ORIGINAL RESEARCH

Expression of Aldehyde Dehydrogenases 1 (ALDH1) - A Stem Cell Marker in Non-Neoplastic and Neoplastic Lesions of Gall Bladder – A Correlative Study

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ABSTRACT

Background: The study aims to analyse expression of stem cell marker aldehyde dehydrogenases 1 (ALDH1) by Immunohistochemistry (IHC) in non-neoplastic and neoplastic lesions of the gall bladder.

Materials and Methods: The study was accomplished at the Department of Pathology Era's Lucknow Medical College and Hospital, Lucknow. A total of 105 cases were enrolled for the study. The paraffin embedded tissue was subjected to haematoxylin and eosin staining. Immunohistochemistry was done further for the expression of ALDH1 stem cell marker, IHC was further done.

Results: Out of 70 non-neoplastic cases, ALDH1 came out to be positive for 12 cases and 15 cases came out to be positive for ALDH1 out of 35 neoplastic cases. The ALDH1 expression was found to be significantly higher in neoplastic than non-neoplastic lesions. Among the non-neoplastic lesions no significant difference seen with age, sex and cholelithiasis, whereas statistical significance was seen in cases with chronic cholecystitis, metaplasia, hyperplasia and low grade dysplasia as well as in ALDH1 expression in neoplastic lesions associated with cholelithiasis and histological grade of tumour.

Conclusion: ALDH1 expression in non - neoplastic & neoplastic gallbladder lesions may support the concept that the stem cells may be primary target of transformation in gallbladder carcinogenesis associated with prolong inflammation.

Keywords: Chronic Cholecystitis, Gall Bladder Carcinoma, Immunohistochemistry, ALDH1.

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INTRODUCTION

Among GI cancer. Gall Bladder cancer (GBCs) is highly aggressive cancer with highest mentality rate. Its prevalence is high in American Indian, Chilean, Japanese women and India

especially North India around gangetic belt.[1,2] A close relationship between chronic inflammation and neoplasia has been established through recent studies.[3] Examples include inflammatory bowel disease and colon carcinoma, Helicobacter pylori–associated gastritis and gastric carcinoma,[4] and Hashimoto disease and the development of papillary thyroid carcinoma.[5]

Cancer is a stem cell based disease as proved through increasing & that tumours originate from the cancer stem cells (CSC).[6] The ability to identify the stem cells by ALDH1 expression, in non-neoplastic & neoplastic gallbladder lesions may support the concept that the stem cells may be a primary target of transformation in gallbladder carcinogenesis associated with prolonging inflammation. Few studies are available on stem cell expression patterns in Non- Neoplastic v/s Neoplastic gallbladder lesions.

The aim of the study is to analyse the expression of stem cell marker ALDH1 by IHC in neoplastic and non-neoplastic lesions of the gall bladder.

MATERIALS AND METHODS

Patients

This cross-sectional study was conducted from June 2018 to October 2020. The initiation work was done after detaining ethical clearance from the Institutional Ethical Committee of the university.

Histopathology

Tissue samples were obtained from patients who had undergone cholecystectomy. For histopathological diagnosis, all tissue samples were collected in 10% buffered formalin and processed for routine histopathology examination; 4mm thick sections from formalin- fixed paraffin embedded (FFPE) blocks were cut and stained with haematoxylins and eosin. After histological confirmation of the diagnosis, (i.e. chronic cholecystitis/metaplasia/hyperplasia/ primary adenocarcinoma of the gallbladder), tumour grade, lympho-vascular invasion, perineural invasion was assessed in the gallbladder carcinoma group after histopathological confirmation of diagnosis.

