Original Article

Evaluation of RAGE (Receptor for Advanced Glycation End-products) Expression in Gastric Carcinoma of Egyptian patients in Relation to Helicobacter pylori Infection

Tarek Aboushousha[1]*, Afkar Badawy[1], Mona Moussa[1], Zeinab Omran[1], Ahmed-Hazem Helmy[2] and MagdyYoussef[3]

Pathology[1], Surgery[2] and Gastrointestinal and Hepatology Medicine[3] departments, Theodor Bilharz Research Institute, Cairo, Egypt.

Keywords: *Gastric Carcinoma, Helicobacter Pylori, Rage.*

ABSTRACT

Background: Gastric cancer is one of the most common malignancies and is the second most common cause of death from cancer worldwide. Gastric cancer is a multistep process that is regulated by intrinsic and extrinsic cellular signals. Extrinsic factors include molecular patterns that are derived from either pathogens or cellular damage, which can promote tumourigenesis. Helicobacter pylori plays an important role in the pathogenesis of chronic gastritis and gastric adenocarcinoma. Receptor for Advanced Glycation End products (RAGE) is a pattern recognition receptor that binds multiple ligands derived from a damaged cell environment, and plays a critical role in promoting the intestinal tumorigenesis. The over-expression of RAGE has been associated with increased invasiveness and metastasis generation in different types of cancer, including gastric cancer. Therefore, the aim of this study was to evaluate the expression of RAGE protein in gastric carcinomas either in cases associated with Helicobacter pylori (Hp) infection or not, so as to predict its value as a target for therapy.

Methods: 51 endoscopic and 19 surgical gastric biopsies including cases of gastric carcinoma, intestinal metaplasia and chronic gastritis were histopathologically and immunohistochemically studied for RAGE expression and were statistically discussed.

Result: RAGE was not expressed in any case of gastritis or signet-ring gastric carcinoma. The RAGE cellular expression parameters were correlated significantly with the stages of gastric adenocarcinoma and lymph node metastasis and non-significantly with the grade of neoplasia. Our results showed no significant correlation between RAGE expression and Hp infection, either in chronic gastritis or malignant cases.

Conclusion: RAGE expression could be identified as a possible marker for target therapy in some types of gastric carcinoma, possibly to control its invasive and metastatic potential, however, its relation to Hp infection was not quite evident in our current study.

***Corresponding author:**
Dr. Tarek Aboushousha, M.D., Professor of Pathology, Theodor Bilharz Research Institute, El-Nile Street, Warrak El-Hadar, Imbaba, P O Box: 30, Giza 12411,Cairo, Egypt
Phone: 00201222186036
Email: taboushousha@gmail.com

Introduction

Gastric cancer is one of the most common malignancies worldwide, with an estimated 934,000 cases reported globally in 2011, and is the second most common cause of death from cancer.[1] Gastric cancer is a multistep process that is regulated by intrinsic and extrinsic cellular signals. Extrinsic factors include molecular patterns that are derived from either pathogens or cellular damage, which can promote tumorigenesis.[2] Helicobacter pylori (*Hp*) is a gram-negative, spiral bacterium that colonizes gastric mucosa and plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[3] Although the vast majority of *Hp* in colonized hosts are free-living, ~20% bind to gastric epithelial cells and adherence is required for prolonged persistence in the stomach and for induction of injury.[4] *RAGE* is a membrane receptor, belonging to the immunoglobulin family, and the over-expression of *RAGE* has been associated with increased invasiveness and metastasis generation in different types of cancer, including gastric cancer.[3] *RAGE* is a pattern recognition receptor that binds multiple ligands derived from a damaged cell environment, and plays a critical role in promoting the intestinal tumorigenesis.[2] *RAGE* is also an important inflammatory mediator that modulates cross-talk between survival pathways and autophagy in tumor cells. It sustains autophagy and limits apoptosis promoting tumor survival.[5] Although expressed at very low levels in normal tissues, chronic inflammatory conditions increase not only *RAGE* expression, but also the formation and release of some *RAGE* ligands.[6,7,8,9]

Therefore, the aim of this study was to evaluate the expression of *RAGE* protein in gastric carcinomas either associated with Hp infestation or not, in order to estimate the value of it as a possible target for therapy.

Methods

Paraffin blocks of gastroscopic biopsies (51) and gasterectomy specimens (19) of 70 cases diagnosed as ; gastric carcinoma (48 cases) , intestinal metaplasia associated with gastritis (16 cases) and chronic gastritis without intestinal metaplasia (6 cases) received in the pathology department of Theodor Bilharz Research institute, Cairo, Egypt in the interval from Jun. 2012 to Apr. 2014 were included in this study. Hematoxylin and eosin staining was done for routine diagnosis, grading and staging. Giemsa stain was used to help detection of Hp in examined gastric mucosal sections, applying the Sydney updated scoring system.[10] Immunohistochemical staining for *RAGE* was done, and its expression was evaluated and discussed.

