

ESTROGEN AND PROGESTERONE RECEPTOR CONTENTS IN HEPATOCELLULAR CARCINOMA

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SUMMARY: Estrogen and progesterone receptor content (ER-PR) were studied by immunohistochemistry in 25 cases with hepatocellular carcinoma (HCC) and 28 cases with chronic liver disease (CLD). 21 of 25 HCC samples were positive for ER and 8 of them had strong staining. 12 of 28 cases with CLD was positive for ER and all of them had weak staining. The result of anti-estrogen-tamoxifen therapy in one case was discussed. This study suggests that ER is found in high incidence in HCC and anti-estrogen therapy must be evaluated in larger study groups.

Key Words: Estrogen receptor, progesterone receptor, hepatocellular carcinoma, chronic liver disease.

INTRODUCTION

Hormonal factors may play a role in the pathogenesis of hepatic neoplasia. Oral contraceptives, at least in some cases, may cause malignant transformation (1-3). Megestrol and/or ethynil estradiol have been shown to cause the HCC in female rats (4). It has also been shown that long term administration of androgenic anabolic steroids may cause HCC (5). Histologically confirmed tumors may regress after the contraceptive use is withdrawn and conversely may recur if it is reused (6) but data about estrogens as an initiative factor of neoplastic growth are controversial (7,8).

In this study we aimed to study the estrogen and progesterone receptor content in HCC and to compare it with other CLDs such as chronic persistent hepatitis (CPH), chronic active hepatitis (CAH) and cirrhosis (C).

MATERIALS AND METHODS

Our study group was consisted of 25 cases with HCC, 10 cases with CPH, 7 cases with CAH and 11 cases with C.

The tissues were obtained either by percutaneous needle biopsy or laparoscopic biopsy. They were fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections (5 micron), which had been de-paraffinized in xylene, were treated by the streptavidin-biotin complex immunohistochemical technique described by the manufacturers.

Rabbit anti-estradiol concentrated antibody (polyclonal primary antibody) (Biogenex, PU038-UP) (dilution 1/40) was used to determine the estradiol content and mouse anti-human progesterone concentrated antibody (monoclonal primary antibody) (Serotec, MCA 397) (dilution 1/50) was used to determine the progesterone content of the biopsy samples.

The intensity of the cytoplasmic staining in carcinoma cells and liver cells was graded as follows: -, no staining; +, weak staining; ++, intermediate staining; +++, strong staining.

RESULTS

ER content was positive in 21 of 25 HCC cases of which 4 had (+), 8 had (++) and 9 had (+++) staining for ER. PR was positive in 16 cases. Patient characteristics and ER-PR content is shown in Table 1. ER content was not high in CLDs and greatest ER content was (+) in these cases. 4 cases with CPH (4/11), 4 cases with CAH (4/7)

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Table 1: Patient characteristics ER and PR contents of the tumor cells.

No	Age	Sex	(ER)	(PR)
1	78	M	(++)	(++)
2	60	M	(+)	(+)
3	30	F	(+++)	(+)
4	59	M	(++)	(+)
5	60	M	(+)	(-)
6	64	M	(+++)	(+++)
7	35	M	(-)	(+)
8	53	F	(+++)	(-)
9	63	M	(++)	(+)
10	62	M	(+++)	(-)
11	70	M	(++)	(++)
12	53	M	(++)	(+++)
13	43	M	(+++)	(++)
14	75	M	(+++)	(+++)
15	40	M	(+++)	(-)
16	60	M	(+++)	(-)
17	14	M	(++)	(-)
18	32	F	(+++)	(+++)
19	60	M	(-)	(+++)
20	10	M	(-)	(-)
21	64	M	(+)	(+)
22	63	M	(-)	(+)
23	53	M	(+)	(+)
24	25	M	(++)	(+++)
25	41	41	(++)	(++)

and 4 cases with C (4/11) had (+) staining. ER and PR content were shown in Figure 1.

There were 2 cases of fibro-lamellar HCC in our study group and both of them had (+++) ER staining and one of them was treated with anti-estrogen-tamoxifen.

Case Report: A thirty two year-old-woman admitted to the hospital in February 1991 due to gastrointestinal bleeding. Physical exam revealed a huge mass filling the right upper quadrant and epigastrium. CT scanning of the abdomen showed giant mass originating from left liver lobe pushing the stomach downward and invading the large abdominal vassels.

Percutaneous liver biopsy was non-diagnostic and laparotomy was performed for diagnosis and for possible resection. Large liver mass was explored but it was unrespectable because of invasion to the large abdominal vassels. Histopathological diagnosis of the preoperative liver biopsy was HCC-fibro-lamellar type.

Chemotherapy with mitoxantrone was given until toxic dosage. Tumor diameter did not decrease by this therapy. In February 1992, 5 Fluorouracil plus interferon alpha was started but she could not tolerate this regimen and it was withdrawn. There was no therapy alternative and ER content was explored. The tumor cells showed strongly positive (+++) staining for ER (Figure 2). Anti-estrogen-tamoxifen was started 20 mg/daily/orally. Three months later mass volume was found to be less than half of the original tumor tomographically but transudative ascites developed due to hypoalbuminemia. Liver biopsy was repeated and was reported as: large hyalinized fibrous tissue areas among which there were small neoplastic cell clusters and normal liver tissue. ER was poorly stained in highly limited areas (Figure 3).