Immunohistochemistry

Section which was four μm thick was cut from routinely paraffin embedded tissues. The staining of ALDH1 was carried out according to the manufacture's protocol, staining of ALDH1 was carried out through a graded series of alcohol and subjected to IHC for ALDH1 after antigen retrieval in buffer using primary polyclonal antibody ALDH1 (Catalog Number:15910-1-AP, Immunogen Catalog Number: AG8665, proteintech ^{TM}). Visualization was obtained by Dako REAL ^{TM} EnVisionTM and then incubated with peroxidase inhibitor (3% H2O2) in the dark for 15 minutes, followed by EDTA-trypsin digestion for 15 minutes. Sections were incubated with primary antibody for 60 minutes, then secondary antibody for 30 min after being soaked with PBS for 3 × 5 minutes. Colour developed using DAB chromogen and hematoxylin counter-staining. The slides were dehydrated with different concentrations (70%–100%) of alcohol and soaked in xylene for 3 × 5 minutes, and finally mounted with neutral balsam. Positive control (Human kidney tissue) will be run with each batch of staining. Development of a cytoplasmic brown colour will be considered positive staining.

Scoring of Immunohistochemistry

Per section, ten fields were examined. The percentage of positively stained cells relative to the total number of cells was determined. Staining strength was rated on a scale of 1 to 3. 2 represents week to moderate staining. 1 represents little to no positive staining. 3 represents moderate to strong staining. A section is determined as positive for msi-1 or ALDH1 when the percent of positively stained cells was 10% and staining strength 2. The few sections where the percent positive staining was 5% to 10% and staining strength was 3 were also regarded as positive.

Statistical analysis

Data were analysed using Statistical Package for Social Sciences, Version 21.0. A Chi-square test was used to compare the data. A 'p' value less than 0.05 was considered as significant.

RESULTS

This study was done to carry forward the analysed of expression of stem cell marker ALDH1 by IHC in non-neoplastic and neoplastic lesions of the gall bladder.

Patient characteristics

Neoplastic group

35 patients in this group. Majority of patients were above 40 years of age. Presented with pain and all had histologically proven primary adenocarcinoma of the gallbladder. Associated cholelithiasis was present in 24 (68.6%) patients.

Non-neoplastic group

70 patients in this group. Above 40 years of age, patients presented with pain. All patients had histologically proven chronic cholecystitis only present in 25 (35.7%) with hyperplasia present in 15 (21.4%) with metaplasia present in 15 (21.4%) and low grade dysplasia present in 15 (21.4%). Associated cholelithiasis was present in 55 (78.6%) patients.

Table 1: Shows group-wise distribution of Study Population

SN.	Group	Description	No. of cases (105)	Percentage
1.	Group 1 (Neoplastic)	Carcinoma gall bladder	35	33.3%
2.	Group 2 (Non-neoplastic)	Chronic cholecystitis with hyperplasia/ metaplasia and low-grade dysplasia	70	66.7%

Table 2: Comparison of Demographic and Clinical Profile of two study groups

SN.	Characteristic	Total (n=105)	Group1 (Neoplastic) (n=35)	Group2 (Non-neoplastic) (n=70)	Statistical significance
1.	Age				
	<40 Years	25 (23.8%)	5 (14.3%)	20 (28.6%)	^{2}=2.62; p=0.105
	≥40 Years	80 (76.2%)	30 (85.7%)	50 (71.4%)	
	Mean Age±SD (Range)	46.53±12.21 (17-75)	50.89±10.96 (25-72)	44.26±12.28 (17-75)	't'=2.721; p=0.008
2.	Sex				
	Male	32 (30.5%)	7 (20.0%)	25 (35.7%)	^{2}=2.72; p=0.099
	Female	73 (69.5%)	28 (80.0%)	45 (64.3%)	
3.	Cholelithiasis	79 (75.2%)	24 (68.6%)	55 (78.6%)	^{2}=1.25; p=0.263
4.	Histopathological type				
	Chronic cholecystitis only (CC)			25 (35.7%)	
	CC with Hyperplasia			15 (21.4%)	

	CC with Metaplasia			15 (21.4%)	
	CC with Low grade dysplasia			15 (21.4%)	
5.	Histopathological grade				
	Well-differentiated		15 (42.9%)		
	Moderately differentiated		10 (28.6%)		
	Poorly differentiated		10 (28.6%)		
6.	Lympho-vascular invasion		14 (40.0%)		
7.	Perineural invasion		9 (25.7%)		

