ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2021; 13(6); 250-255

Original Research Article

Alpha-Methylacyl-CoA-Racemase expression in Gastric Lesions

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Received: 07-08-2021 / Revised: 10-09-2021 / Accepted: 29-11--2021
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Conflict of interest: Nil

Abstract

Background: Alpha-Methyl-CoA racemase (AMACR) is involved in the beta-oxidation of fatty acids and their derivatives. It is expressed in several neoplasms including prostate, colon cancers. The study aimed to determine the rate of expression of AMACR in various gastric lesions.

Methods: This cross-sectional study was conducted in the Department of Pathology, Kamineni Institute of Medical Sciences Narketpally, Nalgonda, Telangana State. Successive tissue samples with gastric lesions and suspected gastric malignancy were taken for the study. The Samples were stained with Hematoxylin and Eosin for morphological details and immunohistochemistry (IHC) was done to check for the expression of AMACR proteins.

Results: A total of n=41 cases of various gastric lesions were included. N=24 cases of gastric carcinoma cases subjected to AMACR staining. N=14 (58.33%) cases were positive and n=10(41.67%) cases were AMACR negative. Out of the n=24 cases, n=18(75%) cases were male and n=6(25%) were females. The n=9 cases of well-differentiated adenocarcinoma n=6 cases were positive and n=3 cases were negative for AMACR. In signet ring carcinoma n=1 case was positive and n=2 cases were negative for AMACR.

Conclusion: The Expression of AMACR is higher in neoplastic tissue as compared to adjacent dysplastic and non-neoplastic tissue of the stomach. The expression is higher in cases of intestinal adenocarcinoma as compared to signet ring carcinoma. Higher levels of expression in poorly differentiated carcinoma were noted.

Keywords: Alpha-Methyl-CoA racemase (AMACR), Gastric Adenocarcinoma, Dysplasia, Gastritis.

Introduction

Among all the cancers, stomach cancer is the 5th leading cause and the third leading cause of cancer of all cancer deaths making about 7% of cases and 9% of all deaths across the world. [1] In the Indian scenario, the rate of gastric cancer reveals it is the 5th common cancer diagnosed in males and the 7th commonly diagnosed cancer in females. [2, 3] The reported annual incidence across the country is 10.6/1 million population the approximate male to female ratio is 2.1: 1. [1] *Helicobacter pylori* is a gram-negative bacterium commonly found in the stomach. Prevalence of *H. pylori* is considered a precancerous lesion in the stomach and has been classified as a group I carcinogen in the Intestinal type of gastric carcinoma. [4] The progression of gastric cancer is multi-staged from the development of precancerous lesions based on environmental factors to risk factors such as age, non-vegetarian food, tobacco and alcohol use, radiation, and family history. [5-9] The signs and symptoms of stomach cancer are non-specific in the initial stages which may be ignored by many patients and generally reported at advanced stages. The 5-year survival rates are expected to be below 30% in developed countries and less than 20% in developing countries. [10] The knowledge of the occurrence and progression of gastric carcinoma is limited therefore, much significance is applied in the prevention, treatment, and prognostic evaluation of gastric cancers. The conversion of the normal gastric cell to gastric cancer involved multiple genetic and epigenetic alterations of oncogenes, tumor suppressor genes, cell cycle regulators, and cell adhesion molecules. [11] Germline truncating mutations of the E-cadherin gene have been found in families with hereditary cancer of the diffuse type. Since the susceptibility was very high some researchers have proposed prophylactic gastrectomy for such individuals. [12-14] Somatic mutations of this gene have been found in approximately 30% of sporadic diffuse-type carcinoma but not in intestinal-type tumors cancers. [15, 16] A closely related aberration is the loss of beta-catenin, which is bound to cadherin as a complex that regulates cell adhesion. Some of the pathways by which these alterations works are by mTOR, Ras/Raf/Kinase pathway. [17] Alpha-Methyl Acyl Co-A Racemase (AMACR) is also known as P504S, one of the several enzymes involved in the regulation of the mTOR pathway which has been involved in gastric cancer. The present study was conducted to determine if AMACR could be used as a diagnostic marker in Gastric adenocarcinoma.

