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Original Research Paper

Pathology

ALPHA-METHYL ACYL CO-A RACEMASE EXPRESSION IN GASTRIC BIOPSIES –A 2 YEARS STUDY AT TERTIARY CARE HOSPITAL

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ABSTRACT

Alpha-methylacyl-CoA racemase (AMACR) is a mitochondrial and peroxisomal enzyme involved in beta-oxidation of dietary branched-chain fatty acids and their derivatives. Recent studies showed that AMACR is expressed in several neoplasms, including prostate and colon cancer.The present study was planned to determine if AMACR can be used as a diagnostic marker in gastric adenocarcinoma similar to prostate cancer.

OBJECTIVES: The main objectives of this study is to diagnose nonneoplastic and neoplastic lesions on H&E sections, to infer rate of positivity and negativity in all cases and to correlate AMACR expression with types and grades of adenocarcinoma.

MATERIALS AND METHODS: The present study is a prospective study of two years from July 2015 to June 2017. A total of 62 cases (gastric biopsies) were received at the Department of Pathology, Mahathma Gandhi Memorial hospital,Warangal constituted the material for the present study.Samples were stained with H&E for morphological details and immunohistochemistry (IHC) was done to check for the expression of AMACR proteins.

Statistical analysis was done using chi square test, Spearman's correlation coefficient and Fisher's exact test. The p-value ≤ 0.05 was taken as critical level of significance.

RESULTS: In this study, Immunohistochemical expression of AMACR in a total of 62 cases consisting of 18 (29%)gastritis, 4(6%) dysplasias, 40 (65%)carcinomas was studied.AMACR expression is absent or grade 0 in all 18 cases of gastritis, 2/4 (50%) of dysplasia cases showed AMACR positivity.34/40 (85%) are intestinal type adenocarcinoma and (6/40)15% are signet ring/diffuse carcinoma. AMACR expression is seen in 24/34(70%) of cases of intestinal adenocarcinoma and 1/6(17%) cases of signet ring carcinoma which is statistically significant ($p<0.025$).

CONCLUSION : The expression of AMACR in neoplastic tissue was significantly higher as compared to adjacent dysplastic or nonneoplastic tissue of stomach and its overexpression is seen in intestinal adenocarcinoma compared to signet ring type and well differentiated adenocarcinoma than poorly differentiated adenocarcinoma.

KEYWORDS : AMACR, Gastric carcinoma, Dysplasia, Gastritis

INTRODUCTION

Gastric carcinoma is the third leading cause of cancer death world wide[1] . It is the 5th most common malignancy in the world[2].In India, gastric cancer is ranked as the fifth and seventh most common cancer in Males and females, respectively[3] and is the second leading cause of death from cancer in both men and women[4].Annual incidence rate of gastric cancer in India is 10.6 /1 million population[1]. Incidence ratio - male to female is 2.1 : 1[1]

Incidence of stomach cancer has declined over last decades in developed countries due to identification of risk factors like Helicobacter pylori and dietary and environmental factors. In contrast with general decline in the incidence of proximal gastric cancer ,i.e. adenocarcinoma of cardia and gastro esophageal junction , and barrettsadeno carcinoma has increased in western world[5]. Increase is associated with hyperacidity, reflux esophagitis, barretts esophagus[6,7,8].The symptoms and signs of stomach cancer are often reported late when disease is already in advanced stages and 5 yr survival is less than 30% in developed countries and around 20% in developing countries[9].

Several gene alterations have been associated with gastric cancer and its specific subtype, but our knowledge of gastric cancer tumorigenesis is still limited.Gastric adenocarcinoma (GAC) has a dismal outcome since a high percentage of cases present with advanced disease, and carcinogenesis is still poorly understood.

Therefore, it is of much significance for the prevention, treatment and prognostic evaluation of gastric cancer to clarify its molecular mechanisms & to find out a good biomarker to indicate its carcinogenesis and subsequent progression.