ALDH1 expression on immunohistochemistry

Table 3: Association of ALDH1 Expression with neoplastic and non-neoplastic lesions

SN	**Group**	**ALDH1 Expression**				**Positive expression rate in each group**
		Present (n=27)		**Absent (n=78)**		
		No.	**%**	**No.**	**%**	
1.	Group 1(Neoplastic) (n=35)	15	55.6	20	25.6	15/35 = 42.9%
2.	Group2(Non-Neoplastic) (n=70)	12	44.4	58	74.4	12/70; 17.1%

^{2}=8.08; p=0.004

ALDH1 expression and clinicopathological correlation

Table 4: Correlation of ALDH1 Expression with different clinicopathological parameters

SN.	**Characteristic**	**Total**	**ALDH1 expression**		**'p' value**
			Present (n=27)	**Absent (n=78)**	
1.	Age				
	<40 Years	25	9 (33.3%)	16 (20.5%)	^{2}=0.958; p=0.328
	≥40 Years	80	18 (66.7%)	62 (79.5%)	
2.	Gender				
	Male	32	7 (25.9%)	25 (32.1%)	^{2}=0.355; p=0.551
	Female	73	20 (74.1%)	53 (67.9%)	
3.	Gallstones				
	Present	79	24 (88.9%)	55 (69.6%)	^{2}=3.64; p=0.057
	Absent	26	3 (11.1%)	23 (30.4%)	
4.	Non-neoplastic lesions	70	12 (44.4%)	58 (74.4%)	^{2}=8.08; p=0.004
	CC only	25	0	25 (43.1%)	^{2}=23.1; p<0.001
	CC with hyperplasia	15	0	15 (25.9%)	
	CC with metaplasia	15	4 (33.3%)	11 (19.0%)	

	CC with dysplasia	15	8 (66.7%)	7 (12.1%)	
5.	Neoplastic lesions (Adenocarcinoma)	35	15 (55.6%)	20 (25.6%)	^{2}=8.08; p=0.004
	WD	15	2 (13.3%)	13 (65.0%)	^{2}=11.2; p=0.004
	MD	10	5 (33.3%)	5 (25.0%)	
	PD	10	8 (53.3%)	2 (10.0%)	
6.	LVI	14	6/15 (40.0%)	8/20 (40.0%)	^{2}=0; p=1
7.	PNI	9	4/15 (26.7%)	5/20 (20.0%)	^{2}=0.013; p=0.911

CC: Chronic cholecystitis, WD: Well differentiated, MD: Moderately differentiated, PD : Poorly differentiated, LVI= Lympho-vascular Invasion, PNI= Perineural invasion

Figure -1: **Gross specimen of Gall Bladder showing thickened wall with lumen containing multiple gall stones.**