Immunohistochemical Method

Anti-RAGE antibody (Santa Cruz Biotechnology) was used for immunohistochemical (IHC) detection of the expression of *RAGE* protein in tissue. Tissue sections were processed for IHC analysis of *RAGE* protein as follows. IHC examinations were carried out on 3 µm thick sections. For anti-RAGE IHC, unmasking was performed with 10 mM sodium citrate buffer, pH 6.0, at 90°C for 30 min. Sections were incubated in 0.03% hydrogen peroxide for 10 min at room temperature, to remove endogenous peroxidase activity, and then in blocking serum (0.04% bovine serum albumin, A2153, Sigma-Aldrich, Shanghai, China, and 0.5% normal goat serum X0907, Dako Corporation, Carpinteria, CA, USA, in PBS) for 30 min at room temperature. Anti-*RAGE* antibody (A11): sc-80652 RAGE Antibody (A11) is a mouse monoclonal IgG_{2a} provided at 200 µg/ml, raised against a truncated extracellular domain of RAGE of human origin (Santa Cruz Biotechnology , USA)

The antibody was used at a dilution of 1:100. The antibody was incubated overnight at 4°C. Sections were then washed three times for 5 min in PBS. Non-specific staining was blocked 5% normal serum for 30 min at room temperature. Finally, staining was developed with diaminobenzidine substrate and sections were counterstained with hematoxylin. PBS replaced RAGE antibody in negative controls.[11]

Quantification of protein expression: The expression of *RAGE* was semiquantitatively estimated as the total membrano-cytoplasmic immunostaining scores, which were calculated as the product of a proportion score and an intensity score. The proportion and intensity of staining was evaluated independently. The proportion score reflected the fraction of positive staining cells (score 0: <5%, score 1: 5%-10%, score 2: 10%-50%, score 3: 50%-75%, score 4: >75%), and the intensity score represented the staining intensity (score 0: no staining, score 1: weak positive, score 2: moderate positive, score 3: strong positive). Finally, a total expression score was given ranging from 0 to 12. Based on the analysis in advance, *RAGE* was regarded as negative expression in gastric cancer tissues if the score <2, and positive expression if the score ≥2.[11]

It is to be noted that expression of *RAGE* in cases of malignancy was estimated only in malignant cells, also, in cases of intestinal metaplasia estimation of *RAGE* expression was estimated in metaplastic cells, not including non-metaplastic gastric epithelial cells.

Statistical analysis:_ Statistical analysis was performed using SPSS.19 software program. For *RAGE* monoclonal

antibody, data were summarized as means and percentage. Means of groups were compared using unpaired t-test. For *RAGE* monoclonal antibody, data were summarized using cross tabulation. correlation tests served in correlating extent, intensity, and pattern of expression of the different *RAGE* parameters with other pathological features (grade, stage, inflammation, *Hp*,...etc).

The interpretation of *p* value:

p value of <0.05 was considered statistically significance. *p* value of <0.01 was considered of high statistical significance.

Results

Seventy cases were examined in this study; of them 57 biopsies were from male patients and 13 were from female patients, with the mean age of 52 years for males (range 34 -72) and 56 years for females (range: 38-84), with no statistically significant difference ($p>0.05$). As regard malignant cases females represent 17 cases, while males represent 31 cases ($p<0.01$). For malignant cases, the mean age for female patients was 58 years while it was 55 years for male patients, with no significant difference ($p>0.05$). (Table 1)

Most of the studied cases were positive for *Hp* (70%). Signet-ring carcinoma cases showed the highest percentage of *Hp* positivity (80%), followed by cases of intestinal metaplasia (68.75%), adenocarcinoma (68.42%) and gastritis (66.67%) without significant differences ($p>0.05$). (Table 2).

On the other hand a significant positive correlation was obtained between *Hp* score and the score of gastritis activity, and significantly inverse correlation with intensity of gastric inflammation ($p<0.05$ and $p<0.01$ respectively).

In all examined cases, the intensity of *RAGE* expression mostly in the cytoplasm of positive cells was inversely correlated with inflammatory activity ($p< 0.05$), while percentage of positive cells expressing *RAGE* was positively correlated with intensity of inflammatory reaction ($p< 0.01$).

The overall *RAGE* score showed positive correlation with the inflammatory intensity ($p< 0.05$) and inverse correlation with the inflammatory activity ($p< 0.01$). (Table 3)

In cases of gastric adenocarcinoma, all *RAGE* expression parameters were non-significantly correlated with tumor grade and *Hp* score ($p>0.05$), while they were correlated positively with tumor stage and inflammatory intensity ($p<0.01$). On the other hand, *Hp* score was correlated positively with the inflammatory activity ($p<0.05$) and correlated inversely with the inflammatory intensity ($p<0.01$) (Table 4)

As regard *RAGE* expression, our study showed that it was absent in non-malignant gastric glandular epithelial cells and in all cases of signet-ring carcinoma. On the contrary, in cases of intestinal metaplasia, *RAGE* was expressed with significant intensity ($p<0.05$) and highly significant percentage ($p<0.01$) and consequently showed higher *RAGE* score compared to cases of adenocarcinoma ($p<0.05$). (Table 5)

In cases of adenocarcinoma, there were non-significantly higher values of *RAGE* intensity and scores in high grade tumors compared to low grade ones, also, there was a non-significant difference between the mean percentage of *RAGE* expression in low and high grade tumors. (Table 6)

RAGE expression parameters (intensity, percentage and score) were non-significantly higher in Hp negative cases of adenocarcinoma compared to *Hp* positive cases (Table 7)

All *RAGE* expression parameters were significantly higher in high stages of gastric carcinoma compared to low ones ($p<0.01$). Also, cases with lymph node metastases (LN+ve) showed significantly higher values of *RAGE* intensity and overall score ($p<0.01$) but a non-significantly higher value of *RAGE* percentage compared to cases without lymph node metastases (LN-ve) ($p>0.05$). (Table 8).