AMACR is a well characterized enzyme that plays a key role in peroxisomal beta-oxidation of dietary branched fatty acids and C27-bile acid intermediates. It catalyzes the conversion of ®-alpha-methyl-branched chain fatty acyl coA esters to their(S) stereoisomers[10.] Only the (S)-stereoisomers can serve as substrates for branched chain acyl-CoA oxidase during their subsequent peroxisomal beta-oxidation .Peroxisomal beta-oxidation generates hydrogen peroxide, a potential source of procarcinogenic oxidative damage.

Emerging evidence indicates that there are several convergent and interconnected signalling pathways that are involved in gastric carcinogenesis and are currently under active investigation. These are the mammalian target of rapamycin (mTOR) pathway, the Ras/Raf/Kinase/ERK pathway and the nuclear factor (NF)-KB pathway[11]. The mTOR pathway is known to regulate protein synthesis, cell-cycle progression, metabolism and angiogenesis. .mTOR pathway is regulated via sequential activation of multiple molecules, including alpha-methylacyl-CoA racemase (AMACR).

Over expression of AMACR has been studied in various cancers like prostate cancer, hepatocellular cancer, breast cancer, colorectal cancer[12].

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. Studies have shown that PPAR-γ is expressed in various human cancer cells, including colon, prostate, breast, and gastric cancer cells[13] Sato et al reported strong expression of PPAR-γ in gastric carcinomas regardless of the tumor differentiation as well as. PPAR-γ expression in gastric antral mucosa with intestinal metaplasia. Thus, AMACR may play a role in the promotion of gastric

cancer cell growth through PPAR- γ activation [13]... Currently, AMACR's potential role as a target for treating gastric cancer seems to be promising. However, confirming this role requires a more thorough understanding of the function of AMACR in gastric tumorigenesis as well as its use as a therapeutic target.

This study aims to examine AMACR expression in gastritis ,dysplasia and gastric adenocarcinoma cases and correlate its expression with types and grades of adenocarcinoma.

MATERIALS AND METHODS:

CASE SELECTION:

The Present Study is a Prospective study of two years from July 2015 to June 2017. A total of 62 cases (gastric biopsies) were received at the Department of Pathology, MahathmaGandhi Memorialhospital (MGM), Warangal, constituted the material for the present study. The tissues were fixed in 10%formalin, processed and embedded in paraffin. The individual case was typed histologically according to WHO classification.The criteria for inclusion in this study were a diagnosis of gastritis, dysplasia, primary gastric adenocarcinoma, no treatment prior to complete surgical resection of the tumor, adequate archival tumor tissue samples available for analysis, and complete clinicopathologic data (age, sex, date of initial diagnosis, histopathologic diagnosias and tumour grade are available on file).

Histologically,Out of 62 cases, 18 (29%)cases were gastritis,4 (6%)cases were dysplasia ,40(65%) cases were gastric carcinoma. . Among 40 gastric carcinomas, 34 cases (85%) are intestinal type and 6 cases (15%) are signet ring /diffuse type.Out of 34 cases,21(52.5%) were well differentiated adenocarcinoma and 6(15%) were moderately differentiated adenocarcinoma and 7(17.5%) were poorly differentiated.age group ranges from 21-80 yrs of age. mostcommon group is 51-60 yrs. 47(76%) of cases constituted by male and 15(24%)of cases are female. Incidence ratio-male to female is **3.1:1.**

IMMUNOHISTOCHEMISTRY: Two sections of 4-5µ thickness were prepared from corresponding paraffin sectionsand coated on poly-L-lysine coated slide for immunohistochemical staining. .The kits for AMACR immunohistochemical staining obtained from DAKO Company were anti-AMACR monoclonal antibody. Staining was done according to the manufacturer's protocol.The sections were de paraffinized, dehydrated & endogenous peroxidase quenching done with 3% H202. Antigen retrieval done by Pressure cooker (HIER, heat induced epitope retrival) with Tris buffer (1.21 g of TrisHydroxymethyl methylamine and 3.75 mg of EDTA in 1000 ml distilled water). incubated with Primary antibody (AMACR) which is ready to use, at room temperature in a humidifier chamber for 30 minutes. The sections were washed with TBS buffer (9.6 g of risHydroxyrnethylmethylaminc and 8.6 g of NaCl in 1000 ml distilled water). Incubated with secondary antibody (Envision HRP) in a humidifier chamber for 30 minutes. Chromogen DAB (DAKO labeled) used for detection of enzymatic activity.Counter staining was done with haematoxylin.Dehydrate in alcohol and xylene and mount with DPX

