Research Article/ Araştırma Makalesi

NEW IMMUNOHİSTOCHEMİCAL METHODS IN THE DİFFERENTIAL DIAGNOSİS OF HEPATOCELLULAR CARCİNOMA, CHOLANGIOCELLULAR CARCİNOMA, AND COLON ADENOCARCINOMA METASTASES

HEPATOSELLÜLER KARSINOM, KOLANJIOSELLÜLER KARSINOM VE KOLON ADENOKARSINOM METASTAZLARININ AYIRICI TANISINDA İMMUNOHISTOKIMYASAL METODLAR

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Abstract

Objectives: Liver is the main organ carrying out metabolic functions in the human body. The cause of death from cancer is the third among cancers in the world. Metastases are the most common malignant tumors in liver. Hepatocellular carcinoma, primary malignant tumors of the liver, is the second (80-90 %), and cholangiocellular carcinoma is the third (10-15 %) most common malignant tumor of liver. Early diagnosis in these tumors is important for early treatment planning. Although late-stage hepatocellular carcinoma is easily detectable, the treatment could not be avaliable in a large proportion and recurrence is frequently observed. Therefore, in the differential diagnosis using more sensitive and specific antibodies can increase the effectiveness of the other markers used in routine practice, and can achieve an earlier diagnosis. **Methods:** In our study, paraffin blocks of the biopsy and resection material of 60 cases were studied immunohistochemically with GPC-3, Agrin/CD34 combination, CDX2. **Results:** HepPar1, AFP, CEA, CD10, CK-19, CK-7, CK-20, CK-8 and CK-18 which are studied previously, were evaluated in combination with GPC-3 in hepatocellular carcinoma, and GPC-3 was found to be more sensitive and specific than HepPar1 and AFP. In the differential diagnosis of hepatocellular carcinoma from cholangiocellular carcinoma and metastatic adenocarcinoma of the colon, GPC-3 was also found to be very useful. Although in hepatocellular carcinoma tissues Agrin was observed in the new areas of vascularity, it was not observed in the areas of cholangiocellular carcinoma and tissues of metastatic colon adenocarcinoma. That has been followed in our attention. CDX2 staining was observed in tissues with metastatic adenocarcinoma of the colon, but it is very little in hepatocellular carcinoma and cholangiocellular carcinoma. This is an important finding in the differential diagnosis. **Conclusion:** Considered a combination of all of our findings, Glypican-3, Agrin/CD34 combination and CDX2 are very sensitive and specific markers of hepatocellular carcinoma. These markers must be applied in routine practice for early diagnosis and treatment, and for the extend of the survey in patients with hepatocellular carcinoma.

Key Words: Hepatocellular Carcinoma, Cholangiocellular Carcinoma, Metastatic Adenocarcinoma of the Colon, Immunohistochemistry, Glypican-3, Agrin/CD34 combination,CDX2

Özet

Giriş: Karaciğer insan vücüdunda metabolizma fonksiyonlarının yürütüldüğü temel organdır. Kanserleri dünyada kanserden ölüm sebebleri arasında 3.sırada yer alır. Metastazları en sık görülen malign tümörleridir. Primer malign tümörlerinden hepatosellüler karsinom (%80-90) ikinci sıklıkta,kolanjiokarsinom da 3. sıklıkta görülen malign tümörleridir (%10-15). Bu

