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Original Research Paper

Pathology

IMMUNOHISTOCHEMICAL EVALUATION OF ALPHA-METHYL ACYL CO-A RACEMASE EXPRESSION IN GASTRIC BIOPSIES AND RESECTED SPECIMENS WITH HISTOPATHOLOGICAL CORRELATION

Dr K.A.T.Mirunalini	Assistant Surgeon,Gh Tambaram.
Dr K.Valarmathi	Professor Of Pathology,Stanley Medical College.
Dr.A.R.Jaswanthini	Assistant Professor Of Pathology,Stanley Medical College.
Dr.N.Kiruthika*	Assistant Professor Of Pathology,Stanley Medical College. *Corresponding Author

ABSTRACT The third most common cause of cancer associated deaths worldwide is due to gastric cancer. Finding specific targets for neoadjuvant therapy is essential which will lead to better survival of the patients. Various molecular pathways are involved in gastric cancer pathogenesis. Of these pathways , deregulation of PI3K/AKT/mTOR pathway plays an important role in cell growth, cell proliferation and metabolism. The mTOR is activated by multiple growth factors including IGF-1R, EGFR and HER2. mTOR is regulated by activation of various molecules one of which isalpha methyl acyl coA racemase (AMACR).

This alpha methyl acyl coA racemase can be tried as a molecular target as it is involved in one of the molecular pathways needed for gastric carcinoma development.

KEYWORDS : gastric carcinimas, amacr , gastric adenocarcinomas, targeted therapy for adenocarcinoma

INTRODUCTION

In developing countries like India, still gastric adenocarcinoma continues to be a menace. This is because patients present mostly during advanced stages with symptoms and signs leading to increased mortality. 5-year survival rate is less than 30% in developed countries and around 20%in developing countries[1].Therefore, it is imperative to find out a good biomarker to indicate its carcinogenesis and subsequent progression and to clarify its molecular mechanisms so that targeted therapy can be initiated early and mortality can be reduced. The two most important preceding events in Intestinal type gastric carcinoma are – chronic gastritis and Intestinal metaplasia.

Gastritis can be H.pylori induced gastritis or Non H.pylori associated gastritis which includes Gastritis in association with Crohn's disease ,Granulomatous gastritis, Autoimmune gastritis, chemical gastritis and other special forms like eosinophilic gastritis, collagenous gastritis.

Intestinal metaplasia can be complete or small intestinal type and incomplete or type II, III.

Dysplasia can be Intestinal type or gastric or Foveolar type of dysplasia.

Gastric Carcinoma :

All gastric carcinomas arise from the stem cells of the foveolae preceded by dysplasia on a background of chronic atrophic gastritis and intestinal metaplasia. It is mostly seen in 4th decade.

Molecular Pathways In Gastric Cancer [2]

Dysregulation of developmental pathways such as Wnt/β-catenin signalling, Hedgehog signalling, Hippo pathway, Notch signalling, nuclear factor-kB, and epidermal growth factor receptor are involved in gastric cancer pathogenesis. Of these pathways , deregulation of PI3K/AKT/mTOR pathway plays an important role in cell growth, cell proliferation and metabolism. mTOR activation leads to cell growth, angiogenesis and regulation of cellular metabolism. mTOR pathway acts through Cyclin D1, which plays a role in cell migration, metabolism and gene transcription. This mTOR is activated in 60-80% of gastric carcinomas[3,4]. It is expressed in both early as well as advanced lesions. The distinct expression of mTOR and p-P70S6K underlays the mechanism about differentiation of diffuse and intestinal type of carcinomas[5]. The mTOR is activated by multiple growth factors including IGF-1R, EGFR and HER2. mTOR is regulated by activation of various molecules one of which isalpha methyl acyl coA racemase (AMACR)[3,5,6]. Hence mTOR inhibitors may play a role in management of cancer.

