The Professional Medical Journal
www.theprofesional.com

DOI: 10.29309/TPMJ/18.4468

ORIGINAL PROF-4468

ESTROGEN RECEPTORS;

IMMUNOHISTOCHEMICAL STUDY OF ESTROGEN RECEPTORS IN HUMAN GALLBLADDER

Munawar Hussain Shah[1], Rana Khalid Mahmood[2], Afra Samad[3], Muhammad Abubakre Khalid[4], Mohammad Usama Shah[5], Muhammad Sulaiman Saeed[6]

1. MBBS, M.Phil.
Associate Professor
Department of Pathology
Nishtar Medical University Multan.
2. MBBS, M.Phil.
Assistant Professor
Department of Pathology
Nishtar Medical University Multan.
3. MBBS, DCP, M.Phil.
Assistant Professor
Department of Pathology
Multan Medical and Dental College
Multan.
4. MBBS
House Officer
Shaikh Zayed Medical College
Rahim Yar Khan.
5. MBBS Final Year Student
Shaikh Zayed Medical College
Rahim Yar Khan.
6. B.Sc MLT, M.Sc. Biochemistry,
M.Phil. Biochemistry
Biochemist
Department of Pathology,
Nishtar Medical University Multan.

Correspondence Address:
Dr. Rana Khalid Mahmood
Assistant Professor
Department of Pathology
Nishtar Medical University Multan.
drkhalid.rana@yahoo.com

Article received on:
30/10/2017
Accepted for publication:
25/05/2018
Received after proof reading:
06/11/2018

ABSTRACT… Objectives: Purpose of study is to determine Estrogen receptor status in inflammatory and neoplastic lesions of human gallbladder and to determine any relation of ER status with age, sex and histological grades of gallbladder carcinoma. **Study Design:** Retrospective observational study. **Setting:** Pathology Department Nishtar Medical College Multan. **Period:** March 2016 to March 2017. **Methodology:** In this study total no. of 50 cases of different gallbladder diseases were included. Out of these 50 cases 30 were of neoplastic lesions and other 20 were of non-neoplastic inflammatory lesions of gallbladder. The gallbladders slides of these 50 cases were randomly selected from the records of Pathology Department. Demographic variables like age and gender were calculated and analyzed. Estrogen positivity was the outcome variable of this study. Data was analyzed using computer software SPSS version 23. Chi square Test was applied to check the association between different variables. **Results:** In this study, 100% (n=50) diagnosed cases of human gallbladder diseases including inflammatory and neoplastic lesions were subjected to immunohistochemical staining for ER expression. 100% (n=20) cases of inflammatory lesions and 100% (n=30) cases of neoplastic lesions were included, in this study. Estrogen positivity was noted in 36.7% (n=11) cases of neoplastic lesions, while it was 25% (n=5) in inflammatory lesions cases. No association was found between estrogen positivity in groups, (χ^2=0.751, p=0.386). (Table1).Histological types of gallbladder carcinoma was noted as adenocarcinomas (well differentiated) in 60% (n=18) cases, adenocarcinomas (moderately differentiated) in 16.7% (n=5) cases, adenocarcinomas (poorly differentiated) in 13.4% (n=4) cases, mucinous adenocarcinomas in 1% (n=3.3) cases, adenosquamus carcinoma in 1% (n=3.3) cases and squamous cell carcinoma in 1% (n=3.3) cases. (Table2).No association was found between gender (p=0.780), stratified age (p=868) and estrogen positivity (p=0.386), after applying the chi-square. **Conclusion:** In this study it was concluded that statistically no significant difference has been found between ER positivity with age, gender and histological type of carcinoma. Presence of ER raises questions regarding the role of estrogen in various gallbladder diseases and whether this hormone is functional or not in neoplastic lesions is also questionable. Techniques more modified may be tried in search of better and more reliable results. It is therefore too early to consider anti-estrogen trials in gallbladder carcinoma until the role of these steroid binding proteins is well characterized.