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tümörlerin erken tanı alması, tedavinin erken planlanması açısından önemlidir. Geç evre hepatosellüler karsinom kolay tespit edilebilmesine rağmen büyük oranda tedavi edilememekte ve nüks izlenmektedir. Bundan dolayı ayırıcı tanıda daha sensitif ve spesifik antikorlar kullanarak, rutin uygulamada kullanılmakta olan diğer belirleyicilerin etkinliğini artırmak, daha erken tanıya ulaşmak gerekmektedir. **Metot:** Çalışmamızda 60 adet olguya ait biyopsi ve rezeksiyon materyallerinin parafin bloklarında immunohistokimyasal olarak Glypican-3, Agrin/CD34 kombinasyonu, CDX2 çalışıldı. **Bulgular:** Daha önce çalışılmış HepPar1, AFP, CEA, CD10, CK-19, CK-7, CK-20, CK-8, CK-18 ile kombine değerlendirildiğinde özellikle GPC-3'ün hepatosellüler karsinomda HepPar1 ve AFP'ye göre daha sensitif ve spesifik olduğu gözlendi. Ayrıca kolanjiosellüler karsinom ve kolon adenokarsinom metastatik tümörlerleriyle ayırıcı tanısında oldukça faydalı olduğu tespit edildi. Agrin'in hepatosellüler karsinomlu dokularda yeni damarlanma alanlarında izlenmesine rağmen kolanjiosellüler karsinom ve kolon adenokarsinom metastazlı dokularda izlenmemesi dikkatimizi çekti. CDX2 ile kolon adenokarsinom metastazlı dokularda boyanma gözlenirken, hepatosellüler karsinom ve kolanjiosellüler karsinomda çok az boyanma izlenmesi de ayırıcı tanıda önemli bir bulguydu. **Sonuç:** Bulgularımızın hepsini bir arada değerlendirdiğimizde, GPC-3, Agrin ve CDX2 oldukça sensitif ve spesifik belirleyicilerdir. Olguların erken tanılanmasını ve daha hızlı tedavi almasını sağlayıp, hastaların surveyini uzatması açısından rutinde de uygulanması gerekir.

Anahtar Kelimeler: Hepatosellüler Karsinom, Kolanjiosellüler Karsinom, Kolon Adenokarsinom Metastazı, İmmunohistokimya, Glypican-3, Agrin, CDX2

1. INTRODUCTION

Hepatocellular carcinoma (HCC) constitutes 80-90% of all primary liver tumors (1). While it's the fifth commonest malignancy, it ranks third among all cancer related death. Although diagnostic methods are very advanced nowadays, there are still difficulties in the definitive diagnosis of liver tumors. In addition to imaging methods for diagnosis, the increase serum alpha feto protein (AFP) level is one of the important parameters (3,4). Ultrasonography guided fine needle aspiration biopsies is used as diagnostic method despite the 10% false positivity. AFP, HepPar1, CEA, CD10 are the most important immunohistochemical antibodies used for diagnosis. Especially AFP and HepPar1 positivity are the most important parameters that supports hepatocellular carcinoma (4). Cytokeratins are antibodies used in the diagnosis of hepatocellular carcinoma (CK8, CK18), cholangiocarcinoma (CK19) and in the differentiation of metastatic lesions, especially colon adenocarcinoma (CK20) (5). Glypican-3 (GPC-3) is from the glypican family, which is Glycosylphosphatidylinositol (GPI)-linked heparansulfate proteoglycan (HSPG). Glypican consists of proteoglycans and core protein that binds two polysaccharide units known as Heparan sulfate glycosaminoglycan (HSGAG). After synthesizing 60-70 kDa core protein, HSGAG is added by glycosylation in the Golgi body. It's located on Xq26.1 in adult tissues. Six glypican family members have been identified in humans.

GPC-3 is highly expressed in embryonal structures and mesoderm-derived tissues. It showed high expression especially in embryonic liver and intestine, while expression level decreased in normal adult tissues. This situation suggests that it acts as an oncofetal protein for these organs. In general, while oncofetal proteins do not play a critical role in tumor progression, they are used as tumor markers or as a potent target for immunotherapy. GPC-3 is involved in cellular growth, differentiation and migration. It shows different expression patterns in tumor progression. It induces apoptosis in some types of tumor cells. It is therefore not surprising that some tumors of different origin are downregulated of GPC3 expression. GPC3 downregulation has been observed in ovarian cancer, breast cancer, lung adenocarcinoma, and cholangiocellular carcinoma (8-11). While it is not expressed in the colon, high expression is observed in colorectal cancers (12-13).