This alpha methyl acyl coA racemase can be tried as a molecular target as it is involved in one of the molecular pathways needed for gastric carcinoma development.This alpha methyl acyl coA racemase enzyme is involved in peroxisomal beta oxidation of branched chain fatty acids and C27 bile acid intermediates. It catalyses the conversion of R-alpha-methyl- branched chain fatty acyl coA esters to their (S) stereoisomers[7]. Only the S isomers can serve as substrate for beta oxidation. The beta oxidation generates hydrogen peroxide which is a source of procarcinogenic oxidative damage.AMACR is also an activator of peroxisome proliferator – activated receptor PPAR- γ. Studied have shown that various cancers including prostate, breast cancer and gastric carcinomaexpress this PPAR- γ. Thus, AMACR may play a role in promotion of gastric cancer cell growth through PPAR- γ activation[8].

AIMS AND OBJECTIVES

- To differentiate neoplastic and non-neoplastic lesions by histopathological examination.
- To observe the expression of AMACR in both neoplastic and non-neoplastic lesions.
- To examine the rate of positivity and negativity in all the lesions.
- To correlate the expression of AMACR in various grades of adenocarcinoma

MATERIALS AND METHODS

This study is a prospective and retrospective study of two years from July 2017 to July 2019. A total of 60 cases received in the Dept of Pathology, Govt. Stanley Medical College were studied. The tissues were fixed in 10% neutral buffered formalin. Tissues were processed and paraffin wax embedded.

All the cases were stained with H and E and typing according to WHO classification. All these cases where studied for AMACR expression.

Inclusion Criteria:

- Cases diagnosed as Gastritis, Dysplasia, Primary Adenocarcinoma were included in the study.

- Cases that have not had any previous treatment.
- Cases that had adequate tissue samples for analysis and those that had complete clinicopathologic data including age, sex were all included in the study.

Exclusion Criteria:
Patients who have received previous chemotherapy or radio therapy were excluded from the study.

METHODS

- Sections of 4-5μ thickness were cut from the paraffin block and taken on charged slides.
- Corresponding H and E sections are also stained.
- AMACR kits obtained from Path in situ company were monoclonalanti-AMACR antibody. Immunohistochemical staining was done as per manufacturer guidelines.
- Positive control – Prostatic adenocarcinomatous tissue was taken as positive control.
- Negative control – Non neoplastic gastric tissue was taken as negative control.

Evaluation Of Amacr Staining

- Staining was evaluated by light microscopy.
- Positive staining was considered when the staining is easily visible on low power examination. The stain should be circumferential, granular, luminal to subluminal and diffuse cytoplasmic stain.
- Intensity was graded as follows based on Luo J and ZhaS et al[10].

Table - Grading Of Intensity

GRADE	STAINING PATTERN
0	Absolutely no staining
1	1-10% of cells in a gland show positivity
2	10-50% of cells in a gland show positivity
3	>50% of cells in a gland show positivity

OBSERVATION AND RESULTS

In this study, a total of 60 gastric biopsies were studied from July 2017 to July 2019 in Govt. Stanley Medical College.

The age group distribution was from 21 years – 90years, commonest age group being 21-30 years with 18 cases and least commonest being 81-90 with 1 case. Out of 60 cases examined, 38/60(63.3%) were males and 22/60(36.7%) were females with a male to female ratio of 1.72:1.

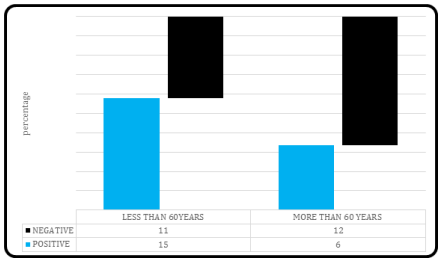
Amacr Expression In Gastritis And Dysplasia
In the present study, there were 14 cases of gastritis and 2 cases of dysplasia. In all 14 cases, AMACR expression was positive in one case. In two cases of dysplasia, one case (50%) showed positivity (circumferential luminal positivity) whereas the other case was negative.