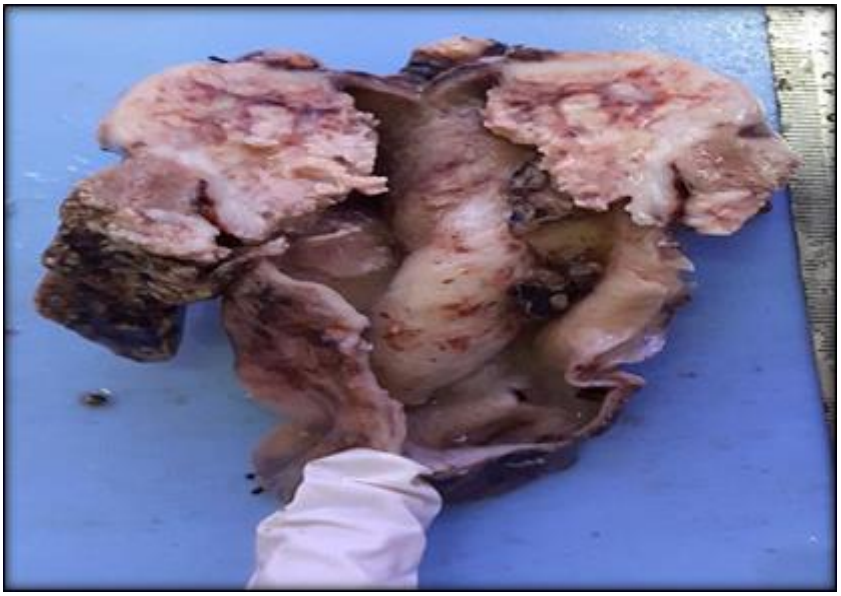

Figure 2: **Gross specimen of Gall Bladder showing a tumour growth a fundus**

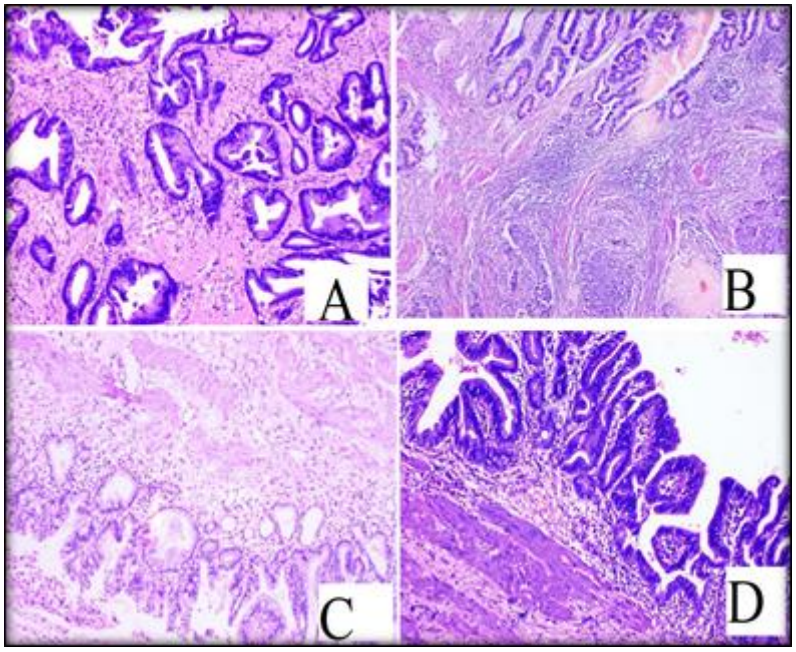

Figure 3: **NON NEOPLASTIC LESION- Gall bladder (H&E)-Photomicrograph showing (A), chronic cholecystitis (H&E,100X) (B), chronic cholecystitis with hyperplasia (H&E, 100X) (C), chronic cholecystitis with mucinous metaplasia (H&E, 100X) (D), chronic cholecystitis with dysplasia (H&E, 100X).**

