Int. j. pathol 2015; 13(4): 159-163

Original Article

Expression Of Cox-2 In Non- Liver Diseases

Mubashira Hashmi* and Shahnaz Imdad Kehar**
Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center Karachi.

Objective: To determine the frequency of liver diseases and evaluate the expression of COX-2 in non-neoplastic and neoplastic liver lesions.

Methodology: This cross sectional study is based on the analysis of liver biopsies received at department of pathology, BMSI, JPMC from 1st January 2010 to 31st December 2012. Approximately 71 cases of formalin fixed liver tissue biopsies were selected and analyzed for morphological features, grading and results of immunohistochemical staining for COX-2. The data feeding and analysis were on computer package SPSS (Statistical Packages of Social Sciences) version 16.0. In all statistical analysis only p-value <0.05 was considered significant.

Results: The most commonly encountered liver disease , out of the total 288 cases ,was chronic liver disease (CLD) including 255 cases (88.54%) out of these 12(4.17%) showed full- fledged cirrhotic nodule. Mean age was 33 yrs. for chronic liver disease. Male female ratio was 1.6:1 for CLD while for hepatocellular carcinoma and bile duct carcinoma M/F ratio was 2.8:1. The etiological distribution of 255 cases of CLD cases revealing that hepatitis C is the most common cause of chronic hepatitis accounting for 70% of cases. On immunostaining using COX-2 antibody for CLD with mild inflammatory grade cases, only 14.2% showed strong staining and all the 19 HCC and bile duct carcinoma 78.94% displayed strong positivity.

Conclusion: There is progressive increase in staining expression of COX-2 in non-neoplastic and neoplastic liver lesions. An early inhibition of COX-2 via selective COX-2 inhibitors may prevent further exacerbation of disease.

Key Wrds: COX-2, liver disease, liver cancer.

Introduction

cancer is the sixth most common cancer in the world, 750000 people worldwide i.e. 6% of the totals were diagnosed for liver cancer (Ferlay et al., 2010).

In Pakistan the data from Shaukat Khanum Cancer Hospital and Research Center (CRCDM) from Dec 1994 to Dec 2011 shows that liver cancer is at number 1 position amongst the top 10 malignancies and accounts 1,926 cases i.e. 8.8% in males while in females it is 697 i.e. 2.97 %. Main causes of liver cancer are hepatitis B and C viruses, alcohol, cirrhosis related to B and C viruses and heavy alcohol smokers, vinyl chloride (occupational exposure) and aflatoxin (Cancer research UK,2009). Chronic inflammation is a known risk factor for carcinogenesis and is thought to play a role in pathogenesis of several types of cancers like cervical, ovarian, esophageal adenocarcinoma, mesothelioma, colorectal cancer, lung cancer and HCC (Balkwill and Mantovani, 2001). As it is postulated that inflammation produces many proinflammatory cytokines, reactive oxygen species and mediators of inflammation like COX-2 that helps in cancer production. Therefore COX-2 could be the next goal for anticancer therapies such as COX-2 inhibitors show anticancer response in many malignant tumors (Cervello and Montalto, 2006). In our study we attempted to observe the expression of COX-2 in non-neoplastic and neoplastic liver disease such as various grades of chronic hepatitis, steatosis, dysplasia, cirrhosis and carcinoma.

Material and Methods

All properly fixed liver biopsies received in department of pathology, BMSI, JPMC from 1st January 2010 to 31st December 2012 were included while Inadequate material metastatic carcinomas (adenocarcinomas) and Cystic lesion (Hydatid cyst) were excluded for evaluation of COX-2 expression . Approximately 71 cases of formalin fixed liver tissue biopsies were selected and analyzed for morphological features, grading and results of immunohistochemical staining for COX-2. Apart from tissue samples we needed Immunohistochemical antibody cox-2 Table:1 (Rabbit monoclonal from tissue culture supernatant diluted in tris saline, PH 7.3-7.7, with protein base and preserved with sodium azide.), Control (positive)

Adenoca-rcinoma colon, Control (negative) Adenocarcinoma colon without primary antibody, Poly-L-lysine coated slides for immunohistochemistry H&E stained slides for all cases, Massons Trichrome stained slides for all cases, PAS and PAS-D stained slides for hepatocellular carcinoma, Clinical surgical, pathological records and detection system.