Material and methods

This cross-sectional study was conducted in the Department of Pathology, Kamineni Institute of Medical Sciences Narketpally, Nalgonda, Telangana State. Successive tissue samples with suspected gastric malignancy were taken for the study. Institutional Ethical permission was obtained from the committee. The biopsy specimens were fixed in 10% formalin, processed, and embedded in paraffin. The histological typing was carried out as per WHO Classification. [18] All the n=41 cases were also subjected to AMACR immunostaining. Two sections of 4-5µ thickness were prepared from corresponding paraffin blocks. One on albumin coated slide for H&E staining and the other on poly- L-lysine coated slide for immunohistochemical staining. The kits for AMACR immunohistochemical staining obtained from DAKO Company were anti-AMACR monoclonal antibodies. Staining was done according to the manufacturer's protocol. Prostate adenocarcinoma tissue sections were used as positive controls. Non-neoplastic gastric tissue samples were used as negative controls. IHC staining was done as per the standard procedure. Slide evaluation was performed by using a Light microscope. AMACR stains a variety of normal and neoplastic tissues. Positive AMACR staining is uniformly described as

being easily visible on low power examination, as circumferential, granular, luminal(apical) to subluminal, and diffusely cytoplasmic in nature. Observed AMACR staining showed the following grades of staining intensity following Luo J et al. [17] (Table 1).

Table 1: Grading of AMACR Intensity

Grade	Staining Pattern
0	when there is absolutely no staining
1	1-10% of cells in a gland show positive staining
2	10-50% of cells in a gland show positive staining
3	>50% of cells in a gland show positive staining

Results

In the present study, the age group ranges from 31-80 yrs. of age the youngest patient was 28-year male, and the oldest case was 74-year female. Of these, the most common group was 51 - 60 yrs. consisting of 39.02% cases followed by age group 41-50 years with 29.27% cases. The male to female ratio is approximately 3:1 detail depicted in (table 2).

Table 2: Age and Sex Distribution

Age in years	Male	Female	Total	Percentage
21-30	0	0	0	0.00
31-40	3	1	4	9.76
41-50	10	2	12	29.27
51-60	11	5	16	39.02
61-70	4	1	5	12.19
71-80	2	2	4	9.76
Total	30	11	41	100

In the present study, the most common symptom of presentation is anorexia in 26.83% of cases, the next most common presentations are weight loss 24.39%, abdominal pain 29.27%, nausea/vomiting 14.63%, dyspepsia 7.31%, the least common presentation was dysphagia 4.87%. Out total n=41 biopsy samples obtained n=32 (78.05%), were from the antrum, and the remaining n=9 (21.95%), were taken either in the cardia, fundus, or body. N=24 cases were gastric carcinoma (table 3) in which n=13 (52.17%), were well-differentiated adenocarcinoma and n=3(12.5%) were moderately differentiated adenocarcinoma and n=4(16.67%), were poorly differentiated and n=4 cases (16.67%) are of signet ring type.

Table 3: Distribution of Lesions According to Histological Type

Histological Type	Frequency	Percentage
Gastritis	14	34.15
Dysplasia	3	7.31
Adenocarcinoma	24	58.54
Total	41	100

AMACR was negative or expressed as focal cytoplasmic positivity in all the cases of gastritis and AMACR staining was observed as circumferential to non-circumferential luminal positivity of grade 2 to 3 in 2/4 (50%) cases of dysplasia and

2/4 (50%) cases are grade 0 (AMACR negative). Out of n=24 cases of gastric carcinoma cases subjected to AMACR staining. N=14 (58.33%) cases were positive and n=10(41.67%) cases were AMACR negative. Out of the n=24 cases, n=18(75%) cases were male and n=6(25%) were females. The n=9 cases of well-differentiated adenocarcinoma n=6 cases were positive and n=3 was negative for AMACR. In signet ring carcinoma n=1 case was positive and the n=2 cases were negative for AMACR the details have been depicted in table 4.