Key words: Immunohistochemical, Estrogen Receptors, Neoplastic, Non-Neoplastic, Gallbladder.

Article Citation: Shah MH, Mahmood RK, Samad A, Khalid MA, Shah MU, Saeed MS. Estrogen receptors; immunohistochemical study of estrogen receptors in human gallbladder. Professional Med J 2018; 25(11):1735-1740.
DOI:10.29309/TPMJ/18.4468

INTRODUCTION

Estrogen receptors and progesterone receptors activity have been identified in a variety of human tissues including, heart, endometrium, liver, myometrium, pancreas, bones and skin, and in several body tumors like breast cancer, endometrial cancer, meningioma, cancers of alimentary tract, thymus tumor, melanoma and pancreas tumor[1,2,3] using dextran coated charcoal method or radio-immunoassay.[4]

On the other hand histological detection of estrogen receptors and progesterone receptors in tumor tissues has been done using fluorescent immunochemical methods or cytochemical methods.[5] However there are some discrepancies in the result between biochemical assay and cyto-chemical methods.[6] Advent of specific

monoclonal antibodies to human estrogen[7] and progesterone receptors has heralded a new era in the histological analysis of these steroid receptors. These monoclonal antibodies are useful for estrogen receptors and progesterone receptors immunohistochemically not only on frozen sections but also on formalin fixed paraffin sections. It is well known fact that gallbladder disease including gall bladder carcinoma is found more frequently in females than males.[8] This fact suggests a possibility that the gall bladder may be a sex hormones responsive tissue. The development of liver adenomas and carcinomas in women who take contraceptive pills and inothers, who take drugs with androgenic side effects, is supportive to the hypothesis that both normal and malignant cells of billiary tract may be very much responsive to hormones. Gall bladder cancer is also more frequently seen in females[9] especially with large number of pregnancies and advancing age. This raises the possibility that sex steroids may influence the pathogenesis of gall bladder cancer either directly or indirectly.

The concentration of estrogen and progesterone receptors in breast carcinomas is well established and as an index predicting the subsequent response of disease to endocrine therapy. The response rate to hormonal therapy has improved from 30% in general breast cancer population to 60% in receptor rich tumors. Highest response rate approximately 80% are found in tumors containing both estrogen and progesterone receptors.[2] Carcinoma of gallbladder is highly lethal disease and five years survival rate after surg ery is minimal, most of the patients die in one year.[10] It is usually diagnosed at an advanced age and response to therapy is poor, therefore survival rate is short resulting in poor prognosis. At the time of the surgery half of the patients have metastasis and the tumor is advanced usually inoperable. For many years, five years survival rate for stage V is zero in spite of the radical surgery. So this study is designed, based on the status of estrogen receptors and progesterone receptors in gallbladder as it might help the patients suffering from carcinoma of gallbladder.

MATERIALS AND METHODS

In this study total no. of 50 cases of different gallbladder diseases were included. Out of these 50 cases 30 were of neoplastic lesions and other 20 were of non neoplastic inflammatory lesions of gallbladder. Study was conducted in Pathology Department Nishtar Medical College Multan from March 2016 to March 2017. The gallbladders slides of these 50 cases were randomly selected from the records of Pathology Department Nishtar Medical College Multan. Slides were reviewed critically and those slides showing poor amount of viable representative tumor and other lesions with a less preserved morphology were excluded from study.

Histologically neoplastic lesions were well differentiated, moderately differentiated and poorly differentiated adenocarcinomas, mucous carcinoma, adenosquamous carcinoma and squamous cell carcinoma. Inflammatory lesions included chronic cholecystitis and its variants. Each of the 50 blocks were re-cut and subjected to haemotoxylin and eosin staining, PAS, Trichrome and Alcian blue staining. Immunohistochemistry on paraffin sections was performed using a modified avidin-biotin alkaline phosphatase method. Rat monoclonal antibody to ER was used for immunohistochemical detection of ER in normal and neoplastic lesions and as atool for prediction of tumor response to endocrine therapy and assessment of prognosis. Demographic variables like age and gender were calculated and analyzed. Estrogen positivity was the outcome variable of this study. Data was analyzed using computer software SPSS version 23. Chi square Test was applied to check the association between different variables.