Clinical history and relevant data were recorded on the request form in the Proforma. Hematoxylin and Eosin staining and trichrome staining were performed. PAS and PAS-D were performed for hepatocellular carcinoma. All the slides were studied under light microscopy using scanner (4x), low power (10x), and high power (40x) lenses. Various parameters were recorded in Proforma. Grading was done in all cases. For the interpretation of grading and staging of all the selected slides we have used the "modified histological activity index" an extension of the original Knodell system. Modified HAI grading or necroinflammatory scores has maximum possible score is 18(1-4=minimal inflammation, 5-8=mild inflammation, 9-12=moderate inflammation and 13-18=marked or severe inflammation). Modified HAI staging, is for extent of fibrosis. The maximum score is 6(0=no fibrosis, to gradual increase in fibrosis up to stage 5 which is early cirrhotic change and then definite cirrhosis which is grade 6). Severity of steatosis is judged from mild (less than one third), moderate (one third to two thirds) to severe (more than two thirds).but in our study we have only included severe steatosis cases. Dysplasia is found in two forms large cell dysplasia and small cell dysplasia (Schwartz, 1998). In our study we had only large cell dysplasia. There were 71 cases selected and the intensity of COX-2 immunostaining was graded on a scale of 0-3 where 0 = no staining, 1 = equivocal staining 2 = moderate to intense staining, 3 = highest intensity staining.The proportion of COX-2 expression in parenchyma cells were assess in various liver lesions includes hepatitis, fatty liver, dysplasia, cirrhosis, and carcinoma. Histo score (Kinsel et al, 1989), the intensity of staining of different areas of section and proportion of positive cells were assessed, the percentage of positive cells and intensity then multiplied to get the high score (H). The intensity was scored according to the overall appearance as judged at different power of magnification, i.e. 0 none (no staining), 1 weak (only visible at high power magnification), 2 moderate (visible at low power magnification) , 3strong (striking even at low power magnification).The total proportion of cells staining positively at any intensity was scored as, 0 (no cell staining), 1 (when 1- 25% cells stained), 2 (when 26-50% cell stained), 3(when 50 -75% cells stained), 4(>75% cells stained).The scores were then together to give a range of 1-12 similar to the immunoreactive score (IRS) by Remmele and Stegner (1987), 9-12 was considered strong immunoreactivity, 5-8 was considered moderate, 1-4 was considered weak, and 0 was scored as negative (Soslow et al., 2000). All the information recorded in the Performa was computerized by using the software SPSS version

Data analysis: The data feeding and analysis was performed on computer package SPSS (Statistical Packages of Social Sciences) version 16.0. Clinical characteristics was summarized in terms of frequencies and percentages for qualitative variables (gender, age grouping, etiology of liver diseases, type of neoplastic lesions, types of malignant and COX-2 immunoreactivity, mean ± S.D for age in years. Statistical comparison of age according to type of liver diseases and histology (non-neoplasm/neoplasm) was performed by student-test/ANOVA and Chi-square/Fisher test (was applied for values<5) for qualitative variables were used to examine the association between COX-2 expression and immunoreactivity (weak, moderate, severe) with liver

Table:1 Distribution of Liver Diseases cases According To Cox –2 (n=71)

Liver diseases	No. of cases	COX-2 immunoreactivity		
		Weak	Moderate	Strong
Mild inflammation	14	7 (50%)	5 (35.7%)	2 (14.2%)
Moderate inflammation	10	-	4 (4.0%)	6 (60.0%)
Marked inflammation	28	2 (7.1%)	4 (14.3%)	22 (78.6%)◊
Steatosis	13	2 (15.4%)	4 (30.8%)	7 (53.8%)
Cirrhosis	08	1 (12.5%)	1 (12.5%)	6 (75.0%)
Dysplasia	07	-	1 (14.2%)	6 (85.7%)
Carcinoma	19	-	4 (21.1%)	15 (78.9%)