Table : AMACR expression in Gastritis and Dysplasia

AMACR EXPRESSION	GASTRITIS	DYSPLASIA
POSITIVE	1	1
	7.1%	50.0%
NEGATIVE	13	1
	92.9%	50.0%
Total	14	2
	100.0%	100.0%

Amacr Expression In Gastric Carcinoma
Out of 44 cases of gastric carcinoma in this present study, 21 cases showed positivity for AMACR expression.

Chart8- Age Wise Amacr Distribution In Gastric Carcinoma



Sex Wise distribution of AMACR Expression in Gastric carcinoma
Out of 44 cases of gastric carcinoma, 21 cases showed positive AMACR expression.

Table: Sex Wise distribution of AMACR Expression in Gastric carcinoma

AMACR expression	Males	Females
Positive	14	7
Negative	15	8
Total	29	15

Grade Wise Expression Of AMACR
Out of 60 cases of gastric tissues examined for AMACR expression, 44 cases were adenocarcinoma. 9 cases of Well differentiated adenocarcinoma were studied, out of which 6 (66.7%) were found to be positive for AMACR expression and 3(33.3%) were negative. Out of 25 cases of moderately differentiated adenocarcinoma, 11(44%) were positive and 14(25%) were negative for AMACR expression. 10 cases of poorly differentiated adenocarcinoma were studied and 4(40%) was found to be positive and remaining 6 (60%) were negative for AMACR expression

Table: Grade Wise Distribution of AMACR

	GRADE 0	GRADE1	GRADE 2	GRADE 3	AMACR POSITIVE	AMACR NEGATIVE
Well Differentiated Adenocarcinoma	3	3	1	2	6	3
Moderately Differentiated Adenocarcinoma	14	5	3	3	11	14
Poorly Differentiated Adenocarcinoma	6	3	0	1	4	6
TOTAL	**23**	**11**	**4**	**6**	**21**	**23**

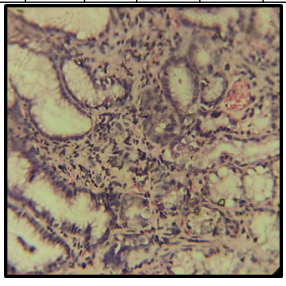


Fig. 1 Dysplasia, H&e, 40 X

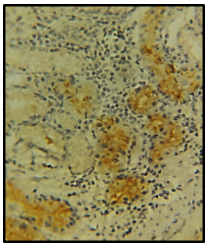


Fig2:dysplasia, AMACR Positive, 40x

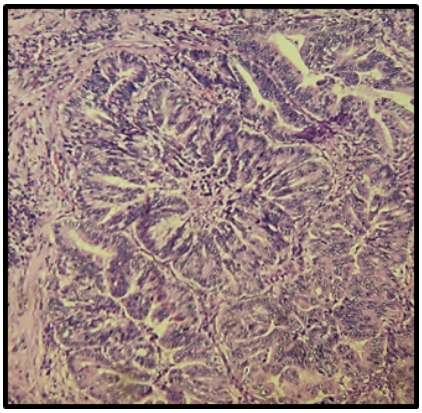


Fig 3 :well Differentiated Adenocarcinoma, H&e, 10x

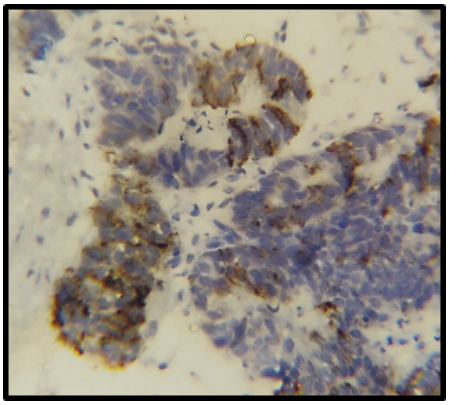


Fig.4 Well Differentiated Adenocarcinoma, Amacr Positive, 40x

Statiscal Analysis

Statistical analysis was done using SPSS. v23 software.

Chi square test was used to compare AMACR expression in gastritis and dysplasia and carcinoma and was found to statistically significant (chi square value – 7.515; P Value – 0.006)

Analysis between well differentiated, moderately differentiated and poorly adenocarcinoma revealed p value of 0.433 which was not statistically significant.