Table 4: Grade Wise Expression of AMACR

	Grade 0	Grade 1	Grade 2	Grade 3	AMACR POSITIVE	AMACR NEGATIVE
Well-differentiated adenocarcinoma	5	2	1	1	6	3
Moderately differentiated adenocarcinoma	3	2	2	1	5	3
Poorly differentiated adenocarcinoma	1	1	1	1	2	2
Signet ring carcinoma	1	1	0	1	1	2
Total	10	6	4	4	14	10

Discussion

In the present study, a total of n=41 gastric biopsies were taken. Out of these n=14(34.15%) were the cases of gastritis none of the gastritis cases showed AMACR positivity. Lee et al., [19] in their study found n= 2(4.5%) out of n=44 non-neoplastic epithelium cases show AMACR positivity. All the cases of non-neoplastic mucosa showing AMACR positivity exhibited moderate immunostaining at the very focal portion of deeper mucosa. Weak staining was mostly noted at the basal portion or proper glands of non-neoplastic mucosa. The possible explanation is it may be due to the presence of abundant endogenous enzymes and also due to differences in epitope recognition or specificity. Cho et al., [20] in a similar study found no AMACR positive in formal gastric mucosa adjacent to adenomas and carcinomas in their n=32 cases. This clear-cut demarcation was AMACR expression was significant. Jiang et al 50, reported that normal stomach expressed very low to non-existent levels of AMACR mRNA using real-time PCR, whereas AMACR mRNA was overexpressed in n=1 of n=7 stomach carcinomas and showed variable copies of the relative expression level of AMACR. Troung et al., [21] reported none of the n=38 non-neoplastic gastric mucosa samples had a detectable expression of AMACR. Our study agrees with the observations of these studies. The difference in expression of AMACR in gastric cancers as compared with non-neoplastic lesions has been studied at mRNA levels. Jiang Z et al., [22] using real-time quantitative PCR demonstrated robust AMACR mRNA expression in gastric carcinoma but very low levels in normal gastric mucosa. In this study n=2(66.67%) cases out of n=3 cases of dysplasia showed AMACR positivity which is comparable with other similar studies where the rate of positivity in dysplasia was 75 – 83%. [61] Huang et al., [23] results showed that AMACR was not expressed in the gastric mucosal specimens with negative and indefinite for dysplasia, but it was observed in 40.8% of gastric biopsy specimens with dysplasia, which suggested that AMACR

may be a useful immunohistochemical marker for detecting dysplasia. In the present study, AMACR expression is seen in 61% of adenocarcinomas and 33.33% of signet ring carcinoma cases. Thus, results are consistent with Lee et al., [19] study regarding expression in intestinal-type adenocarcinoma where 66% of intestinal adenocarcinoma cases were positive and AMACR expression is seen in 37% of signet ring carcinomas. In a study done by Jindal et al., [24] AMACR positivity was seen in 88% in intestinal-type and 78% in signet ring type. Thus, the results of this present study are not consistent with the study done by Jindal et al., [24] Emerging evidence has suggested that one possible function of AMACR in gastric cancer is via its ability to act as an activator of peroxisome proliferator-activated receptor (PPAR)-γ, an enzyme that is predominantly expressed in adipose tissue and has an important function in triggering adipocyte differentiation. [21] Studies have shown that PPAR-γ is expressed in various human cancer cells, including colon, prostate, breast, and gastric cancer cells. Sato et al., [25] observed substantial expression of PPAR-γ in gastric carcinomas disregarding the tumor differentiation as well as PPAR-γ expression in gastric antral mucosa with intestinal metaplasia. Therefore, it appears that AMACR plays an important role in the promotion of gastric cell growth through PPAR- γ activation.

Conclusion

The present study concludes that the Expression of AMACR is higher in neoplastic tissue as compared to adjacent dysplastic and non-neoplastic tissue of the stomach. The expression is higher in cases of intestinal adenocarcinoma as compared to signet ring carcinoma. Higher levels of expression in poorly differentiated carcinoma were noted. Thus, AMACR may be used for differentiating the cases of reactive atypia from early gastric neoplastic lesions.

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