

Immuno-Histochemical Analysis of Estrogen Receptors on the Malignant Gallbladder Tissue

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Abstract

Sex steroids play an important part in the functioning of normal gallbladder, formation of gallstones and possibly in the pathogenesis of gallbladder cancer. Steroid receptors have been previously demonstrated on normal and malignant gall bladder tissues. To study this phenomenon further, we correlated clinicopathological features and survival with estrogen receptor (ER) status of the tumour in 30 patients with histologically proven adenocarcinoma of the gallbladder. Estrogen receptor assay was performed immuno-histochemically utilizing Universal Immunoperoxidase Staining Kit. Tumor tissue was obtained either surgically or with fine needle aspiration of the gallbladder mass. There were 27 females and 3 males. Eighteen patients had estrogen receptors expressed on the malignant tissue, 12 were negative. Comparison of clinicopathological characteristics and survival between the two groups demonstrated no significant difference in gender, mean age, marital status and parity. Similarly, presence of gallstones, histologic grade or survival did not correlate with the estrogen receptor status. There is, however, a trend in favour of poorly differentiated tumors being more often receptor negative. Further studies are necessary to elucidate the biologic significance of these receptors (JPMA 48:123, 1998).

Introduction

Several studies have suggested the importance of female sex hormones in the pathogenesis of gallbladder disease. Sex steroids have been demonstrated to influence the functioning of normal gallbladder¹⁻⁶. Gallbladder emptying is impaired during luteal phase of the menstrual cycle and pregnancy. Development of cholelithiasis has been associated with the use of oral contraceptive drugs. Furthermore, gallstones are more frequently observed in women and are particularly common in multiparous females⁷. This suggests a significant role played by the sex steroids in the functioning of normal gallbladder and the development of gallstones.

Gallbladder cancer is an uncommon malignancy. It accounts for less than 1% of all cancers in the United States⁸. Incidence of gallbladder cancer, however, exhibits marked geographic variability^{9,10}. This cancer is more frequently observed in Israel, Central and Eastern Europe, Japan and Latin America. Particularly high incidence has been reported in Hispanics and Native Indians¹¹⁻¹⁵. In Pakistani females, gallbladder cancer is the second commonest malignancy of gastrointestinal tract origin¹⁶. This makes gallbladder cancer, a formidable health problem, at least in certain population. Gallbladder cancer is more frequently observed in women. Risk increases with the number of pregnancies. Additionally, gallstones are present in majority of the patients with gallbladder cancer¹⁷. This raises the possibility that sex steroids may influence the pathogenesis of gallbladder cancer. Sex steroids have been demonstrated to influence the growth of tumors arising from the target tissues such as breast and prostate. Biologic effect of sex steroids is mediated by the receptors present on the

target cells¹. Presence of these receptors on the malignant mammary tissue influences the biologic behaviour of breast cancer. Therapeutic responses to anti-estrogens are more frequently observed in tumors with higher expression of the steroid receptors^{18,19}.

Gallbladder cancer carries a very poor prognosis. It is usually diagnosed at an advanced stage, response to therapy is poor and survival is short¹⁷. Due to the high incidence of gallbladder cancer in Pakistan, lack of any effective therapy, poor prognosis and a likelihood that sex steroids may influence the pathogenesis and management of gallbladder cancer, we investigated 30 patients with gallbladder cancer for estrogen receptor (ER) expression on the malignant tissue and correlated it with the clinicopathological characteristics and survival.

Patients and Methods

This study was carried out on 30 consecutive patients who were histologically confirmed to have adenocarcinoma of the gallbladder. Twenty patients had tissue obtained at the time of laparotomy. Other ten had diagnosis based upon fine needle aspiration of the gallbladder mass. In all cases, samples were obtained from the primary site and enough tissue was available for histopathologic examination and ER analysis. Tissue was immediately fixed and embedded. Receptors were assayed subsequently, usually within two weeks of obtaining the tissue.

