

Role of cholecystokinin receptors in carcinoma gallbladder.

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Abstract

Cancer of the Gallbladder commonly occurs in elderly population and is fifth common cancer in women. The aetiology of cancer gallbladder is not well understood. The present study was conducted to compare expression of the CCK receptors in the blood and gallbladder tissue in cases of cholelithiasis, cancer gallbladder and normal gallbladder. Forty- nine cases comprising 22 cancer gallbladders, 19 cholelithiasis and 8 normal gallbladders obtained from patients operated for biliary trauma or Whipple's procedure. RNA extraction and cDNA formation for CCK-A and CCK-B receptors were carried out. Real Time PCR was performed. Both CCK-A and CCK-B receptors were expressed in all tissues and blood samples studied in the normal, cholelithiasis or carcinoma gallbladder. Although there was higher expression of CCK-A receptor in the cholelithiasis group as compared to normal group and decreased expression of CCK-A receptors in the carcinoma gallbladder group as compared to normal group yet this difference was not statistically significant. Conclusion: This is a preliminary study reported a comparative expression of cholecystolinin receptors of gallbladder tissues and blood samples from patients with normal gallbladder, cholelithiasis, and carcinoma gall bladder. The resulted insignificant data could possibly be due to small sample size. So far previous reports are not available to compare our results.

Key words: Cholecystokinin, receptor, gallbladder

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Introduction

Biliary malignancy and gallbladder stone disease are very common in some parts of India . Cholelithiasis and cholecystitis are found in 10 to 15% cases [1]. The Cancer of the Gallbladder commonly occurs in elderly population and is fifth common cancer in women [2]. The aetiology of cancer gallbladder is not well understood though impaired gallbladder emptying has been reported in patients with gallbladder stone disease due to defect in the cholecystokinin receptors [3].

Cholecystokinin receptors (CCK) are widely distributed in the human body especially in the gastrointestinal system and are of two types namely, CCK-A and CCK-B. Gallbladder muscle has predominant expression of CCK-A which controls its contraction and relaxation [4]. Studies have shown that CCK-B receptors are also predomi-

nantly expressed on the polymorphonuclear cells in the human blood.

Whether defects of the CCK receptors play a role in the development of gallbladder stone disease and malignancy of the gallbladder or increased expression of one type of CCK receptor/s is the effect of the above diseases, remains speculative. Enough data are not available yet the subject excites interest. The present study was conducted to find out expression of the CCK receptors in the blood and gallbladder tissue in cases of gallbladder stone disease and gallbladder cancer compared to normal gallbladder.

Methods

Forty-nine cases were recruited, after informed consent and approval of the ETHICAL committee of the institution, which comprised of 22 cancer gallbladder, 19 chole-

cystitis with cholelithiasis. Eight normal gallbladders were also obtained from patients operated for trauma of the biliary system or Whipple's procedure, which served as control.

RNA extraction and cDNA formation for CCK-A and CCK-B receptors were carried out. Real Time PCR was performed on cDNA and threshold cycle (Ct) value of each sample was obtained and ΔCt was calculated.

ΔCt (sample) = Ct (Target) - Ct (Reference) Where Target-receptor, Reference- β actin. The PCR products were subjected to electrophoreses on 2% agarose Tris-boric acid EDTA gel.

Mean and SD were calculated for quantitative data. Three-way ANOVA test was used to compare expressions of CCK receptors in the normal and diseased gall bladders. Chi-square test was applied for comparing two groups and ANOVA test for comparing multiple. Tukey's and Dunnet-C test were used to find out significance at 5% ($p < 0.05$).

Results

In our study, a total of 49 cases were recruited of which 8 cases of normal gallbladder, 19 cases of cholecystitis with cholelithiasis, 22 cases of carcinoma gallbladder and 4 cases were of advanced carcinoma gallbladder where only blood sample was analysed for CCK receptors.

The mean age of the subjects was 45.12, whereas mean age in normal gallbladder group was 43.25, in cholelithiasis group was 37.42 and in the carcinoma gallbladder group it was 52.43. Significant difference was seen between cholelithiasis and carcinoma gallbladder ($p = 0.001$). There were 33 female subjects out of a total of 49 subjects ($p = 0.08$).

In gallbladder tissue samples, all patients of normal gallbladder group had RNA with expressions of both CCK-A and CCK-B receptors, however CCK-B receptors were expressed in most cases (63%). Similar trend was observed in the patients of cholecystitis with cholelithiasis where CCK-B receptors were expressed in 56% (9/16) cases. In gallbladder cancer, again the expression of CCK-B was the most 73% (13/18).

In blood, all cases of normal gallbladder group had RNA with expressions of both CCK-A and CCK-B receptors but CCK-A receptors were expressed more 62% (5/8). CCK receptors were analysed in the blood samples of 15 patients of cholecystitis with cholelithiasis. All samples showed RNA with both CCK-A and CCK-B receptors but CCK-A were expressed predominantly in 15 patients. In the gallbladder cancer group, 19 patients out of 22 were studied for CCK receptors in blood. Four patients out of 19 were suffering from advanced carcinoma gallbladder. All 19 patients showed RNA in which both CCK-A and

CCK-B receptors were expressed almost equally in 10 and 9 patients, respectively.

Discussion

Both CCK-A and CCK-B receptors were expressed irrespective of its origin in all tissues samples studied. Although there was higher expression of CCK-A receptor (44%) in Cholelithiasis group as compared to normal/control group (37%) and a decreased expression of CCK-A receptors in Carcinoma gallbladder group (27%) as compared to normal/control group (37%), yet this difference was not found to be statistically significant ($p = 0.27$). In all blood samples so far studied, higher expression of CCK-A receptor (87%) in Cholelithiasis group as compared to normal group (63%) and an attenuated expression of CCK-A receptors in Carcinoma gallbladder group (53%) as compared to normal group (63%) were not statistically significant ($p = 0.17$).

This is a preliminary study done to evaluate the expression of cholecystokinin receptors in gallbladder tissues and blood samples of patients having normal gallbladder, cholelithiasis, and carcinoma gall bladder. Limitation of our study is primarily insufficient sample size. Moreover, the lack of literature in our area of research restricted us to compare our finding with others. Other parameters like genetic makeup, different food habits, racial differences of a larger population are to be considered to make a definitive conclusion on the pathogenesis of biliary carcinoma and gallbladder stone disease.

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