Table 1: Demographic data for studied cases:

Diagnosis	Sex		Total
	f	m	
Adenocarcinoma	14	24	38
Signet-ring carcinoma	3	7	10
Int. Metaplasia*	1	15	16
Gastritis	5	1	6
Total	**23**	**47**	**70**

*Intestinal metaplasia of gastric mucosa

Table 2: Histopathological Diagnosis versus Helicobacter pylori infection:

Histopathology		Hp		Total
		Negative	Positive	
Diagnosis	Adeno Ca	12(31.58%)	26(68.42%)	38(100%)
	Signet-ring Ca	2(20%)	8(80%)	10(100%)
	Metaplasia	5(31.25%)	11(68.75%)	16(100%)
	Gastritis	2(33.33%)	4(66.67%)	6(100%)
Total		**21(30%)**	**49(70%)**	**70(100%)**

Non-significant differences (p> 0.05)

Table 3: Spearman's correlation (rho) in the all-studied cases:

	Hp (score)	Inflammatory Intensity	Inflammatory Activity
RAGE score	-0.068	0.301*	-0.392**
RAGE %	0.067	0.336**	-0.105
RAGE Intensity	-0.064	0.175	-0.408**
Inflammatory Activity	0.526*	0.077	
Inflammatory Intensity	-0.428**		

**p<0.05 significant correlation ; **p<0.01 highly significant correlation*

Table 4: Spearman's correlation (rho) in adenocarcinoma cases:

	Tumor Grade	Tumor Stage	Hp (score)	Inflammatory Intensity	Inflammatory Activity
RAGE score	0.188	0.840**	-0.206	0.535**	-0.314
RAGE %	0.030	0.687**	0.004	0.587**	0.148
RAGE Int.	0.285	0.765**	-0.171	0.331*	-0.316
Inf. Act.	-0.192	-0.454	0.351*	0.023	
Inf. Int.	-0.231	0.313	-0.419**		
Hp (score)	0.110	-0.099			

*p<0.05 significant correlation ; **p<0.01 highly significant correlation

Table 5: Differences in means of *RAGE* parameters between Adenocarcinoma and Intestinal metaplasia:

Diagnosis (Number)	RAGE Intensity Mean±SE	*RAGE* (%) Mean±SE	*RAGE* Score Mean±SE
Adenocarcinoma (38)	1.76±0.71	73.95±20.47	6.42±3.24
Metaplasia (16)	1.95±0.36	91.52±8.26	10.25±2.11
P value	NS	*p*< 0.01	*p*< 0.05

NS: nonsignificant difference (p>0.05)

Table 6: Difference in RAGE parameters in relation to grades of adenocarcinoma:

Grade of AdenoCa (N)	RAGE Intensity Mean±SEM	RAGE % Mean±SEM	RAGE Score Mean±SEM
Low Grdae (22)	1.59 ± 0.14	74.09 ± 4.25	5.91 ± 0.69
High Grade (16)	2.03 ± 0.18	73.75 ± 5.47	7.13 ± 0.81

No significant difference between groups (p>0.05)

Table 7: RAGE expression in relation to Hp infection in cases of gastric adenocarcinoma:

Hp detection (N)	*RAGE* Intensity Mean±SEM	*RAGE* % Mean±SEM	*RAGE* Score Mean±SEM
Hp -ve (12)	2.03 ± 0.25	76.67 ± 3.55	7.50 ± 1.06
Hp +ve (26)	1.65 ± 0.12	72.69 ± 4.59	5.92 ± 0.58

No significant difference between groups (p>0.05)

Table 8: RAGE parameters in studied adenocarcinoma cases as regards histopathological stage and lymph nodes metastasis:

RAGE Parameter	Stage of Malignancy Mean±SEM		LN metastasis Mean±SEM	
	Low (7)	High(12)	-ve(4)	+ve(6)
RAGE Intensity	1.29±0.18	2.50**±0.15	1.00±0 .00	2.50**±0.22
RAGE %	67.14±5.22	89.17**±0.29	85.00± 2.89	93.33± 3.33
RAGE Score	3.71±0.47	10.00**±0.55	3.75±0.25	10.33**± 0.95