Histopathologic analysis was carried out on tissue stained with hematoxylin and eosin. Degree of differentiation of the tumor was noted. ER assay was performed immuno-histochemically utilizing Universal Immunoperoxidase Staining Kit (Signet Laboratories, Dedham, MA)²⁰. For this purpose, tissue sections were deparaffinized, rehydrated and separately incubated with two blocking agents (hydrogen peroxide and normal saline) to reduce non-specific background staining. Monoclonal antibody against estradiol was used to demonstrate cytoplasmic binding to the receptors. Peroxidase-antiperoxidase (PAP) method was used. Two negative and positive control slides were run in each case. Antibody reactivity was observed in the cytoplasm and scored as negative, mild, moderate or strongly positive depending upon the percentage of positive cells and intensity of staining. These values were expressed numerically.

Statistical analysis was performed using Fisher's exact test. Unless otherwise specified all values are expressed as the mean \pm one standard deviation. A probability value of 0.05 or less was considered significant.

Results

Thirty patients were included in this study. Clinical characteristics are provided in Table I.

Table I. Clinicopathological characteristics.

	Number	%
Sex		
Male	3	10
Female	27	90
Age (years±SD)		53.07±11.64
Female patients (n=27)		
Marital status		
Married	27	100
Parity (mean±SD)	5.8±2.73	
Menopausal status		
Pre	6	22.2
Post	21	77.8
Presence of gallstones	24	80
Degree of differentiation		
Well	4	13.3
Moderate	19	63.3
Poor	7	23.3
Stage of disease		
II	3	10
III	2	6.7
IV	5	16.7
Va	3	10
Vb	2	6.7
Vc	15	50
Estrogen receptor status		
Positive	18	60
Negative	12	40
Estrogen receptor score		
Mild to moderate	13	43.3
Strong	5	16.7
Present status		
Alive	5	16.7
Dead	25	83.3
Survival in months (Mean±SD)	5.97±4.88	

There were 27 females. Most of them were married, multiparous and post-menopausal. Majority (80%) had gallstones. Most of the patients had moderately differentiated tumors and two-thirds presented with stage V disease (Table I). Nevin's staging system was used²¹. Eighteen tumors had cytoplasmic expression of estrogen receptors, 12 were negative (Table II).

Table II. Clinicopathological characteristics stratified according to the ER status.

	ER Positive n=18	ER Negative n=12	p Value
Sex			
Male	2	1	0.806
Female	16	11	
Age (Years±SD)	52.7±10.6	53.6±13.5	0.840
Female patients (n=27)			
Marital status	All married	All married	
Parity (mean±SD)	6.0±2.8	5.6±2.8	
Menopausal status			
Pre	4 (25)	2 (18)	0.691
Post	12 (75)	9 (82)	0.739
Associate gallstones	16 (89)	8 (67)	0.142
Degree of differentiation of tumor			
Well differentiated	3 (17)	1 (8)	0.82
Moderately differentiated	13 (72)	6 (50)	
Poorly differentiated	2 (11)	5 (42)	
Stage of disease			
II	3 (17)	0 (0)	0.124
III	1 (5.5)	1 (8)	
IV	4 (22)	1 (8)	
Va	1 (5.5)	2 (17)	
Vb	0 (0)	2 (17)	
Vc	9 (50)	6 (50)	
Present status			
Alive	4	1	0.317
Dead	14	11	

Percentages are given in parenthesis.

Table II provides a comparison of clinico-pathological characteristics and survival between the two groups. There was no significant difference in gender, mean age at the time of presentation, marital status and parity. Most of the patients in both groups were post-menopausal. Menopause, however, did not influence ER status. Vast majority of the patients in both the groups had cholelithiasis. ER positive tumors were more frequently well-to moderately-well differentiated (89% vs 58%). This difference, however, did not achieve statistical significant. Most of the ER negative patients had advanced disease. Overall survival was poor and similar between the two groups.