Interestingly, although there is no expression of GPC3 in the liver, Glypican is up-regulated in the vast majority of tumors and its expression decreases during tumor progression. In addition, serum GPC3 levels were found to be significantly higher in patients with HCC compared to healthy people and non-malignant liver patients (12-14).

Glypican-3(GPC-3) is used to differentiate HCC from dysplastic changes in cirrhotic liver (15-17). It is also useful in seperating benign liver nodules from malignant nodules.

Agrin is from the heparan sulfate proteoglycan (HSPG) family, which is found on the surface of cells and in the extracellular matrix. It is activated by myofibroblasts, vascular smooth muscle cells and biliary epithelial cells in human and rat liver. It is not found in the wall of lymphatic vessels.

It is strongly found in vascular structures and peribiliary basement membranes in HCC. When the lesion becomes malignant, expression of agrin increases because of formation of new blood vessels increases. Combination with CD34 was found highly sensitive and specific and express more diffusely than GPC-3. It doesn't stain sinusoidal walls of regenerative nodules in cirrhosis. When benign and malignant lesions of the liver were compared, it was determined that the sensitivity of Agrin in favor of malignant lesions was 93.1% and its specificity was 92.6%, when combined with CD34 (18-21).

Agrin is a recently discovered antibody used to detect microvasculature in hepatocellular carcinomas. Combined evaluation of Agrin with CD34 was found to be highly sensitive and specific in the diagnosis of HCC (22).

CDX2 is responsible for regulation of cellular proliferation, differentiation and aging, regeneration of intestinal epithelium and preservation of tissue structure (22-23). It is widely expressed in the fetal and embryonic period. It becomes to localized since the early neonatal period. CDX2 regulates the

transcription of intestinal specific genes and functions as a tumor suppressor gene (23-24). In cases where CDX2 expression is low in cancer cells, CDX2 insertion increases the sensitivity of apoptosis in the cells and the growth rate slows down. CDX2 is down-regulated in 85% of colorectal adenocarcinomas. As a result, there is an increase in proliferation in tumorogenesis. In addition, replication errors due to small mutations of CDX2 have been observed in colorectal carcinomas. CDX2 is expressed in the nuclei of intestinal epithelial cells from the duodenum to the rectum, as well as neuroendocrine cells and pancreatic islet cells. The expression of CDX2 is observed at different levels in different parts of the gastrointestinal tract. It is highest in the small intestine and cecum, but less expressed in the distal colon. It is observed in goblet cells, enteroendocrine cells and absorptive enterocytes in the villi on the surface of the intestinal epithelium (23,25-31).

Although late-stage HCC can be detected easily, it is substantially incurable and relapses. Only 25% of cases receive appropriate curative treatment. Inoperable cases can survive for only a few months (9). Therefore, early diagnosis and treatment are important. In order to provide appropriate treatment as well as early diagnosis, neuroendocrine tumors, especially cholangiocarcinoma (CCA), and metastatic liver diseases should be differentiated from hepatocellular carcinoma.

In our study, we aimed to determine the role of immunohistochemistry in the differential diagnosis of HCC from CCC and metastatic cancer. We emphasized the importance of antibodies such as GPC-3, the combination of Agrin and CD34 and CDX2 in differential diagnosis, that they can be used routinely and that when evaluated in combination with other antibodies, they help us to make the diagnosis even earlier and more precisely.