IMMUNOHISTOCHEMICAL ASSESMENT AND STASTICAL ANALYSIS

Prostate adenocarcinoma tissue sections were used as positive controls. Nonneoplastic gastric tissue samples were used as negative controls

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. AMACR staining showed following grades of staining intensity in accordance with Luo J and ZhaS et al[14].Grade 0-no absolute staining, grade 1-1-10% of cells in a gland show positive staining ,grade 2-10-50% of cells in a gland show positive staining ,grade 3->50% of cells in a gland show positivity.

Statistical analysis was done using chi square test, Spearman's correlation coefficient and Fisher's exact test. The p-value ≤ 0.05 was taken as critical level of significance.

RESULTS: AMACR EXPRESSION IN GASTRITIS AND DYSPLASIA

In present study, there are 18 cases of gastritis and 4 cases of dysplasia. AMACR was negative or expressed as focal cytoplasmic positivity in all the cases of gastritis and AMACR staining 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).

AMACR EXPRESSION IN GASTRIC CARCINOMA

In present study, total of 40 cases of GAC cases are subjected to AMACR staining. out of these 25 cases showed positive staining these include 15 (60%)cases in <60 yrs of age and 10(40%) cases in >60 yrs of age.15 cases shows negative staining, of these 7(46%) cases were <60 yrs of age and 8 (54%)cases were >60 yrs .out of which 17(68%) cases were males and 8(32%) cases were females.15 cases showed AMACR negative staining, include 11(73%) male and 4 (37%)female.

Among 40 cases of GAC,34 (85%) are intestinal type(21 well differentiated,6 moderately differentiated and 7 poorly differentiated) and 6 (15%) are signet ring type.24/34 (70%)cases of intestinal adenocarcinoma show AMACR positivity and 1/6(17%)of signet ring type (SRCC)shows AMACR positivity.Out of 21 cases of well differentiated adenocarcinoma (WD)cases, 18 (85%)cases were AMACR positive and 3 (15%)were negative. Out of 6 cases of moderately differentiated adenocarcinoma(MD), 4(66%) were AMACR positive and 2(37%) were negative. Out of 7 cases of poorly differentiated adenocarcinoma(PD), 2 (28%)were AMACR positive and 5(72%) were AMACR negative.

GRADE WISE EXPRESSION OF AMACR

	Grade 0	Grade 1	Grade 2	Grade 3	AMACR POSITIVE	AMACR NEGATIVE
Well differentiated adenocarcinoma	3	9	5	4	18	3
Moderately differentiated adenocarcinoma	2	2	1	1	4	2
Poorly differentiated adenocarcinoma	5	2	-	-	2	5
Signet ring carcinoma	5	1	-	-	1	5
TOTAL	15	14	6	5	25	15

Clinicopathological Features and AMACR expression of Gastric Carcinoma

		Positive	Negative	TOTAL	P-VALUE
GENDER	MALE	17	11	28	<0.75
	FEMALE	8	4	12	
AGE	< 60	15	7	22	<0.5
	>60	10	8	18	
Grade	WD	18	3	21	<0.00336
	MD	4	2	6	
	PD	2	5	7	
	SRCC	1	5	6	
Type	Intestinal	24	10	34	<0.025
	Diffuse	1	5	6	