Discussion

It has previously been demonstrated that sex steroids influence the formation and growth of tumors arising from their target organs. This effect is mediated through some specific receptors on the malignant cells. In breast cancer, ER status is an important determinant of response to anti-estrogenic therapy and survival^{18,19}. Estrogen receptors have also been demonstrated on other hormone-dependent

tumors such as ovarian and endometrial cancer^{22,23}. Interestingly, a variety of tumors arising from tissues not generally considered hormone-dependent also express these receptors. This includes tumors of pancreas, stomach, colon, liver, thymus, thyroid, brain, kidney and skin²⁴⁻³⁰. The patho-physiologic significance and clinical relevance of these receptors, however, remain uncertain³¹⁻³⁴.

Steroid receptors have been demonstrated on benign and malignant gallbladder tissue. Singletary et al observed the presence of both estrogen and progesterone receptors in the cytosolic and nuclear fractions of benign gallbladder tissue obtained from patients undergoing cholecystectomy during liver transplantation or for cholelithiasis. These were high-affinity receptors and specific for their respective ligands³⁵. Daignault et al reported the presence of progesterone receptors in 60% of patients who had cholelithiasis³⁶. More recently, similar results were reported by others³⁷⁻³⁸. These studies suggest that estrogen and progesterone receptors are present on the benign gallbladder tissue obtained from patients with cholelithiasis. Since estrogenic effect is required for the expression of progesterone receptors, these studies also suggest that these receptors are functional³⁹. Very few studies have been performed on the malignant gallbladder tissue. Nakamura et al studied 21 patients with gallbladder cancer⁴⁰. Immuno-histochemical analyses revealed that 58.6% of the tumors had cytoplasmic and 52.4% had nuclear ER expression. In this study, moderately-well and poorly-differentiated tumors were more often ER positive. This difference, however, was not statistically significant. Similarly, gender did not influence the receptor status. Yamamoto et al demonstrated that 23% of the patients with gallbladder cancer had ER positive tumors⁴¹. Well differentiated tumors were more often ER positive. Receptor status, however, did not influence survival. Our study demonstrates the presence of cytoplasmic estrogen receptors in 60% of patients with gallbladder cancer. These figures are similar to Nakamura et al but more frequent than the nuclear receptor positivity reported by Yamamoto et al. Variability of ER expression has been previously documented³⁵⁻³⁸. This may reflect heterogeneity in the patient population, methodology employed to determine ER status and interpretation of the histologic findings. Source of the malignant tissue for histologic examination i.e. primary site versus metastases may also play an important role. In breast carcinoma, it has been demonstrated that metastatic sites are less frequently ER positive as compared to the primary site¹⁹. All patients in our study had tissues obtained from the primary site. We utilized anti-estradiol antibodies to demonstrate cytoplasmic receptor binding. Although less specific than anti ER antibodies, it was the only method available at the Aga Khan University Hospital at that time. Additionally, other authors have used similar methods and demonstrated cytosolic binding^{36,37,40}. Even the more widely used dextran-coated charcoal method demonstrates cytosolic binding^{35,36,38}. However, using more specific monoclonal antibody against nuclear estrogenic receptors may be more advantageous. Such a study is in progress.

In this study, we observed no correlation between ER status and age, parity, menopausal status, presence of cholelithiasis, degree of differentiation of tumor or stage of the disease. Overall survival is also not influenced by the receptor status. Although these observations are similar to the previous two reports, number of patients is small. Larger studies are required to substantiate these preliminary findings.

Biologic significance of these receptors remains unknown. Only a few studies have utilized anti-estrogenic therapy in patients with tumors arising from the "non-target" organs³⁰. In general, such trials have been negative. This may be due to several reasons. Level of ER expression in "non-target" tissues is generally low. Degree of positivity is an important determinant of response to anti-estrogenic therapy. It is also possible that, at least in some cases, these receptors are not functional. This has been demonstrated in patients with melanoma^{42,43}. Furthermore, improper selection of patients may have contributed to the poor results. Restricting the use of anti-estrogens to individuals with ER positive tumors may be more appropriate to evaluate the worth of such an approach. Anti-estrogen therapy has

not been previously evaluated in patients with gallbladder cancer. A well controlled prospective clinical trial of hormonal therapy in patients with ER positive gallbladder cancer may be the best way to evaluate the pathophysiological significance of these receptors. Such a trial is in progress at our institution.

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