** Significant difference between both groups (p< 0.05).*
** High significant difference between both groups (p< 0.01).*

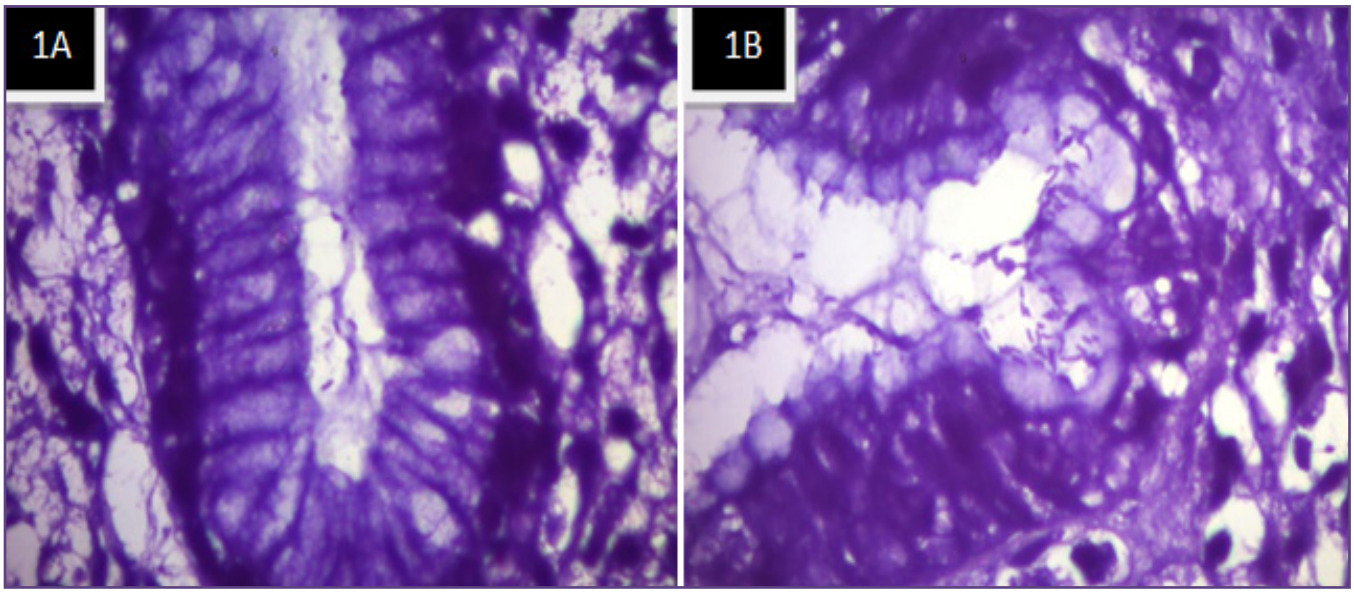


Fig.1: Sections in gastritis cases showing low(1A) and high (1B) scores of Hp infection within superficial mucosal glands. (Giemsa stain, X400).

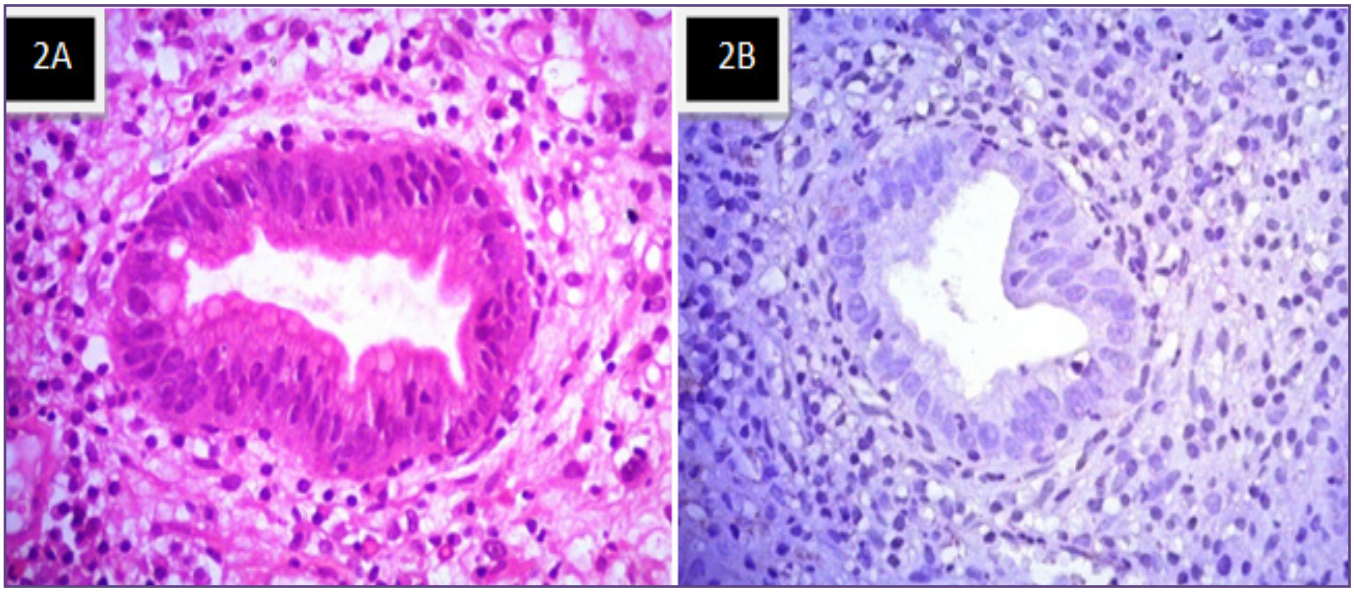


Fig. 2: Sections in case of gastritis showing (2A) moderate inflammatory intensity and activity (H&E stain, X200) and (2B) negative expression of RAGE (Immunohistochemical stain for RAGE, X200).