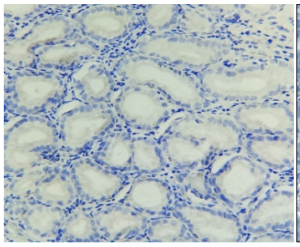

Fig.1.AMACR expression in normal gastric mucosa-grade 0

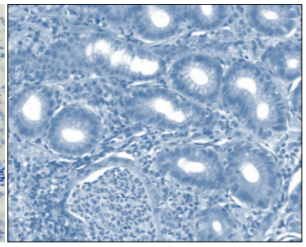

Fig 2.AMACR expression in gastritis grade 0

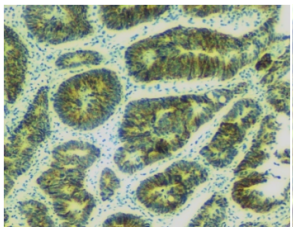

Fig 3.AMACR expression in well differentiated adenocarcinoma grade 3

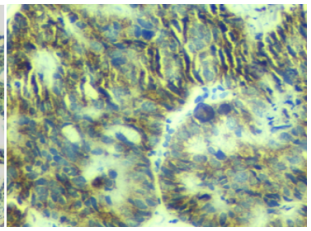

Fig 4 AMACR expression in moderately differentiated adenocarcinoma grade 2

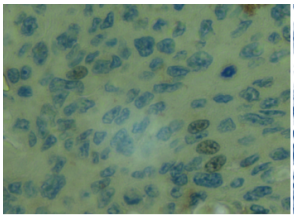

Fig 5 poorly differentiated adenocarcinoma grade 0

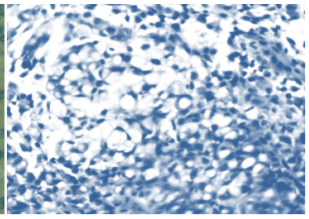

Fig 6 signet ring carcinoma grade 0

DISCUSSION

AMACR EXPRESSION IN GASTRITIS

In present study, total of 62 gastric biopsies were taken. Out of these,18 (29%) cases are gastritis. None of the gastritis cases showed AMACR positivity.

In study done by Lee et al [15], only 2 of 44 (4.5%) non-neoplastic epithelium cases shows positivity.The difference in AMACR expression between adenomas or adenocarcinomas and non-neoplastic mucosa was statistically significant. (p<0.0001).

One possible explanation for AMACR positivity in a study done by Lee et al [15] is nonspecific protein binding in gastric tissue, a known phenomenon due to its abundant endogenous enzymes. In addition, the authors used an AMACR antibody produced by a different bio company, suggesting possible differences in epitope recognition or specificity. In a study done by Cho et al [16], immunoreactivity for AMACR was not found in the normal gastric mucosa adjacent to adenomas and carcinomas in any of the 32 cases. Gastric mucosa with intestinal metaplasia, adenomas and adenocarcinomas were positive in 7.7% (2/26), 79.3%(23/29), and 62.9% (83/132) of cases, respectively The difference in AMACR expression between adenomas or adenocarcinomas and non-neoplastic mucosa was statistically significant.(p_0.0001). Present study findings are more in agreement with those reported by Cho et al [16].

AMACR EXPRESSION IN DYSPLASIA

In present study,2/4 (50%)cases of dysplasia showed strong AMACR positivity as compared to other studies which showed 75-83% positivity in dysplasia.

In a study done by **Lee et al [15]** A significantly high frequency of AMACR expression was found in 40 of 48 (83.3%) cases of dysplasia and 34 of 66(51.5%) carcinoma cases compared with cases of non-neoplastic epithelium (p< 0.05).

The frequency of AMACR expression was significantly higher in dysplasia than in carcinoma cases (p <0.005). They concluded that AMACR immunostaining aids in distinguishing malignant or precancerous lesions from reactive epithelial atypia in gastric biopsy specimens

In a study done by **Huang et al [17]** 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% gastric biopsy specimens with dysplasia, which suggested that AMACR may be a useful immunohisto chemical marker for detecting dysplasia. They also found that AMACR expression was detected in only one biopsy with low-grade dysplasia, suggesting that AMACR had a limited usefulness in discriminating negative for dysplasia and indefinite for dysplasia from low-grade dysplasia. Interestingly, however, AMACR expression was detected in a majority of gastric specimens with high-grade dysplasia (76%) and intestinal-type adenocarcinoma (52.9%). It suggest that AMACR immunohistochemical staining may be useful for distinguishing high-grade dysplasia from low-grade dysplasia and indefinite for dysplasia.