RESULTS

In this study100% (n=20) cases of inflammatory lesions and 100% (n=30) cases of neoplastic lesions were includedand subjected to immunohistochemical staining for ER expression. The mean age of the patients of neoplastic lesions was 53.60±14.20 years ($\chi^2 = 0.028$, p=0.868). In neoplastic lesions cases, there were 23.3% (n=7) males and 76.7% (n=23) females. While, the mean age of the patients of inflammatory lesions

was 54.10±17.36 years. In inflammatory lesions cases, there were 20% (n=4) males and 80% (n=16) females. (Table-I).

Estrogen positivity was noted in 36.7% (n=11) cases of neoplastic lesions (Figure-1), while it was 25% (n=5) in inflammatory lesions cases (Figure-2). No association was found between estrogen positivity in groups, (χ^2=0.751, p=0.386). (Table-I). Histological types of gallbladder carcinoma was noted as adenocarcinomas (well differentiated) in 60% (n=18) cases (Figure-3), adenocarcinomas (moderately differentiated) in 16.7% (n=5) cases, adenocarcinomas (poorly differentiated) in 13.4% (n=4) cases (Figure-4), mucinous adenocarcinomas in 1% (n=3.3) cases, adenosquamus carcinoma in 1% (n=3.3) cases and squamous cell carcinoma in 1% (n=3.3) cases. (Table-II). No association was found between gender (p=0.780), stratified age (p=868) and estrogen positivity (p=0.386) after applying the chi-square. (Table-III).

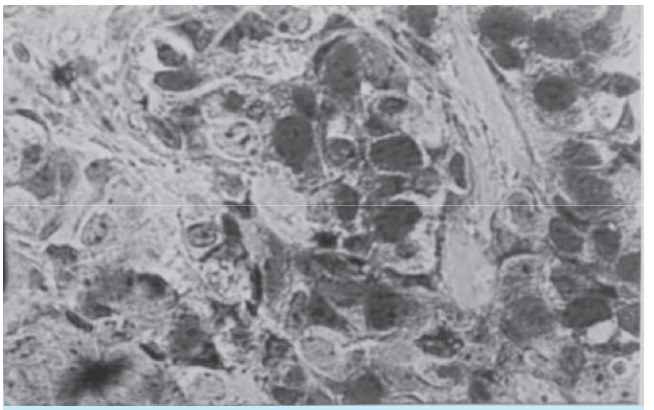

Figure-1. Breast carcinoma with intra-nuclear estrogen receptor positivity. Immunostaining X400.

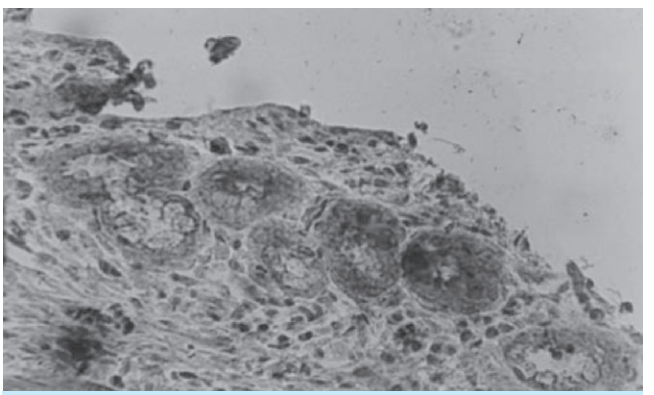

Figure-2. Chronic cholecystitis with intra-nuclear estrogen receptor positivity. Immunostaining X200.