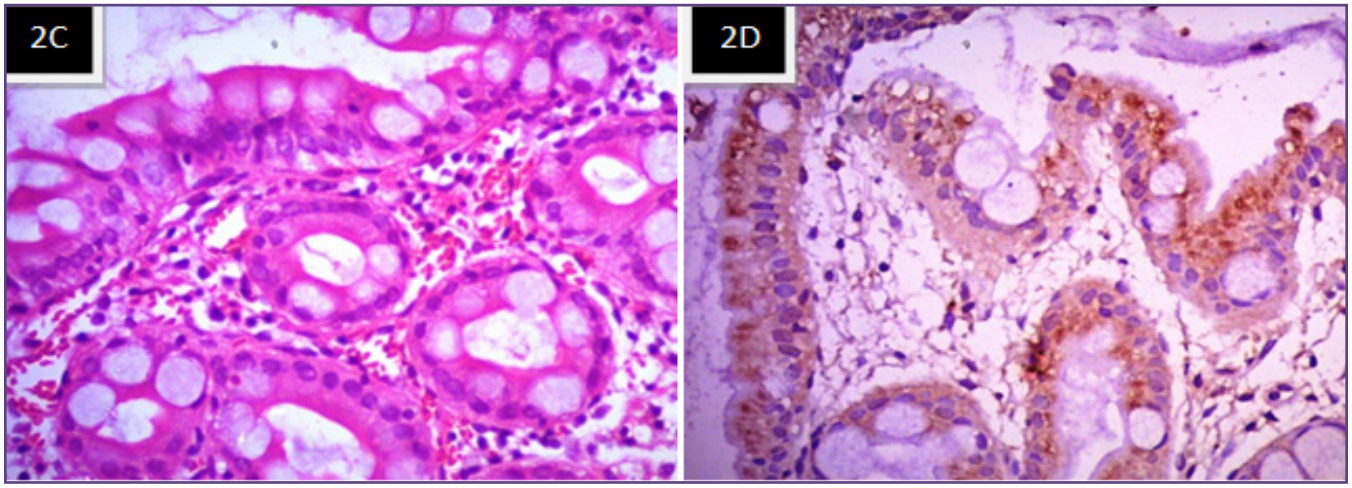


Fig. 2C: Sections in case of gastric intestinal metaplasia. (H&E stain, X200). Fig. (2D): High scores of RAGE expression in case of intestinal metaplasia. (Immunohistochemical stain for RAGE, X200).

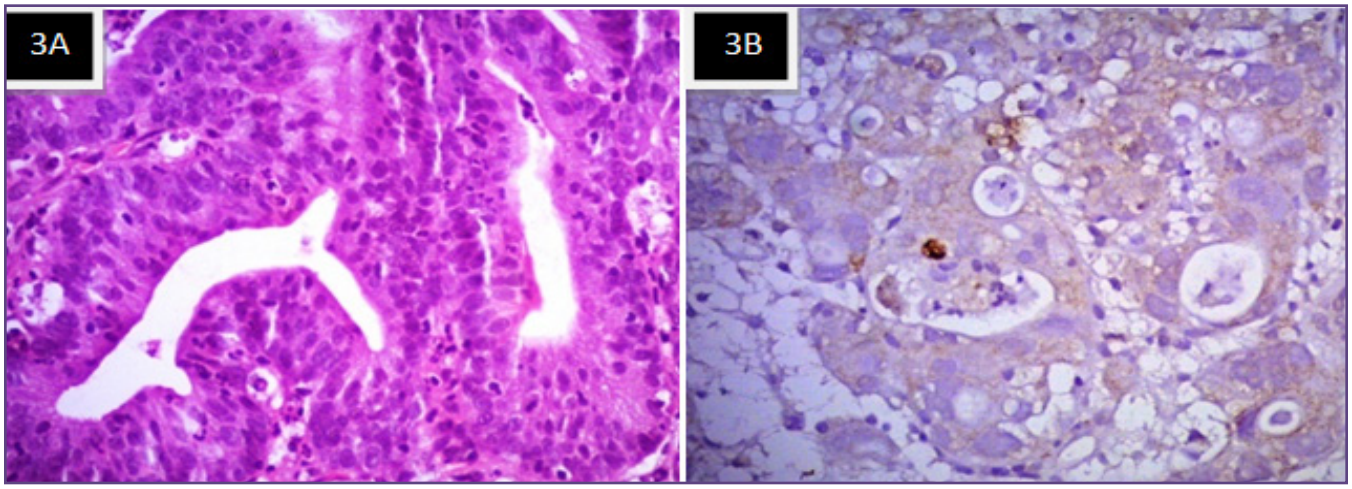


Fig. 3: Sections in cases of low grade gastric superficial adenocarcinoma (3A) exhibiting acinar pattern (H&E stain,X200) showing mild RAGE expression (3B). (immunohistochemical stain, X200)

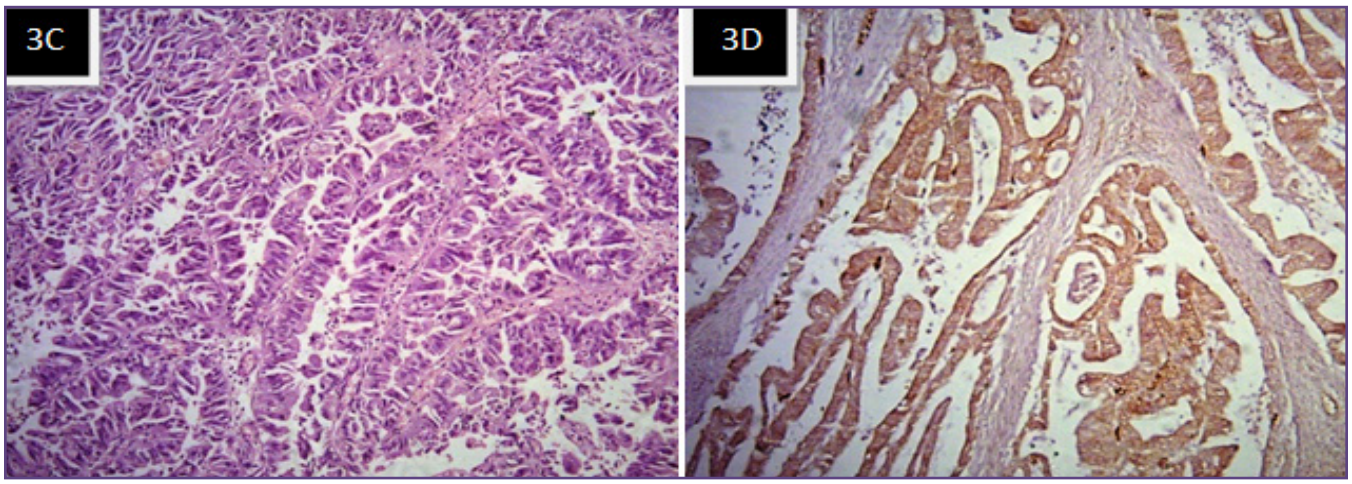


Fig. 3C: Invasive papillary carcinoma (H&Estain, X100) showing high expression of RAGE (3D) by immunohistochemistry (X100).