Table:-P Value – Carcinoma/Dysplasia and Gastritis

AMACR EXPRESSION	Diagnosis		Total	Pearson Chi-Square	P value
	CARCINOMA /DYSPLASIA	GASTRITIS			
POSITIVE	22	1	23	7.515	0.006
	47.8%	7.1%	38.3%		
NEGATIVE	24	13	37		
	52.2%	92.9%	61.7%		
Total	46	14	60		
	100.0%	100.0%	100.0%		

Table:- P Value Between Grades of Adenocarcinoma

AMACR EXPRESSION	HPE DIAGNOSIS			Total	Pearson Chi-Square	P value
	MOD. DIFFERENTIATED ADENOCARCINOMA	POORLY DIFFERENTIATED	WELL DIFFERENTIATED			
POSITIVE	11	4	6	21	1.673	0.433
	44.0%	40.0%	66.7%	47.7%		
NEGATIVE	14	6	3	23		
	56.0%	60.0%	33.3%	52.3%		
Total	25	10	9	44		
	100.0%	100.0%	100.0%	100.0%		

DISCUSSION:

AMACR Expression In Gastritis

Table.- Comparison of Expression of AMACR in Gastritis with Other studies

Study	Cases Positive/ Total Cases	Percentage
Cho et al[11]	0/32	0%
Truong et al[9]	0/38	0%
Lee et al[12]	2/44	4.5%*
Present study	1/14	7.14%*

In Cho et al study[11], expression was negative in normal gastric mucosa in all 32 cases. Gastric mucosa with metaplasia showed 7.7% positivity; adenoma showed 79.3% and adenocarcinoma showed 62.9% positivity for AMACR expression.

In Lee et al study[12], out of 44 cases, 2 (4.5 %) showed positivity. The positive cells were mainly in the deeper mucosa and were focal. Weak staining was also noted at the basal portion of glands of normal mucosa.

This vast difference in expression of AMACR by neoplastic and non-neoplastic gastric tissues is evident in even mRNA level. A real time quantitative PCR study demonstrated that AMACR mRNA was very low to undetectable in normal gastric mucosa whereas, levels were high in gastric carcinomas. Possible explanation for this positivity was presence of nonspecific protein binding in gastric tissue due to the presence of endogenous enzymes.

Our study was similar to Lee et al[12] study.

AMACR Expression In DYSPLASIA

Table: Comparison of AMACR expression in Dysplasia with other studies

Study	Positive Cases/ Total Cases	Percentage
Lee et al [12]	40/48	83%
Truong et al [9]	23/29	79%
Huang et al[13]	19/25	76%*
Present study	01/02	50%*

Lee et al[12] study showed a significant expression of AMACR in 83.3% (40/48) cases of dysplasia. 51.5% (34/66) cases of carcinoma also showed positive expression of AMACR.

With a $p < 0.005$, it was shown in their study that AMACR expression was significantly higher in dysplasia than in carcinoma and they state that immunohistochemical examination with AMACR can help in distinguishing precancerous lesions like dysplasia and malignant lesions from reactive epithelial atypia in gastric specimens.

Thus, AMACR expression helps in identifying the presence of dysplasia when it's doubtful. This is important as identifying dysplasia helps in better follow up of the patient who are at higher risk of gastric carcinoma development at a later stage.

Present study is similar to Huang et al study[13].

AMACR EXPRESSION IN GASTRIC CARCINOMA

AGE distribution:

In this present study, 44 cases were gastric carcinoma out of the total 60 cases. Study population included ages range of 20 years to 81 years. The most common age group was 40-60years. Lee et al[12] study and Mroz et al[14] study also showed the common age group for gastric carcinoma as 5^{th} – 6^{th} decade.

SEX distribution:

Out of 44 cases of gastric adenocarcinoma examined, 30 (68.2%) were male and 14 (31.8%) were female patients. Male to female ratio was 2.14:1. This is in accordance with Lee et al[12] study and Mroz et al[14] study which showed male to female ratio of 2.5:1.