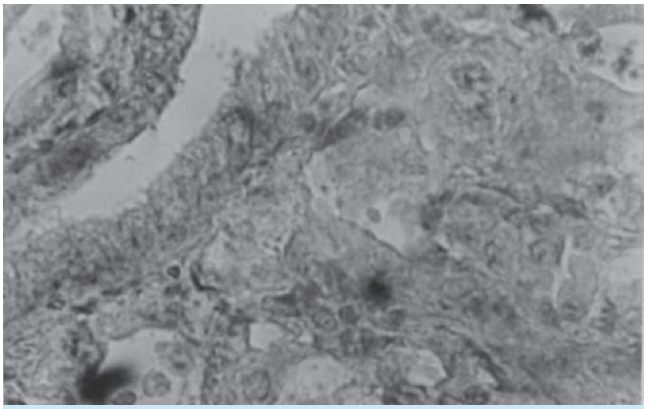

Figure-3. Adenocarcinoma (Well differentiated). Immunostaining X 400 ER Positive.

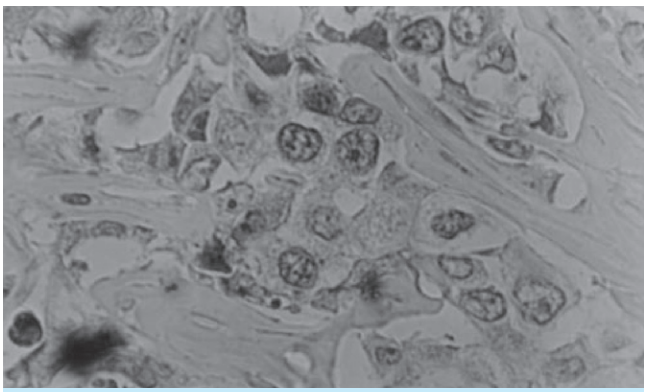

Figure-4. Adenocarcinoma (Poorly differentiated). Immunostaining X 400 ER Positive.

Variable	Neoplastic	Non-Neoplastic	Test of Sig.
Gender	M=23.3% , F=76.7%	M=20%, F=80%	χ^2 = 0.078 p=0.780
Age	53.60±14.20 years	54.10±17.36 years	χ^2 = 0.028 p=0.868
Estrogen positivity	36.7% (n=11)	25% (n=5)	χ^2 = 0.751 p=0.386

Table-I. Demographic variables

Histological Type	Frequency	Percentage
Adenocarcinoma (well differentiated)	18	60.0
Adenocarcinoma (moderately differentiated)	5	16.7
Adenocarcinoma (poorly differentiated)	4	13.4
Mucinous adenocarcinoma	1	3.3
Adenosquamus carcinoma	1	3.3
Squamous cell carcinoma	1	3.3
Total	30	100.0

Table-II. Histological types of gallbladder carcinoma

Variable		Groups		Total	P-value
		Neoplastic	Non-Neoplastic		
Gender	Male	7	4	11	0.780
	Female	23	16	39	
Total		30	20	50	
Stratified Age	10-35 Years	4	3	7	0.868
	36-90 Years	26	17	43	
Total		30	20	50	
Estrogen Positivity	Yes	11	5	16	0.386
	No	19	15	34	
Total		30	20	50	

Table-III. Association of neoplastic and non-neoplastic with gender, age and estrogen positivity

DISCUSSION

In a previous study it was concluded that estrogen receptors are present not only in the mucosa of gallbladder in multiple disease states but are also suggestive of the fact that estrogen receptors are related to metaplasia of gallbladder's mucosa.[11] In another study, immunohistochemical method (Universal Immunoperoxidase Staining Kit) was used to perform estrogen receptors assay. The results of that study showed no significant difference in mean age, gender, parity and marital status upon comparison of clinicopathological properties and survival between the two understudy groups. Likewise, estrogen receptor status was not associated with presence of gallstones, survival or histological grade.[12] A study was performed in the past, which concluded that despite of previous reports, in very small percentage of gallbladder carcinomas, weak estrogen receptor staining occurs. Moreover, even though it's unclear that minimum amount of estrogen receptors is required for optimum therapeutic benefit, hormonal therapy recommendations for gallbladder cancer is very unlikely to change the outcome of this neoplastic condition.[13]