2. MATERIAL AND METHODS

In our study, we include 20 patients from each of the 3 groups were diagnosed as Hepatocellular Carcinoma, Cholangiocellular Carcinoma, Colon Adenocarcinoma Metastasis with underwent tru-cut (fine needle) biopsy, wedge resection, liver lobectomy and hepatectomy between 2000-2011 in Atatürk University Faculty of Medicine Department of Pathology. Paraffin blocks, previous hematoxylin-eosin, immunohistochemical stained slides and pathological diagnosis reports of each case were removed from the archive of our department and re-evaluated. Among these, the most suitable paraffin blocks were selected for immunohistochemical staining from hematoxylin-eosin glasses which the pathological diagnosis was most accurate.

2.1. Immunohistochemical methods

Glypican-3 (CM 396A, B; Bio Care Medical), the combination of Agrin and CD34 (Anti antibody agrin; ab85174) and CDX2 (Leica; AMT28) antibody dyes were applied immunohistochemically to the paraffin blocks selected for our research using the streptavidin biotin peroxidase method. 4 microns thicked sections from paraffin blocks were put on positively charged slides and then were placed in BOND-MAX Fully Automated IHC System.

2.2. Immunohıstochemıcal evaluatıon

Immunohistochemical stained preparation were first evaluated by two different pathologists under the light microscope without using clinical and previous pathological data. Considering the literature for GPC3 in stained tissue sections, the presence of cytoplasmic staining was evaluated in terms of prevalence and severity. All tumoral cells in tissue sections were checked for extensity. While it is negative if there is no staining (Figure 1), if less than 10% of the tumoral cells are stained (1+) (Figure 2), if there is staining in 10-50% of the tumoral cells (2+) If there was staining in more than 50% tumoral cells, it was evaluated as (3+) (Figure 3). For Agrin/CD34, according to the literature, if 10% stained weakly the vascular structures around the tumoral cell (1+), if 10-50% stained the vascular structures around the tumoral cell more strongly (2+)(Figure 4), the vascular structures around the tumoral cell above 50% it was considered as (3+) (Figure 5) if it stained diffusely and darkly. For CDX2, the presence of nuclear staining was evaluated in terms of extensity and severity. Considering the cells with staining, if less than 10% of the tumoral cells are stained (1+), 10-50% staining in the tumoral cells (2+), if there is staining in more than 50% tumoral cells, it was evaluated as (3+) (Figure 6). Cytoplasmic staining for HepPar1 was evaluated for extensity and severity. Considering the cells with staining, the reactivity in tumoral cells was evaluated as scattered sporadic positivity (1+), focal positivity (2+) , diffuse positivity (3+) (Figure 7).

Cytoplasmic staining for AFP was evaluated for extensity and severity. Considering the cells with staining, focal staining in tumoral cells was evaluated as (1+), moderate staining (2+), diffuse staining (3+) (Figure 8). Fiber staining around tumor cells with CD10 was evaluated as (-), diffuse staining (1+), focal staining (2+) , diffuse staining (3+). The presence of cytoplasmic staining in tumor cells with CEA was evaluated in terms of extensity and severity. Considering the cells with staining, if there is no staining in tumor cells was evaluated as (-), focal staining (1+), moderate staining (2+), diffuse staining (3+). Brown staining of cytoplasmic and cytoplasmic membranes in tumor cells for cytokeratins (CK7, CK20, CK19, CK8, CK18) was evaluated in terms of extensity and severity. If there was no staining in tumor cells, it was evaluated as (-), focal weak staining (1+), moderate staining (2+), diffuse staining (3+).

2.3. Statistical evaluation

Statistical transactions were performed on computer using the 'SPSS 22' (Scientific Package for Social Sciences) program. For this purpose, 3 groups were created for cancer type and 4 groups for each of Glypican-3, Agrin, CDX2, AFP, Heppar1, CD10, CEA, CK7, CK20, CK19, CK8, CK18 markers. Since we used categorical data and more than two groups in our study, we used the Chi-Square test. Since at the end of the study, at least 80% of the expected frequencies should be 5 or greater than 5, we combined the group 0 as 1 and group 1, 2, and 3 as 2 and performed the test again. If the probability coefficient (p) value was less than 0.05, it was considered statistically significant.