Age Wise And Sex Wise AMACR Expression

AMACR expression was seen in 21/44 cases. Out of 21 cases, 15 cases (71.4%) were less than 60 years and 6 (28.6%) were more than 60 years of age.

When comparing AMACR expression in males and females, there was male preponderance in expression of AMACR with 14 (66.7%) and females who showed positive expression for AMACR were 7 (33.3%) with a male to female ratio of 2:1.

AMACR Expression In Gastric Adenocarcinoma

In our study, 44 cases of gastric carcinoma were subjected to immunohistochemical examination for AMACR. 21 cases out of 44 cases showed positive staining which was graded as per criteria already mentioned earlier. This amounts to 47.7% which was closely related to the studies done by Lee et al[12] and Huang et al[13].

Table- Comparison of AMACR expression in gastric adenocarcinoma in present study with other studies

STUDY	POSITIVE CASES/ TOTAL CASES	PERCENTAGE
Lee et al [12]	34/66	52%*
Huang et al[13]	18/34	52%*
Truong et al[9]	83/132	63%
Jindal et al[15]	40/50	80%
Present study	21/44	47.7%*

Table- Comparison Of Amacr Expression According To Histological Type With Other Studies

STUDY	INTESTINAL TYPE	DIFFUSE TYPE
Lee et al [12]	22/33 (66%)	12/32(37%)
Mroz et al[14]	74/122(60%) *	20/42(47%) *
Jindal et al[15]	8/9(88%)	32/41(78%)
Present study	17/34 (50%) *	4/10 (40%) *

AMACR expression according to Grade of Gastric adenocarcinoma

In our present study comprising of 44 cases of gastric carcinoma, 9 cases were well differentiated adenocarcinoma, 25 cases were moderately differentiated and remaining 10 cases were poorly differentiated. Out of 44 cases, 21 showed positivity for AMACR expression.

Of the 9 cases of well differentiated adenocarcinoma studies, 6 (66.7%) showed positive expression. Out of 25 cases of moderately differentiated adenocarcinoma cases, 11 (44%) cases expressed AMACR. Out of 10 cases of poorly differentiated adenocarcinoma studied, 4 (40%) showed positivity for AMACR.

Table- Comparison of grade wise AMACR expression in gastric carcinoma to other studies

Study	Well differentiated	Moderately differentiated	Poorly differentiated
Cho et al[11]	34/42 (81%)	29/49 (59.2%)	20/41(48.8%)
Truong et al[9]	61/79 (77%)	61/141(43%)	
Present study	6/9(66.7%)	11/25(44%)	4/10(40%)

The present study was similar to Lee et al12 and Truong et al[13] study.

Table. Comparison of association between AMACR Expression and grade of gastric carcinoma in various studies

Study	AMACR association with grade
Mroz et al[14]	No significant association*
Panwar et al[16]	No significant association*
Cho et al[11]	Significant association
Present study	No significant association*

In the present study, there was increased expression of AMACR with increasing histological differentiation but it was not statistically significant(p value 0.43).

CONCLUSION

The third most common cause of cancer associated deaths worldwide is due to gastric cancer. Most of the patients present at an advanced stage of disease. Radical surgical resection along with adjuvant chemotherapy and radiotherapy has led to improved prognoses.

Finding specific targets for neoadjuvant therapy is essential which will lead to better survival of the patients.

AMACR can be considered as one such candidate for targeted therapy as its expression is significantly higher in neoplastic tissue compared to non-neoplastic tissue of stomach. Over expression is also seen in intestinal type of adenocarcinoma when compared with diffuse / signet ring type of adenocar cinoma.

Increased expression of AMACR is also seen in well different iated and moderately differentiated adenoca rcinomas than in poorly differentiated adenoc arcinoma. All this proves that AMACR may have a role in gastric tumorigenesis and differentiation.

Hence further studies may be done to find out the exact role of AMACR in tumorigenesis. This can help in developing newer therapeutic modalities or preventive targets which will help the patients suffering from gastric carcinoma.