Ranelletti et al performed a study to find out the concentration of estrogen and progesterone in the gallbladders of patients with gallstones and they found that there was a statistically significant difference present among male and female genders while analyzing the proportion of gallbladders with positive estrogen receptors. There were 19 % male and 69% female patients. The results of the study showed that a positive correlation is evident between the sex difference and the co-expression of estrogen and progesterone receptors. It concludes that there is certainly higher rate of gallstones in females than in males.[14] Multiple studies have provided the evidence that gallbladder contains both estrogen and progesterone receptors and it might be possible that many steroid hormones interact with these receptors to carry out certain aspects of gallbladder function.[15] Similarly, higher frequency of gallstones in females of reproductive age is suggestive of the fact that sex hormones play role in pathogenesis of cholelithiasis. In a pilot study higher concentration of estrogen and estrogen receptors was found in the gallbladder of the persons treated for cholelithiasis. These findings and many other previous findings on the presence of estrogen receptors in several human tissues, support the hypothesis, which says that development of gallbladder diseases, like stones and carcinoma is somehow influenced by presence of estrogen and its receptors.[16]

While studying the role of estrogen in gallbladder cancer, Baskaran et al found that female sex hormones might be related to the pathogenesis of cholelithiasis and also that progesterone receptors have a prognostic value. They recommended that larger studies must be conducted to seek out this problem, so that gallbladder cancer can be treated with suitable sex hormone therapy.[17] In a study estrogen and progesterone receptor expression was analyzed using automated immunohistochemical method in both malignant and benign gallbladder tissues. The results showed that these female sex hormones play a considerably significant role in carcinogenesis of the gallbladder. In about one

third of the patients estrogen receptors were found and in half of them progesterone receptors were found, suggesting their prognostic value as well as their potential role in anti-hormonal therapy.[18] Similarly studies have also shown that impairment of gallbladder emptying might be associated the presence of progesterone receptors in gallbladder mucosa of cholelithiasis patients.[19] An assessment of estrogen receptors expression in cancers of gallbladder might be significant in identification of bad prognostic group of gallbladder carcinomas.[20]

CONCLUSION

Estrogen receptors are found in inflammatory as well as neoplastic lesions of human gall bladder. There is no association between ER positivity with age, gender and histological type of the carcinoma. It is not clear whether ER are functional and involved in the development of the gall bladder diseases. Further studies using more advanced techniques are required to establish the role of ER in gall bladder lesions.

REFERENCES

1. Shao R, Cao S, Wang X, Feng Y, Billig H. **The elusive and controversial roles of estrogen and progesterone receptors in human endometriosis.** Am J Transl Res. 2014 Jan 15; 6(2):104-13.

2. Huang B, Warner M, Gustafsson JÅ. **Estrogen receptors in breast carcinogenesis and endocrine therapy.** Mol Cell Endocrinol. 2015 Dec 15; 418 Pt 3:240-4.

3. Caiazza F, Ryan EJ, Doherty G, Winter DC, Sheahan K. **Estrogen receptors and their implications in colorectal carcinogenesis.** FrontOncol. 2015; 5:19.

4. Skenandore CS, Pineda A, Bahr JM, Newell-Fugate AE, Cardoso FC. **Evaluation of a commercially available radioimmunoassay and enzyme immunoassay for the analysis of progesterone and estradiol and the comparison of two extraction efficiency methods.** Domest Anim Endocrinol. 2017 Jul; 60:61-66.

5. Ruijtenbeek R, Hilhorst MH, Umar A, Foekens JA, Martens JW, inventors; Pamgene BV, assignee. **Method for determining the estrogen receptor status of breast cancer.** United States Patent US. 9,075,061. 2015 Jul 7.

6. Reiner A, Neumeister B, Spona J, Reiner G, Schemper M, Jakesz R. **Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer.** CancerRes. 1990 Nov 1; 50(21):7057-61.