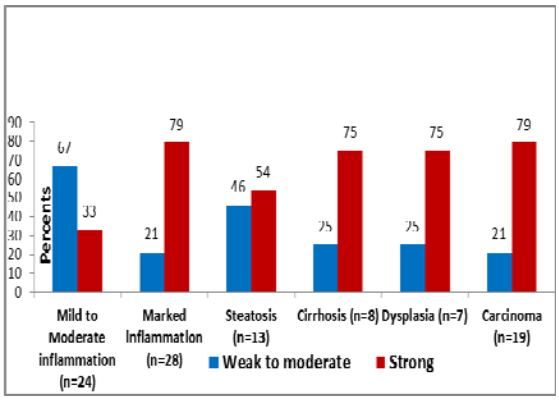
Significant as compared to mild to moderate inflammation $p<0.05$

diseases. In all statistical analysis only p-value <0.05 was considered significant

Results

A total of 71 liver biopsies were included in the study, during a period of January 2010-December 2012 Table shows COX-2 immunoreactivity in non-neoplastic and neoplastic liver diseases. Correlation of necroinflammatory scores with COX-2 shows that amongst 14 cases of mild inflammation only 2 cases shows strong immunoreactivity, 10 cases of moderate inflammation 7 shows strong reactivity and in same way strong inflammation out of 28 cases 22 shows strong reactivity. Out of 13 cases of steatosis 7 shows strong reactivity and 4 moderate .8 cases of cirrhosis and 7 cases of dysplasia shows strong reactivity in 75% & 85.7% of cases respectively. In 19 cases of liver cancer 15 (78.9%) shows strong reactivity in which moderately differentiated, undifferentiated and cholangiocarcinoma catrgories included. (Figure 1-3, table 2)

Table:2 1 Non-neoplastic and neoplastic liver diseases according to cox-2 immunoreactivity in selected cases (n=71)

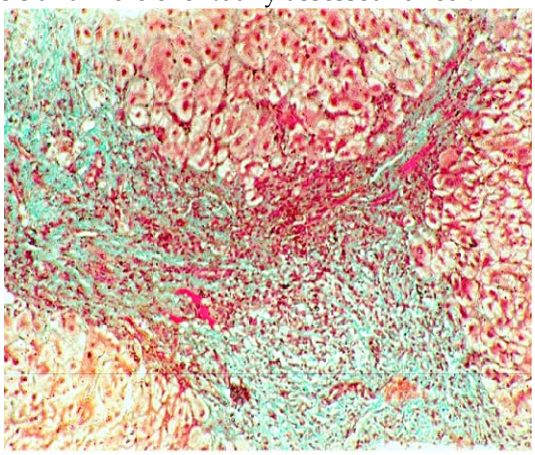


Discussion

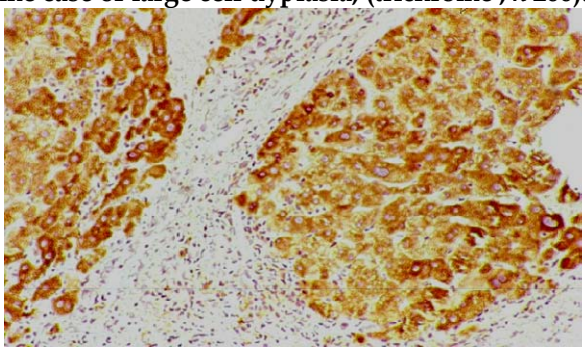
On immunostaining using cox-2 antibody for CLD with mild inflammatory grade cases, only 14.2% showed strong staining. While cases with moderate and severe inflammatory grade 60% and 78.57% gives strong reactivity respectively.

In our study, cases showing severe steatosis with mostly mild & marked inflammation show strong to moderate cox-2 immunoreactivity with 53.84% and 30.76% respectively. Our study is well correlated with the hypothesis of Yu et al. (2006) that Hepatic COX-2 expression is increased in metabolic form of steatohepatitis. Celecoxib (selective cox-2 inhibitors) may protect against the development of steatohepatitis induced by high fat diet (Chen et al., 2006).

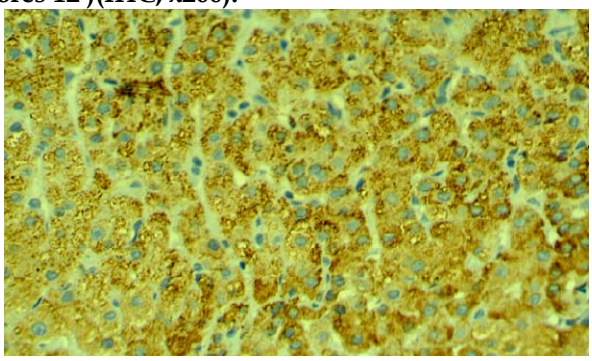
All CLD cases in the present series were evaluated for fibrosis and were eventually assessed for cox-2



Same case of large cell dyplasia, (trichrome , x 200).