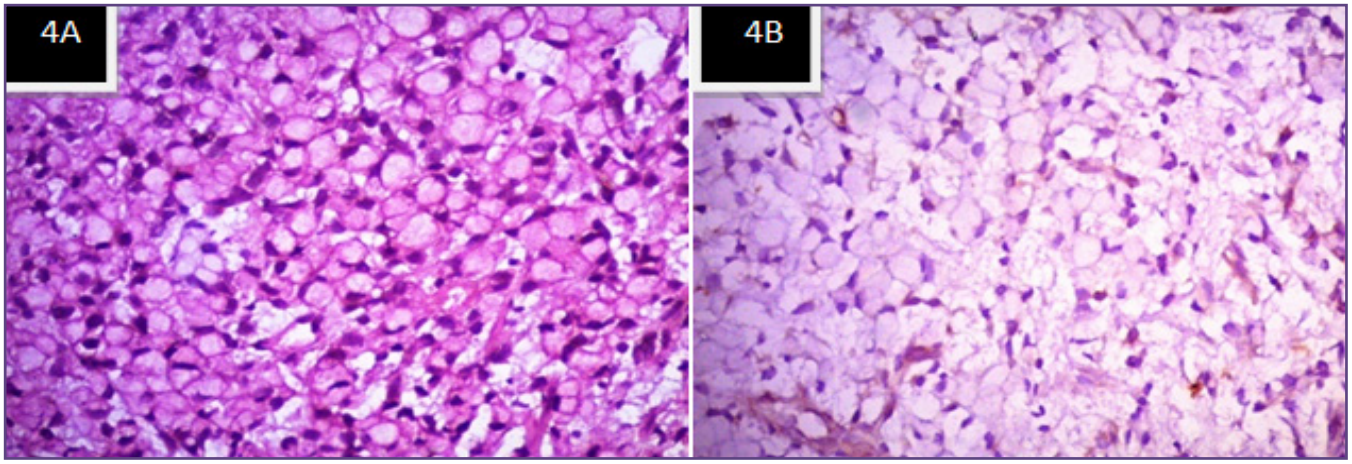


Fig. 4: Sections in signet-ring gastric carcinoma (H&E stain,X200) (4A), showing negative expression for RAGE by immunohistochemistry X200(4B).

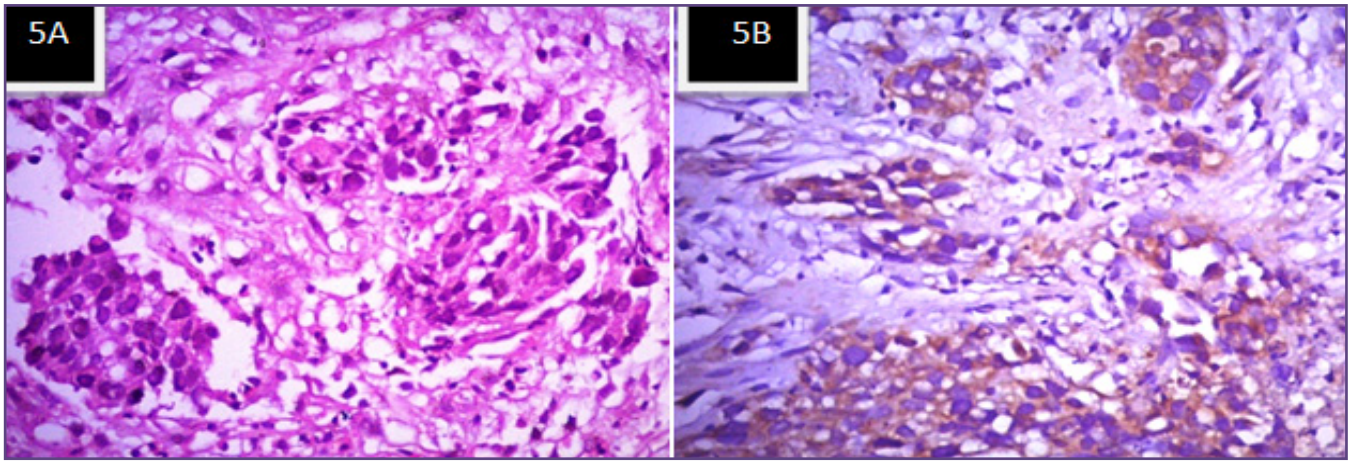
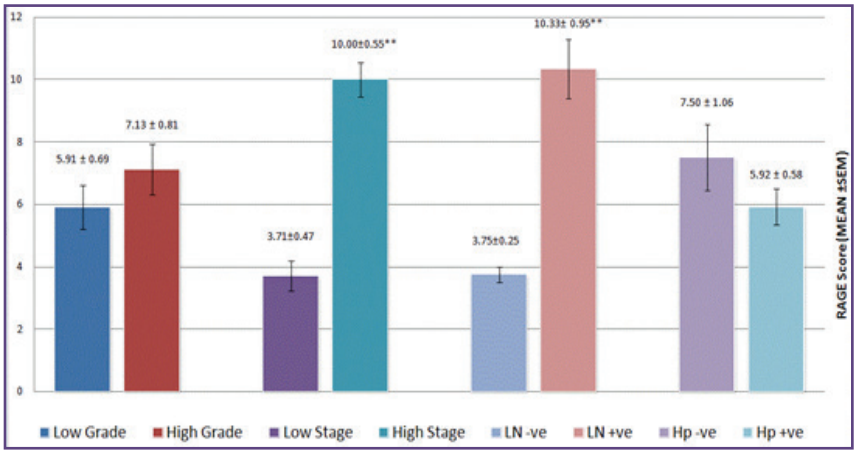


Fig. 5: Sections in grade 3 invasive gastric adenocarcinoma, H&E stain, X200(5A), showing high RAGE expression stained by immunohistochemistry, X200 (5B).