DISCUSSION

Human liver contains estrogen receptors and render it sensitive to estrogen (4). Estrogen and its receptors and androgen may play a role in pathogenesis of hepatic neo-

Figure 1: ER and PR contents in HCC and CLDs.

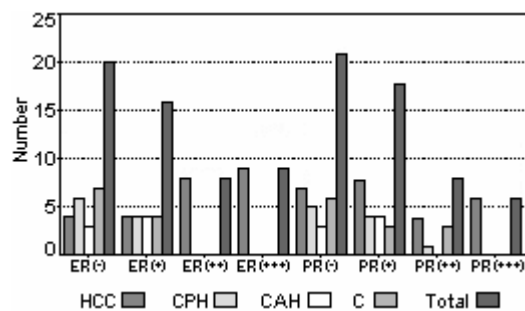
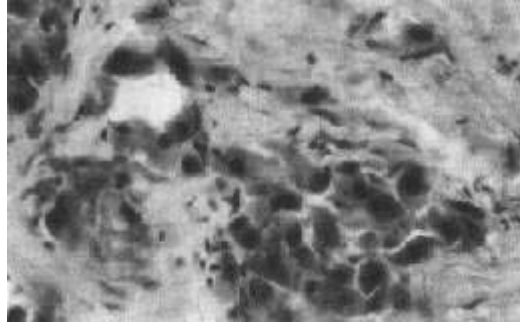


Figure 2: (+++) ER staining before treatment.



plasia (8,9). Estrogen and androgens have been shown to stimulate sex hormone binding globulin secretion in HCC cell-line. Estrogen and androgen regulate sex hormone binding globulin synthesis in man by specific probably receptor mediated effects on hepatocytes (10). Alteration of the estrogen to androgen ratio in cirrhotics with HCC may contribute to the carcinogenesis (11). ER like material exists in the cytosol of the human digestive tumors and normal liver tissue (12). It is widely accepted that liver neoplasms may be induced or inhibited with hormonal agents (1-8,13-15). Oral contraceptives may cause to malignant hepatic tumors (1-3,6,16).

Specific intracellular receptor proteins are involved in the estrogenic regulation of gene expression and growth in reproductive tissues and both cytosol and nuclear forms of the receptor protein may reside in the nuclear compartment (17). It has been found that nuclear ER levels in neoplastic tissue are greater than those in normal tissue. Differences in binding capacity suggest a potential for greater hormone responsiveness in neoplastic liver tissue (4). In another study it has been shown that estradiol bind-

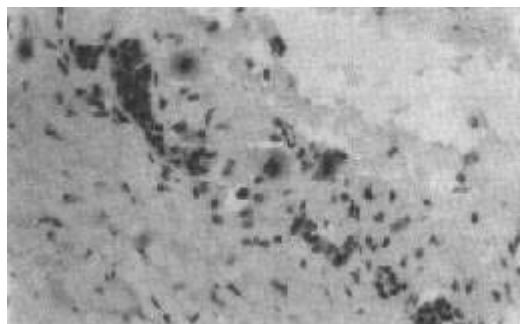
ing activity is similar in normal human liver and HCC (18).

In our study group ER content was found to be in high incidence and strongly positive in HCC group than other CLDs. This result may suggest the role of estrogen in liver carcinogenesis. In 9 cases PR positiveness accompanied to ER in the tumor cells but we could not find available information about PR positivity and we do not know the importance of PR positivity in HCC.

The incidence of ER content is highly variable according to the different authors, but study groups are not large enough. Largest study group containing 66 HCC cases, ER content was found in 26 (19). Among other study groups containing 30, 25, 19, 16 cases ER was found to be positive in 12, 10, 7 and 3 cases respectively (12, 20-22). In our study ER was found to be positive in 84% of the patients which is higher than other groups. This may be due to differences of methods. In other series ER content has been measured directly with charcoal assay. We studied ER content by immunohistochemistry and it is known that ER like material exists in normal tissue (12).

In cirrhotic cases ER was found in 13 of 28 and 11 of

Figure 3: Poor ER staining after treatment.



25 cases (27, 28) and was similar to our results. We could not find a report about CPH and CAH, and could not compare them.

The presence or absence of ERs in human HCC correlates to neither biologic nor pathologic characteristics (19,20,22) and ER content does not correlate with AFP, CEA, HBV (20).

Therapy results associated with anti-estrogen in HCC are not sufficient, and they are controversial. In one study it has been found that hormonal therapy for HCC especially in cases which show high concentrations of ER and/or AR is effective while in another one endocrine therapy was found to be ineffective (9,23). Largest study associated with anti-estrogen-tamoxifen in HCC consisting of 59 cases showed that response to cytotoxic chemotherapy was 11% while cytotoxic chemotherapy plus tamoxifen was 16% and this study suggested that tamoxifen may have a role in maintenance of doxorubicin induced remissions (24). Our experience with anti-estrogen therapy in HCC is highly limited; but the clear evidence of regression of the tumor may suggest the anti-estrogen therapy in HCC especially if the ER is found to be positive. ER content and anti-estrogen therapy must be studied in larger study groups and the subject deserves further investigations.

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