Large cell dysplasia with strong cox-2 expression(scores 12)(IHC, x200).



Hepatocellular carcinoma and strong cox-2 expression, (score 9) (IHC x 400).

expression .The intensity of cox-2 expression gradually increases with the progression of grade of fibrosis .None of the cases show strong immunoreactivity in stage 0 and 1, while strong reactivity was higher in fibrotic stages i.e. 88% in stage 5 & 75% in stage 6.Our findings are in close conformity with the study of Kondo et al. (1999) who reported that 81% of cirrhotic livers displayed marked COX-2 expression. Therefore we can elucidate that cox-2 is playing some role in disease progression. As Kondo et al. (1999) declared

that the level of COX-2 increased from normal liver to chronic hepatitis to cirrhosis.

85.7% of dysplastic cases in our study show strong immunoreactivity. Kondo et al. (1999) had the similar values i.e. 88% moderate or strong cox-2 positivity in dysplastic cases.

Likewise all the 19 HCC and bile duct carcinoma 78.94% displayed strong positivity and 21.5% were moderately positive for cox-2.The cases of carcinoma which shows moderate immunoreactivity, were well differentiated hepatocellular carcinoma. According to Kondo et al. (1999), 54% of HCC samples exhibit high to moderate expression of COX-2 expression.

Our findings related to cox-2 expression in non-neoplastic and neoplastic hepatic lesions strongly suggests that cox-2 expression is an early event and its progressive increase in increasing grade of inflammation, fibrosis and in preneoplastic changes (dysplasia), must have an important role in developing hepatocellular carcinoma. Therefore we are suggesting an early inhibition of cox-2 via selective cox-2 inhibitors, might prevent further exacerbation of disease.