REFERENCES:

1. Mohandas KM, Jagannath P. Epidemiology of digestive tract cancers in India. VI. Projected burden in the new millennium and the need for primary prevention. Indian J Gastroenterol Off J Indian Soc Gastroenterol. 2000 .
2. Molaei F,Forghanifard MM,Fahim.molecular signalling in tumorigenesis of Gastric Cancer.Iranian Biomedical Journal 22 (4):217-230 July 2018M.
3. Feng W, Brown RE, Trung CD, Li W, Wang L, Khoury T, et al. Morphoproteomic Profile of mTOR, Ras/Raf Kinase/ERK, and NF- B Pathways in Human Gastric Adenocarcinoma. Ann Clin Lab Sci. 2008 Jun 20;38(3):195–209.
4. Lang SA, Gaumann A, Koehl GE, Seidel U, Bataille F, Klein D, et al. Mammalian target of rapamycin is activated in human gastric cancer and serves as a target for therapy in an experimental model. Int J Cancer. 2007 Apr 15;120(8):1803–10.
5. Xiao L, Wang YC, Li WS, Du Y. The role of mTOR and phospho-p70S6K in pathogenesis and progression of gastric carcinomas: an immuno histochemical study on tissue microarray. J Exp Clin Cancer Res CR. 2009 Dec 13; 28(1):152.
6. Kim MA, Lee HS, Lee HE, Jeon YK, Yang HK, Kim WHEGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. Histopathology 52: 738-746
7. Evans AJ. α-Methylacyl CoA racemase (P504S): overview and potential uses in diagnostic pathology as applied to prostate needle biopsies. J Clin Pathol. 2003 Dec;56(12):892–7.
8. Sato H, Ishihara S, Kawashima K, Moriyama N, Suetsugu H, Kazumori H, et al. Expression of peroxisome proliferator-activated receptor (PPAR) in gastric cancer and inhibitory effects of PPAR agonists. Br J Cancer. 2000 Nov;83(10):1394–400
9. Truong CD, Wei Li, Wei Feng, Philip Cagle, ThaerKhoury, SadirAlrawi, KepingXie, James Yao and Dongfeng Tan. Alpha methyl acyl CoA racemase expression is upregulated in Gastric carcinoma: Int JClinExpPathol 2008;1(6):518-523.
10. Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, et al. Alpha-methyl acyl-CoA racemase: a new molecular marker for prostate cancer. Cancer Res. 2002 Apr 15;62(8):2220–6.
11. Cho EY, Kim K-M, Park CK, Kim JJ, Sohn TS, Kim DW. AMACR is highly expressed in gastric adenomas and intestinal-type carcinomas. APMIS Acta PatholMicrobiol Immunol Scand. 2007 Jun;115(6):713–8.
12. Won Ae Lee et al. α-Methylacyl-CoA-Racemase Expression in Adenocarcinoma, Dysplasia and Non-Neoplastic Epithelium of the Stomach JI of Oncology 2006,71(3);10:246-50.
13. Huang W, Zhao J, Li L, Huang Y, et al. A-Methylacyl coenzyme A racemase is highly expressed in the intestinal-type adenocarcinoma and high-grade dysplasia lesions of the stomach. HistoPathol.2008 Nov;23(11):1315-1320.
14. Mroz A, Kiedrowski M, Lewandowski Z. -Methylacyl-CoA Racemase (AMACR) in Gastric Cancer: Correlation with Clinicopathologic Data and Disease-free Survival. AppIImmunohistochem Mol Morphol. 2013 Jul; 21(4): 313.
15. Jindal Y, Singh A, Kumar R, Varma K, Misra V, Misra SP, et al. Expression of Alpha Methylacyl CoA Racemase (AMACR) in Gastric Adenocarcinoma and Its Correlation with Helicobacter pylori Infection. J Clin Diagn Res JCDR. 2016 Oct;10(10):EC10–2.
16. Panwar R, Gupta V, Kumar Y et al.: significance of AMACR expression in Gastrointestinal adenocarcinoma 2016;(3),25-31.