Graph. (1): Comparison between RAGE scores in different grades, stages, LN metastasis and Hp detection status in cases of gastric adenocarcinoma.



**: high significant difference between relevant groups ($p<0.01$

Discussion

Worldwide, gastric cancer (GC) is the 5th most common malignancy in both sexes.[11] In Egypt, GC is the 12th most common cancer in both sexes. The median age of GC in the Egyptians is 56 years.[12] This was in accordance with our results in which there was a significantly higher percentage of male patients with GC than females. Within this group, the difference in the mean age for female and male patients, was non-significant. *Hp* causes gastritis and peptic ulceration and it is an important risk factor for gastric adenocarcinoma, the second highest cause of cancer deaths worldwide. The disease process is thought to have a multifactorial etiology; bacterial strain type, pattern of gastritis, and environmental conditions, are all thought to contribute.[13] Seventy percent of our all studied cases were positive for Hp. Signet-ring carcinoma cases showed the highest percentage of Hp positivity (80%). It was found by some authors that the best established risk factors for GC were Hp infection, male sex, a family history of GC, and smoking. Dietary risk factors are related to diet type and food preservation.[14]

All strains of *Hp* induce a marked inflammation in the gastric mucosa which is characterized by neutrophil, lymphocyte and other inflammatory cell infiltration. While antral-predominant gastritis leads to increased acid production from the uninflamed corpus and predisposes to duodenal ulceration, corpus-predominant gastritis leads to hypochlorhydria and predisposes to gastric ulceration and adenocarcinoma.[15] Our study showed a significant positive correlation between Hp score and the scores of gastritis activity, and significantly inverse correlation with intensity of gastric inflammation. Fundamental characteristic of infection with *Hp* is chronic inflammation of the gastric mucosa, being the role of inflammation as a factor favoring tumor growth of widely recognized neoplastic lesions [16,17,18] and in recent years associated with tumorigenesis related to multiligand / *RAGE* axis. [19,20]

Activation of *RAGE* axis also plays a particular role in the malignant transformation of gastric glandular epithelium. [3] In our study *RAGE* expression was absent in cases of gastritis without intestinal metaplasia, but was upregulated in association with intestinal metaplasia as well as cases of gastric adenocarcinoma. Xu et al. (2013) found that the positive expression of *RAGE* protein was detected in the cytoplasm of gastric cancer cells and was increased in gastric cancer tissues compared with the adjacent non-cancerous tissues (ANCT).[11] Immunoexpression observing a nuclear level *RAGE* is a rare event that needs to be corroborated by other studies to confirm their presence at this level and assess the functional significance of this finding.[3]

We found that the overall *RAGE* score showed significantly positive correlation with the inflammatory intensity and inverse correlation with the inflammatory activity. Aso, we found that all *RAGE* expression parameters (intensity, percentage and score) were non-significantly higher in *Hp* negative cases of adenocarcinoma compared to *Hp* positive cases.

Studies in which gastritis has been followed up over a lengthy period by endoscopy and biopsy after eradication of *Hp* infection have been able to show that the neutrophil infiltrate disappeared completely, while infiltration of the mucosa with lymphocytes and plasma cells persisted, albeit only to a very slight degree. However, other parameters such as intestinal metaplasia or lymphoid follicles, which are often formed in association with *Hp* infection, may still be found in the mucosa several years after eradication of *Hp*.[21,22] These, together with othe factors, occasionally make the differential diagnosis vis-a-vis chemically induced/reactive gastritis somewhat difficult.[10]

These observations could explain -at least partially- why we could not found a valuable correlation between *Hp* infection with other parameters of inflammatory mucosal reaction and *RAGE* expression. Another important point is that *Hp* is not the sole factor exciting gastric mucosal inflammatory reaction and consequently carcinoma. Other factors may be of equal or more importance in Egyptian patients, like diet, toxins, or even therapeutic agents, that should be studied intensively.

Kuniyasu et al.(2002) have reported that, *RAGE* expression is closely associated with the invasion and metastasis in GC patients, which provides us a basis for the immuno-histopathological study of *RAGE* in gastric cancer.[23]

In our work, we found positive correlation of *RAGE* expression parameters with grades of gastric adenocarcinoma, although these correlations were non-significant. However, there was non-significantly higher values of *RAGE* intensity and scores in high grade tumors compared to low grade ones, also, there was a non-significant difference between the mean percentage of *RAGE* expression in low and high grade tumors. Zhang et al. (2015), confirmed in his study that *RAGE* receptor activation is required for gastric cancer cell proliferation.[24]

RAGE expression parameters were correlating positively and significantly with ascending stages of malignancy. All *RAGE* expression parameters were significantly higher in high stages of gastric carcinoma compared to low ones.

