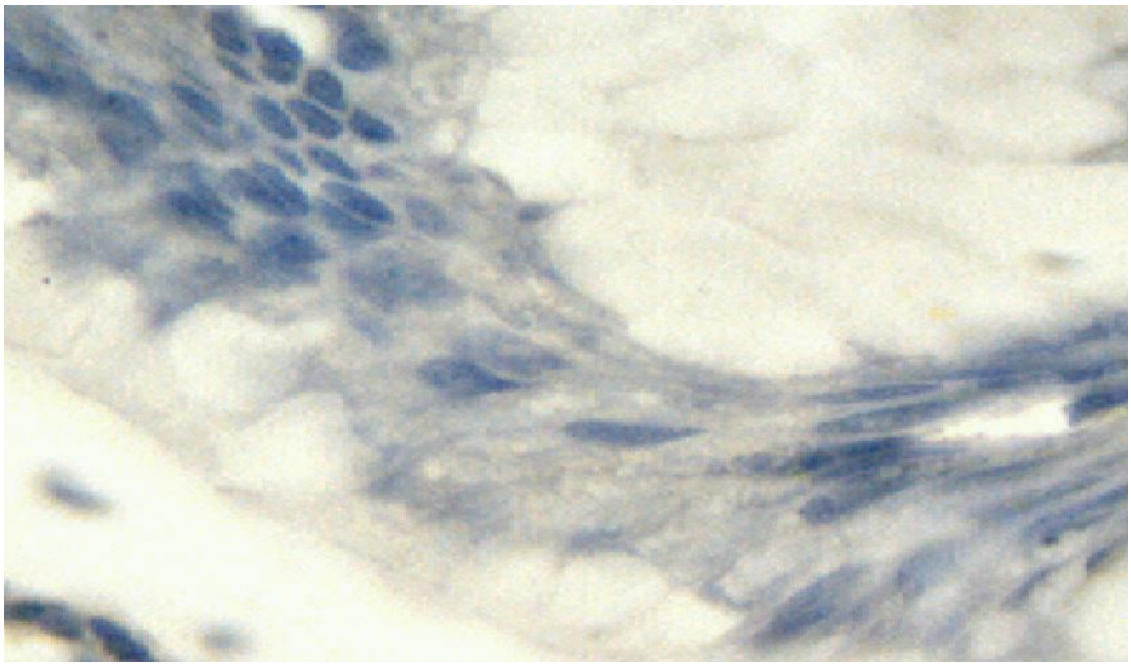

Figure 1. Reduction of E-cadherin expression on gastric adenocarcinoma (Original magnification 100x).

Figure 2: Expression of E-cadherin on gastric adenocarcinoma (Original magnification 100x).

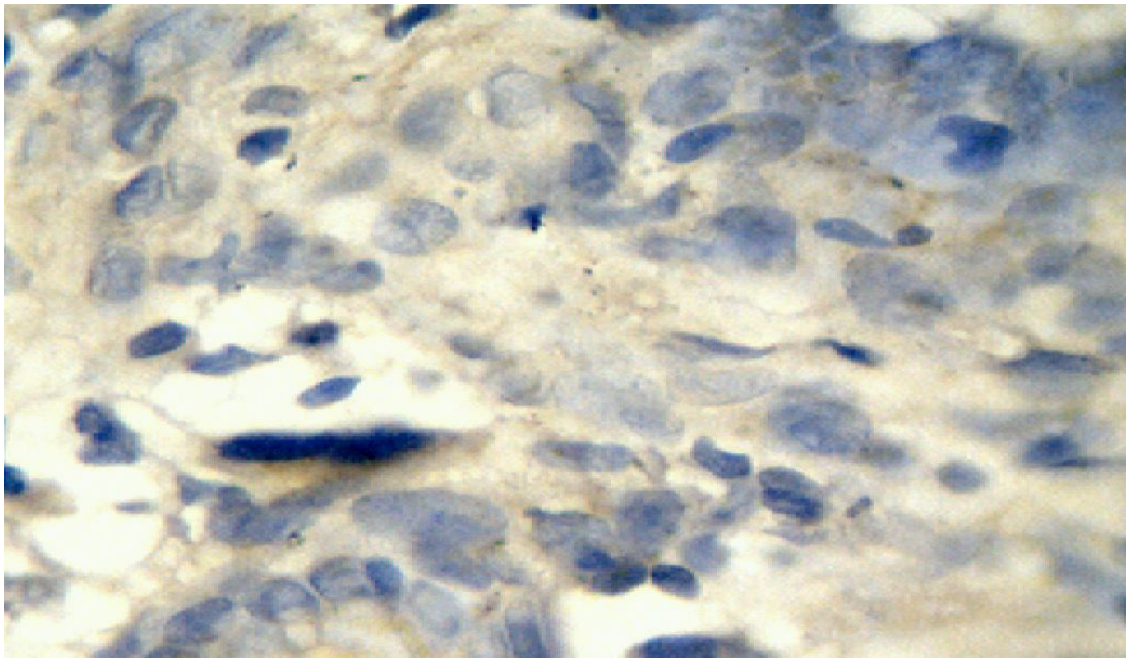

Figure 3: *H.pylori* expression on gastric adenocarcinoma (original magnification 100x)