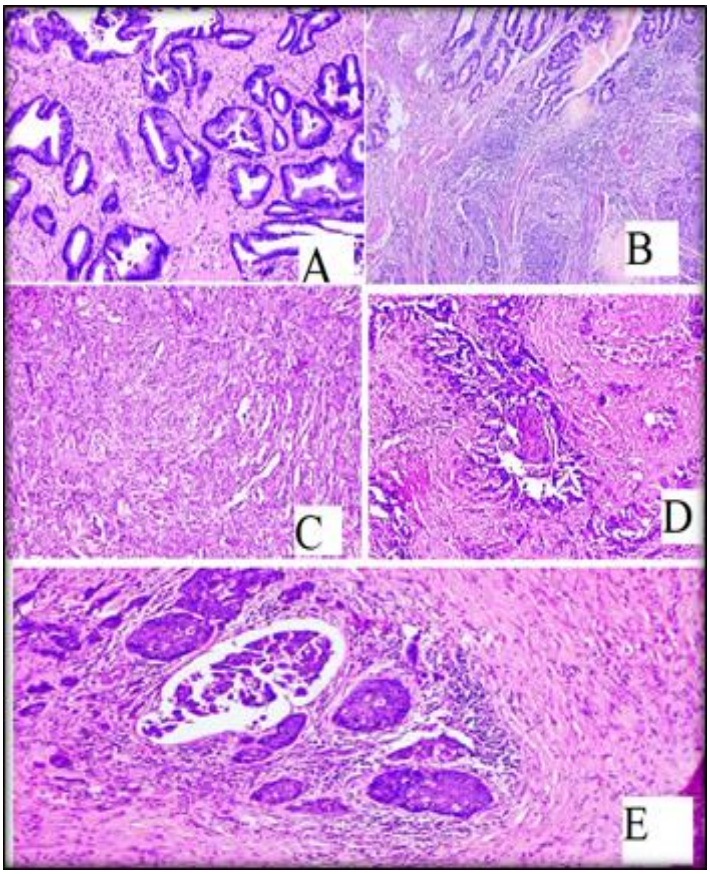

Figure 4: **NEOPLASTIC LESIONS- Gall bladder (H&E)- Photomicrograph showing adenocarcinoma gallbladder (A), Well Diff erentiated Adenocarcinoma (H&E, 400X) (B), Moderately diff erentiated Adenocarcinoma (H&E, 100X) (C), Poorly diff erentiated (H&E, 100X) (D), Adenocarcinoma with Perineural invasion (PNI) (H&E, 400X) (E), Adenocarcinoma with Lympho-vascular invasion (LVI) (H & E, 400X)**

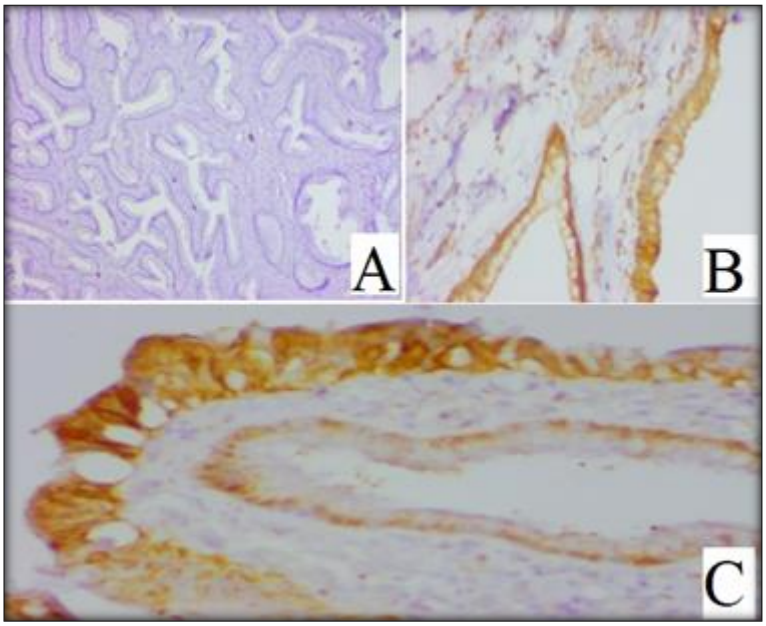

Figure 5: NON NEOPLASTIC LESIONS- Gall bladder (IHC) -ALDH1 expression in non-neoplastic lesions of gallbladder showing (A), No cytoplasmic positivity in Chronic Cholecystitis with hyperplasia (IHC for ALDH1, 400X) (B), Moderate cytoplasmic positivity in Chronic Cholecystitis with Metaplasia (IHC for ALDH1, 400X) (B), Moderate to strong cytoplasmic positivity in Chronic Cholecystitis with Dysplasia (IHC for ALDH1, 400X).In non-neoplastic cases of gallbladder hyperplasia shows no positivity for ALDH1, however metaplasia showed moderate cytoplasmic positivity and dysplasia showed Moderate to strong cytoplasmic positivity.