7. Hill DA, Barry M, Wiggins C, Nibbe A, Royce M, Prossnitz E, et al. **Estrogen receptor quantitative measures and breast cancer survival.** Breast Cancer Res Treat. 2017 Aug 19:doi: 10.1007/s10549-017-4439-6.

8. Hundal R, Shaffer EA. **Gallbladder cancer: epidemiology and outcome.** ClinEpidemiol. 2014; 6:99-109.

9. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet□Tieulent J, Jemal A. **Global cancer statistics, 2012.** CA Cancer JClin. 2015 Mar 1; 65(2):87-108.

10. Lu W, Wang N, Chu Y, Zhou L, Li M, Huang T, et al. **CLIC1 antibody conjugated nanoscale contrast agent as a sensitive and targeted molecular imaging probe for gallbladder cancer diagnosis.** RSC Advances. 2016; 6(29):24104-10. DOI: 10.1039/C5RA26593B.

11. Yamamoto M, Nakajo S, Tahara E. **Immunohistochemical analysis of estrogen receptors in human gallbladder.** ActaPatholJpn. 1990 Jan; 40(1):14-21.

12. Malik IA, Abbas Z, Shamsi Z, Daudi I, Shah HA, Moid I, et al. **Immuno-histochemical analysis of estrogen receptors on the malignant gallbladder tissue.** JPMA. J Pak Med Assoc. 1998 May; 48(5):123-6.

13. Ko CY, Schmit P, Cheng L, Thompson JE. **Estrogen receptors in gallbladder cancer: detection by an improved immunohistochemical assay.** Am Surg. 1995 Oct; 61(10):930-3.

14. Ranelletti FO, Piantelli M, Zanella E, Capelli A, Farinon AM. **Estrogen and progesterone receptors in the gallbladders from patients with gallstones.** Hepatology. 1991 Oct; 14(4 Pt 1):608-12.

15. Singletary BK, van Thiel DH, Eagon PK. **Estrogen and progesterone receptors in human gallbladder.** Hepatology. 1986 Jul 1; 6(4):574-8.

16. Chen A, Huminer D. **The role of estrogen receptors in the development of gallstones and gallbladder cancer.** MedHypotheses. 1991 Nov 30; 36(3):259-60.

17. Baskaran V, Vij U, Sahni P, Tandon RK, Nundy S. **Do the progesterone receptors have a role to play in gallbladder cancer?.** IntJGastrointestCancer. 2005 Feb 1; 35(1):61-8.

18. Gupta P, Agarwal A, Gupta V, Singh PK, Pantola C, Amit S. **Expression and clinicopathological significance of estrogen and progesterone receptors in gallbladder cancer.** Gastrointest Cancer Res. 2012 Mar; 5(2):41-7.

19. Dalgnault PG, Fazekas AG, Rosenthall L, Fried GM. **Relationship between gallbladder contraction and progesterone receptors in patients will gallstones.** AmJSurg. 1988 Jan 1; 155(1):147-51.

20. Park JS, Jung WH, Kim JK, Hwang HK, Cho SI, Yoon DS, et al. **Estrogen receptor α, estrogen receptor β, and progesterone receptor as possible prognostic factor in radically resected gallbladder carcinoma.** J Surg Res. 2009 Mar 31; 152(1):104-10.

Challenges are what make life interesting and overcoming them is what makes life meaningful.

– Joshua J. Marine –

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Munawar Hussain Shah	Study planning, designing, coordination in data collection, Paper writing and editing.	
2	Rana Khalid Mahmood	Study planning, designing, data entry, manuscript writing and editing.	
3	Afra Samad	Study planning, manuscript writing and editing, final proof reading.	Afra Samad.
4	M. Abubakre Khalid	Coordination in data collection, data analysis and data entry.	Abubakr
5	M. Usama Shah	Coordination in data collection, data analysis and data entry.	
6	M. Sulaiman Saeed	Study planning, manuscript writing and editing, Final proof reading.	