Table 2: Distribution GC histopathological diagnosis among study studied population

Grade	Frequency	Percent
WDAC	4	8%
MDAC	18	36%
PDAC	13	27%
GISTs	15	29 %
Total	50	100%

Table 3: Cross-tabulation of E-cadherin expression in the different grades of GCs and GISTs.

The marker	Grade				
E-cadherin	WDAC	MDAC	PDAC	GISTs	Total
Positive	4	5	3	6	18
Negative	0	13	10	9	32

Total	4	18	13	15	50

P=0.020 (Chi Square Test)

Table 4: The cross-tabulation of *H.pylori* expression in different grades of GCs and GISTs.

The marker	Grade				
H.pylori	WDAC	MDAC	PDAC	GISTs	Total
Positive	0	3	5	0	8
Negative	4	15	8	15	42
Total	4	18	13	15	50

P=0.015 (Chi Square Test)

Table 5: Cross-tabulation between *H.pylori* and E-cadherin expression

E-cadherin	*H.pylori* expression	Total

	positive	Negative	
Positive	2	16	18
Negative	6	26	32
Total	8	42	50

P=0.391 (Chi Square Test)

Discussion:

Based on the study findings, there are no differences in distribution of age and sex of GCs patients in Sudan compared with that known worldwide. GCs is rare below the age of 55 years old (23, 24) and the proportion of females with GCs showed a decreasing tendency than that of males (25, 26, 24).

H. pylori expression was highly correlated with grade of cancer, mainly in MDAC and PDAC. Our findings might be supported by Machado et al., in 2009 who pointed out that *H.pylori* colonization is in part responsible of gradual development of gastritis, then to later stage of gastric carcinoma (27). It is known that long-term infection with *H. pylori* induces gastric adenocarcinoma in humans (28, 29), however, here *H. pylori* was detected in small number of samples and were mainly associated with advanced grades. This, in a way, can assist the relation of *H. pylori* with the gradual change of cells to cancer cells. Although *H.pylori* has mechanistic effect on metastatic GISTs but it was not expressed in GISTs samples in this study.

The under-expression of E-cardherin was correlated with GCs grades as well as with GISTs. The decreased membranous expression of E-cadherin was previously detected in GCs by

Shimoyama & Hirohash in 1991 and Mayer et al., in 1993 (30, 31). The under-expression of E-cardherin in the study samples was noticed in moderately as well as advanced grades of GCs. This indicates that the role of under-expression of E-cadherin in progression of GCs. However, there was lack of information on metastasis, in GCs. Therefore, and because it is associated with the gradual developmental grading of cancer in this study we can say that this grading can end to metastatic stage. In addition, GISTs also showed significant association with under-expression of E-cadherin, but, we lack data about metastasis. However, low E-cadherin expression was significantly correlated with metastasis in GISTs (32). Although of the lack of information of metastasis; this study showed the role of under-expression of E-cadherin in GISTs. Moreover, there was no significant association between under-expression of E-cadherin and *H pylori*-positive tumors. These findings contradict with the known role of *H. pylori* infection in down-regulation of E-cadherin, which probably results in the reduction in cell adhesion which results in tissue damage (22). This contradicting result might be due to the limited positive samples for *H.pylori* protein expression on the surface of the tumor tissue. It might also show that GCs development and progression is not necessary depend on infection with *H.pylori* and that the role of under expression of E-cadherin is the sole mechanism responsible for the tumor development and progression.

Conclusion:

Progression of gastric adenocarcinomas is mainly dependant on under-expression of E-cadherin, while expression *H.pylori* might rather play a role in tumor development. Although development of gastrointestional stromal carcinomas is associated with E-Cadherin under- expression, *H.pylori* on the other hand is not associated with gastrointestional stromal carcinomas. Therefore, it is essential to test the expression of

E-cadherin as a biomarker for detection of development of gastric adenocarcinomas and gastrointestional stromal carcinomas.

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