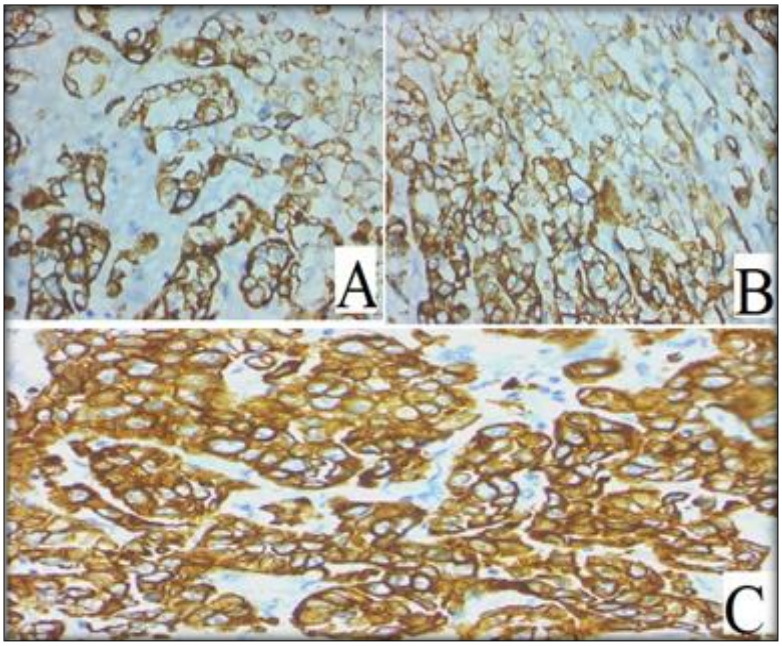

Figure 6: NEOPLASTIC LESIONS- Gall bladder (IHC)-ALDH1 expression in neoplastic lesions of gallbladder showing (A), Moderate cytoplasmic positivity in Well Diff erentiated Adenocarcinoma (IHC for ALDH1, 400X) (B), Moderate to strong cytoplasmic positivity in Moderately diff erentiated Adenocarcinoma (IHC for ALDH1, 400X) (C), Strong cytoplasmic positivity Poorly diff erentiated (IHC for ALDH1, 400X). There is increasing trend of expression of ALDH1 positivity from well to moderately to poorly diff erentiated adenocarcinoma gallbladder

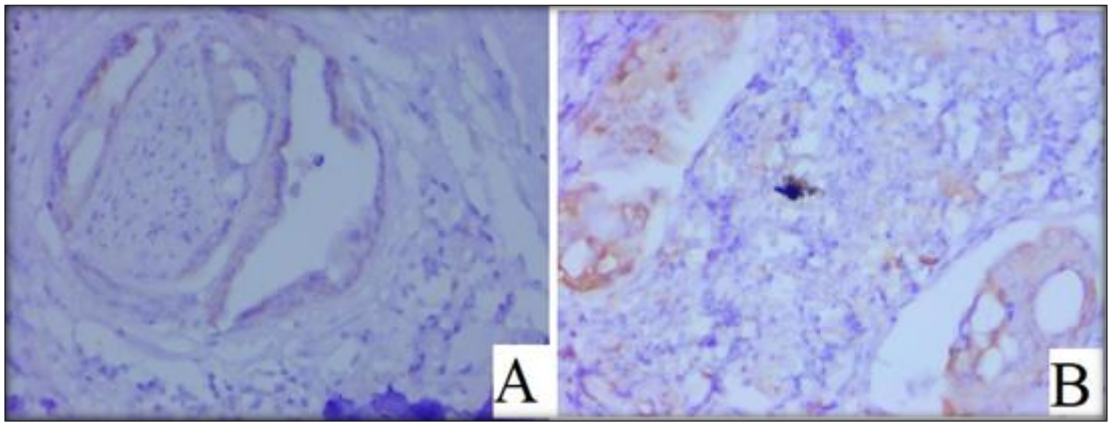

Figure 7: NEOPLASTIC LESIONS- Gall bladder (IHC)-ALDH1 expression in neoplastic lesions showing (A), Moderate cytoplasmic positivity in adenocarcinoma with Perineural invasion (PNI) (IHC for ALDH1, 400X) (B), moderate to strong cytoplasmic positivity in adenocarcinoma with Lympho-vascular invasion (IHC for ALDH1, 400X).