Thus, the recognition of dysplasia in biopsy specimens is of great importance in giving warning of possibility of coexisting carcinoma and indicating that patient may be at higher risk for subsequent development of gastric cancer

AMACR EXPRESSION IN GASTRIC CARCINOMA

In present study,40/62(65%) cases include gastric carcinomas. age group ranges from 21-80 yrs of age. most common age group is 51-60 yrs of age. This is consistent with studies done by Lee et al and Mroz et al [18]. AMACR expression shows no relation with age(p value <0.5) or sex(p value<0.75)which is similar to study done by Troung et al [19] and Mroz et al [18].

In present study, total of 40 gastric carcinoma cases were subjected to AMACR staining. 25/40 (62.5%) cases shows AMACR positivity. These results are consistent with study done by **Trounget al [19]** where 83/132(63%) cases are AMACR positive. AMACR expression is seen in 70% of intestinal type adenocarcinomas and 17% of signet ring carcinoma cases. Thus, results are consistent with Lee et al [6] study regarding expression in intestinal type adenocarcinoma where 22/33(66%)of intestinal adenocarcinoma cases were positive and 12/32(37%) cases of signet ring type are positive.There is significant overexpression of AMACR in intestinal type adenocarcinoma than signet ring variety in studies done by both Lee et al [15] and Troung et al [19]. In present study, there is significant association between AMACR expression and histological type(intestinal or diffuse).Results are consistent with studies done by Yuziro et al [20] ,Lee et al [15] and Jindal et al [21].

In present study, out of 21 well differentiated cases, 18 were AMACR positive(85%). out of 6 moderately differentiated cases,4(66%) cases shows AMACR positivity. out of 7 poorly differentiated, 2/7(28%) cases shows AMACR positivity. Our study is similar to study done by Cho et al [16].

In study done by Cho et al [16], 34 of 42 (81.0%) well-differentiated adenocarcinomas, 29 of 49 (59.2%) moderately differentiated adenocarcinomas, and 20 of 41 (48.8%) poorly differentiated adenocarcinomas over expressed AMACR. this difference was statistically significant (p<0.01).AMACR expression was less frequently observed in poorly differentiated than in differentiated adenocarcinomas. .In a study done by Troung et al [19,] 61/79 (77%) cases of well/moderately differentiated cases AMACR positivity, 61/141 (43%) cases of poorly differentiated adenocarcinoma cases shows AMACR positivity. Present study results are consistent with studies done by both Lee et al [15] and Troung et al [19.]

In present study, AMACR shows increased positivity with increasing grade of differentiation (p<0.003)which is consistent with studies done by Yuziro et al [20] and Cho et al [16].

CONCLUSION

The expression of AMACR in neoplastic tissue was significantly higher as compared to adjacent dysplastic or nonneoplastic tissue of stomach and its overexpression is seen in intestinal adenocarcinoma compared to signet ring type and well differentiated adenocarcinoma than poorly differentiated adenocarcinoma. Thus, AMACR may have role in gastric tumorigenesis and differentiation.

Gastric cancer is the third most common cause of cancer-associated deaths worldwide. The majority of patients with gastric cancer have advance-stage disease at the time of diagnosis. Radical surgical resection has been the main treatment modality for resectable disease. Recently, use of adjuvant chemotherapy and radiotherapy has led to reduced locoregional relapse rates, thus improving prognoses for gastric cancers. Therefore, finding more specific targets for neoadjuvant therapy for gastric cancer is essential.

Despite our limitedunderstanding of the role of AMACR during tumorigenesis, our results suggest that furtherstudies on AMACR may contribute to the developmentof new therapeutic or preventive targetsin gastric carcinomas in the nearfuture.

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