Also, cases with lymph node metastases (LN+ve) showed significantly higher values of *RAGE* intensity and score ($p<0.01$) and non-significant higher value of *RAGE*

percentage (p>0.05) compared to cases without lymph node metastases (LN-ve). We found also that *RAGE* intensity and score of expression were significantly higher in lymph node positive cases than in lymph nodes negative ones . A finding that was similar to the results achieved also by Xu et al. (2013).[11]

Previous study found that upregulation of *RAGE* expression was significantly associated with poor clinicopathological characteristics and poor overall survival, suggesting that it may contribute to the malignant potential of GC. Therefore, *RAGE* could therefore serve as a valuable novel biomarker for predicting prognosis and a potential therapeutic target for patients with GC. However, further studies are warranted to clarify the underlying mechanisms of *RAGE* overexpression, thereby contributing to better understanding and further developing of its potential use.[21]

Conclusion

The present study indicated that *RAGE* was evidently upregulated in gastric cancer and pre-cancerous lesions namely intestinal metaplasia, but the correlation of *RAGE* expression with *Hp* infection was indefinite. There may be unexplained variation in the distribution of virulence factors and gastritis patterns that fail to explain the discordance between *Hp* infection rates and the variations in *RAGE* expression in gastric cancer in Egyptian patients. Other carcinogenic dietary, genetic and therapeutic factors should be intensively studied in these patients. However, we have reported that *RAGE* expression is closely associated with increased tumor grade, stage and lymph node metastasis, so, it could be used as a predictor prognostic factor. However, further work is needed to estimate the reliability of *RAGE* expression in relation to variable etiological factors and for being a target for possible therapy in prophylaxis as well as early intervention in high risk patients.

Acknowledgement

We acknowledge Tech. Nadia Abdullah for her kind help in preparing histopathological and immunohistochemical sections.

Ethical Statement

All our biopsy specimens were archival un-identified paraffin preserved materials from the Pathology Department of Theodor Bilharz Research Institute.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90
2. Heijmans J, Büller NV, Hoff E, Dihal AA, van der Poll T, van Zoelen MA, et al. Rage signalling promotes intestinal tumourigenesis. Oncogene 2013;32:1202-6
3. Morales M E, Rojas R A, Monasterio A V, González B I, Figueroa C I, Manques M B, Romero E J, Llanos L J, Valdés M E, Cofré L C. Expression of RAGE in Helicobacter pylori infested gastric biopsies. Rev Med Chil. 2013 Oct;141(10):1240-8.
4. O'Brien DP, Romero-Gallo J, Schneider BG, et al. Regulation of the *Helicobacter pylori* Cellular Receptor Decay-accelerating Factor. J Biol Chem 2008; v.283(35)
5. Kang R, Tang D, Schapiro NE, Livesey KM, Farkas A, Loughran P, et al. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. Cell Death Differ. 2010;17:666-76
6. Sparvero LJ, Asafu-Adjei D, Kang R, et al., RAGE (Receptor for Advanced Glycation Endproducts), RAGE Ligands, and their role in Cancer and Inflammation J Transl Med. 2009; 7: 17.
7. Odenbreit S. Adherence properties of Helicobacter pylori: impact on pathogenesis and adaptation to the host, Int. J. Med. Microbiol. 2005; 295317-324.
8. Schmidt AM, Yan SD, Yan SF, Stern MD. The multiligand receptor RAGE is a progression factor amplifying immune and inflammatory responses, J. Clin. Invest. 2001; 108 949e955
9. Donato R. RAGE: a single receptor for several ligands and different cellular responses: the case of certain S100 proteins, Curr. Mol. Med. 7(2007) 711e724
10. Stolte M, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. Can J Gastroenterol. 2001; 15 No 9,
11. Xu XC, Abuduhadeer X, Zhang WB, T. Li, Gao H, Wang YH. Knockdown of RAGE Inhibits Growth and Invasion of Gastric Cancer Cells. Published online (2013) Nov 18.
12. Gharbiah Population Based Cancer Registry, Egypt. Ministry of Health and Population. Cancer in Egypt, triennial report of 2000–2002. (2007). MOH: Tanta, Egypt.
13. Peek RM Jr, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer. 2002;2:28–37.
14. Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. Methods Mol Biol Clifton NJ, 2009;472:467–477
15. Asaka M, Kato M, Kudo M, et al. Atrophic changes of gastric mucosa are caused by Helicobacter pylori

infection rather than aging: studies in asymptomatic Japanese adults. Helicobacter. 1996 ;1:52–56.

16. Bornschein J, Kandulski A, Selgrad M, Malfertheiner P. From gastric inflammation to gastric cancer. Dig Dis. 2010; 28 (4-5): 609-14.
17. Blakwill F, Montovani A. Inflammation and cancer: back to Virchow? Lancet. 2001; 237: 539-45.
18. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008, 454, 436-44.
19. Rojas A, Figueroa H, Morales E. Fueling inflammation at tumor microenvironment: the role of multiligand / rage axis. Carcinogenesis .2010; 31: 334-41.
20. Riehl A, J Nemeth, Angel P, Hess J. The RAGE receptor: Bridging inflammation and cancer. Cell Communication and Signaling . 2009, 7: 12. Available at: www.biosignaling.com.
21. Wang D, Li T, YeG, Shen Z, Hu Y, Mou T, et al. Overexpression of the Receptor for Advanced Glycation Endproducts (RAGE) Is Associated with Poor Prognosis in Gastric Cancer. PLoSONE10. 2015 ; 4.
22. Stolte, M, Eidt, S. Lymphoid follicles in antral mucosa: immune response to Campylobacter pylori J. Clin. Pathol. 1991: 42, 1269-1271
23. Kuniyasu H, Oue N, Wakikawa A, et al. Expression of receptors for advanced glycation end-products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. The Journal of pathology. 2002;196(2):163–70.
24. QIu-Yu ZHANG, LIN-QING Wu, TAO ZHANG, YAN-FEI HAN, Xu LIN. Autophagy-mediated HMGB1 release promotes gastric cancercell survival via RAGE activation of extracellular signal-regulated kinases 1/2. ONCOLOGY REPORTS 2015; 33: 1630-1638.