References

1. Ahmed W, Qureshi H, Arif A And Alam SE .Changing trend of viral hepatitis—" A twenty one year report from Pakistan Medical Research Council Research Centre, Jinnah Postgraduate Medical Centre, Karachi". J Pak Med Assoc 2010; 60.2.p.86-89.
2. Bae S H, Jung E S, Park Y M, Kim BS, Kim BK, Kim D G and Ryu W S. Expression of cyclooxigenase-2 (COX-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a COX-2 inhibitor, NS-398. Clin Cancer Res .2001. 7:1410-1418.
3. Balkwill F and Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001; 357:539-545
4. Bhurgri Y,Bhurgri A,Nishter S,Ahmed A,UsmanA,Perviz S and et al. Pakistan- country profile of cancer and cancer control.1995-2004.JPMA 2006; Vol. 56, No. 3 pg 124-130.
5. Bosch F X, Ribes J, Díaz M & Cléries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004; 127(5 Suppl 1), S5.
6. Cancer Registry Clinical Data Management (CRCDM)-Shukat Khanum Memorial Cancer Hospital Research Center (SKMH&RC) based on cancer cases registered at SKMCH&RC from Dec .1994-Dec.2011 and in 2011.Released June, 2012. Available at (www.shukatkhanum.org.pk).Report.
7. Cancer Research UK.2009. Cancer stats-key facts, liver cancer. Available at website http://info.cancerresearchuk.org/cancerstats.
8. Cervello M, Montalato G. Cycloxygenases in hepatocellular carcinoma.World J Gastroenterol 2006; 12 (32):5113-5121.
9. Chen SL, Morgan TR. The Natural History of Hepatitis C Virus (HCV)Infection. Int J Med Sci 2006; 3(2):47-52. doi:10.7150/ijms.3.47.Available from http://www.medsci.org/v03p0047.htm.
10. Cheng AS,Chan HL,Leung WK,To KF,Go MY,Chan JY,Liew CT,Sung JJ. Expression of HBX Cox –2 In chronic hepatitis B, cirrhosis and hepatocellular carcinoma; implication of HBx in upregulation of COX 2. Mod Pathol 2004; 17:1169-1179.
11. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. Globocan 2008 v2.0 (accessed Aug 2012), Cancer incidence and mortality worldwide: IARC Cancer Base No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from http://globocan.iarc.fr/.
12. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet. 2012; 31, 379(9822):1245-55.
13. Hung JH, Su D, Lei HY, Wang HC, Lin WC, Chang WT and et al. Endoplasmic reticulum stress stimulates the expression of cycloxegenase-2 through activation of NF-kappa B and pp38 mitogen activated protein kinase. J Biol Chem 2004; 279:46384-46392.
14. Kern MA, Schubert D, Sahi D, Schoneweiss MM, Moll I, Haugg AM and et al. Proapoptotic and antiproliferative potential of selective cyclooxygenase-2 inhibitors in human liver tumor cells. Hepatology 2002; 36: 885-894.
15. Kinsel LB, Szabo E, Greene GL, Konrath J, Leight GS and McCarty KS jr . Immunocytochemical analysis of estrogen receptors as a predictor of prognosis in breast cancer patints:comparison with quantitative biochemical methods. Cancer Res 1989; 49, 1052-1056.
16. Khokhar N. Spectrum of chronic liver disease in a tertiary care hospital. J Pak Med Assoc 2002; 52(2):56–8.
17. Koga H, Sakisaka S, Ohishi M, Kawaguchi T, Taniguchi E, Sasatomi K and et al. Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. Hepatology 1999; 29:688-696.
18. Kondo M, Yamamoto H, Nagano H, Okami J, Ito Y, Shimizu J and et al. Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. Clin. Cancer Res. 1999; 5:4005-4012.
19. Lampiasi N, Fodera D, D'Alessandro N, Cusimano A, Azzolina A, Tripodo C and et al. The selective cyclooxygenase-1 inhibitor SC-560 suppresses cell proliferation and induces apoptosis in human hepatocellular carcinoma cells. Int J Mol Med 2006; 17: 245-252.
20. Lara-Pezzi E, Gomez-Gaviro MV, Galvez BG, Mira E, niguez MA, Fresno M and et al .The hepatitis B virus X protein promotes tumor cell invasion by inducing membrane-type martrix metalloproteinase-1 and cyclooxegenase-2 expression. J Clin Invest 2002;110:1831-1838.
21. Leng J, Han C, Demetris AJ, Michalopoulos GK and Wu T. Cyclooxygenase- 2 promote hepatocellular carcinoma cell growth through Akt activation: evidence for Akt inhibition in celecoxib- induced apoptosis. Hepatology 2003; 38: 756-768.
22. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E and et al .Suppression of intestinal polyposis in Apc delta716 knockout mice by

inhibition of cyclooxygenase 2 (COX-2). Cell 1996; 87: 803-80.
23. Parkin DM, Bray F, Ferlay J and Pisani P. Estimating the world cancer burden: Globocan 2000. Int J. Cancer 2001; 94:153–156. Schwartz MR. Liver cell dysplasia and other atypical lesions: new insights and applications.adv Anat Pathol 1998; 5:99-105.
24. Remmele W and Stegner HE .immunhistochemischer nachweis von ostrogenerezeptoren (ER-ICA) im mammakariziriomgewbe:vorschlag zur einheitlichen bewertung des untersuchungsbefundes. Frauenarzt 1987; 28.41-43.
25. Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, and et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 2000; Dec 15;89(12):2637-45.
26. Schwartz MR. Liver cell dysplasia and other atypical lesions: new insights and applications.adv Anat Pathol 1998; 5:99-105.
27. Waris G, Siddiqui A. Hepatitis C virus stimulates the expression of cycloxegenase-2 via oxidative stress:role of prostaglandin E2 in RNA replication. J Virol 2005; 79:9725-9734.
28. Yu J, Ip E, Peña A D, Hou, JY, Sesha J, Pera N, Hall P, Kirsch R, Leclercq I. and Farrell G C.COX-2 induction in mice with experimental nutritional steatohepatitis: Role as pro-inflammatory mediator. Hepatology 2006; 43: 826–836. doi: 10.1002/hep.21108.

Contribution of Authors:

Mubashira Hashmi and Shahnaz Imdad Kehar has Planed and carried out the entire study. Shahnaz Imdad Kehar guided and supervised and also prepared the manuscript

.