DISCUSSION

Stem cells are being increasingly recognized to have an important role in different types of cancers.

Aldehyde dehydrogenase (ALDH) play an important role in detoxifying endogenous and exogenous aldehyde substrates through $NAD(P)^+$-dependent oxidation.[7] Immuno-expression of ALDH1 has been declared as a marker of cancer stem cells.

With this background, the present study was done for the detection of presence of cancer stem cell markers labelled by ALDH1 in neoplastic and non-neoplastic lesions of the gall bladder.

For this purpose, a total of 105 tissue specimens of gall bladder lesions (70 non-neoplastic and 35 neoplastic) were subjected to an immunohistochemical evaluation for the presence of cancer stem cell marker ALDH1 and to correlate this immunohistochemical expression with clinicopathological profile of lesions.

In present study, positive ALDH1 expression rate was significantly higher in neoplastic lesions (42.9%) as compared to non-neoplastic lesions (17.1%). This finding is similar to study done by Liu et al,[8] and Yang et al.[9]

In present study, with a systematic sampling and adequate representation of all the histopathological differentiation grades, we were able to derive a significant association between positive ALDH1 expression and histopathological grade, and thus endorsed the relationship between aggressiveness of cancer and positive ALDH1 expression as expounded in previous studies.[10-17]

Among neoplastic lesions, moderate and poorly differentiated were significantly associated with higher prevalence of positive expression (33.3% and 53.3%) whereas well differentiated type was significantly associated with higher prevalence of negative expression (65%) ($p=0.004$). Liu et al,[8] similar to present study also found a significant incremental trend of ALDH1 expression with increasing histopathological grade. A similar observation was also made by Yang et al,[9] in their study for adenocarcinoma cases.

To the best of our knowledge, this is the first study comparing the expression of ALDH1 in different types of non-neoplastic lesions. Within the non-neoplastic lesion group, ALDH1 positive expression as compared to negative expression was higher in metaplasia (33.3% vs 19.0%) and dysplasia (66.7% vs 12.1%) while the proportion of those with positive expression as compared to negative expression was significantly lower in chronic cholecystitis only (0% vs 43.1%) and hyperplasia (0% vs 25.9%) respectively ($p<0.001$).

Sun et al,[18] in their study found ALDH1 expression in normal and benign ovarian tumour is significantly lower as compared to that in ovarian cancer tissues. In another study, Pan et al.[19] too found positive ALDH1 expression in 61% of breast cancer cases as compared to 14.3% of benign breast disease cases. Kunju et al.[20] in their study reported in their study that ALDH1 is expressed in epithelial and stromal cells in benign breast tissues, and discovered that ALDH1 positivity in breast epithelial cells associated with increased risk of breast cancer. Abdullah et al.[21] observed positive ALDH1 expression in 38.2% of non-neoplastic colon tissue and 13.9% of normal colon tissue and linked them with their possible malignancy potential.

The findings of present study show that ALDH1 could be considered as a marker of active cancer stem cells involved in tumorigenesis and its progression. Owing to these features ALDH1 seems to hold a prognostic significance both in neoplastic as well as non-neoplastic lesions.

CONCLUSION

In summary, after the evaluation of the expression of ALDH1 immunoexpression seems to be associated with gall bladder carcinoma. Positive immunoexpression of ALDH1 in metaplastic and low grade dysplasia in non-neoplastic group indicated a possible role of ALDH1 with cancer stem cells leading to progression from non-neoplastic to neoplastic condition.

LIMITATIONS

Studies on a larger sample size with variable clinical and histopathological characteristics could help in understanding the relationship of ALDH1 with gall bladder cancer in a better way. Further studies on a larger sample size with inclusion of more variables are recommended

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