3. RESULTS

In our study, 60 cases diagnosed between 2000 and 2011 who had liver tru-cut (cutting-needle) biopsy, wedge resection and hepatectomy were included. Of these 60 cases, 20 had hepatocellular carcinoma, 20 had cholangiocellular carcinoma, and 20 had colon adenocarcinoma metastasis.

Of the 60 cases, 40 (66.7%) were male and 20 (33.3%) were female. Of the hepatocellular carcinoma cases, 16 (80%) were men, 4 (20%) were women; of the cholangiocellular carcinomas, 13 (65%) were men and 7 (35%) were women; and of the colon adenocarcinoma metastases, 11 (55%) were male, and 9 (45%) were female. The age range of the cases was between 33 and 86, with a mean age of 63. The age range of patients with hepatocellular carcinoma was 33-74 (mean age 64.5 years), 45-86 years (mean age 59.5 years) in patients with cholangiocellular carcinoma, and 40-84 years (mean age 64 years) in patients with colon adenocarcinoma metastases. Immunohistochemical evaluations were evaluated by two different pathologists at the same time and classified in tables.

In the comparison between Glypican-3 expression and cancer types, expression was detected in 17 (85%) cases with HCC, 3 (15%) cases with CCC, and 2 (10%) cases with colon adenocarcinoma; however, expression was not observed in 3 (15%) cases with HCC, 17 (85%) cases with CCC, and 18 (90%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and Glypican3 (Pearson p:0.003, p<005).**(Table 1)(Figure 9)**

In the comparison between Agrin expression and cancer types, expression was detected in 19 (95%) cases of HCC, 10 (50%) cases with CCC, and 1 (5%) case with colon adenocarcinoma; whereas any expression was not observed in 1 (5%) case with HCC, 10 (50%) cases with CCC, and 19 (95%) cases with colon adenocarcinoma. These findings showed that there was a statistically significant relationship between cancer type and Agrin (Pearson p:0.00, 4p<005).(Table 2)(Figue 10)

In the comparison between CDX2 expression and cancer types; while expression was detected in 5 (25%) cases with HCC, 1 (5%) case with CCC, and 18 (90%) cases with colon adenocarcinoma; expression was not observed in 15 (75%) cases with HCC, 19 (95%) cases with CCC, and 2 (10%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and CDX2 (Pearson p:0.000, p<005). (Table 3)(Figue 11).

In the comparison between HepPar1 expression and cancer types, expression was detected in 19 (95%) cases with HCC and in 2 (90%) cases with CCC; while expression was not observed in 1 (5%) case with HCC, 18 (90%) cases with CCC, and 20 (100%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and HepPar1 (Pearson p:0.000, p<005).

In the comparison between AFP expression and cancer types, expression was detected in 15 (75%) cases with HCC, 2 (10%) cases with CCC, and 1 (5%) case with colon adenocarcinoma; yet no expression was observed in 5 (25%) cases with HCC, 18 (90%) cases with CCC, and 19 (95%) cases with colon adenocarcinoma. These findings showed that there was a statistically significant relationship between cancer type and AFP (Pearson p:0.000, p<005).

In the comparison between CEA expression and cancer types, expression was detected in 2 (90%) cases with HCC, 16 (80%) cases with CCC, and 18 (90%) cases with colon adenocarcinoma; while expression was not observed in 18 (90%) cases with HCC, 4 (20%) cases with CCC, and 2 (10%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and CEA (Pearson p:0.000, p<005).

In the comparison between CD10 expression and cancer types, expression was detected in 15 (75%) cases with HCC, 4 (20%) cases with CCC, and 4 (20%) cases with colon adenocarcinoma; whilst expression was not observed in 5 (25%) cases with HCC, 16 (80%) cases with CCC, and 16 (80%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and CD10 (Pearson p:0.000, p<005).

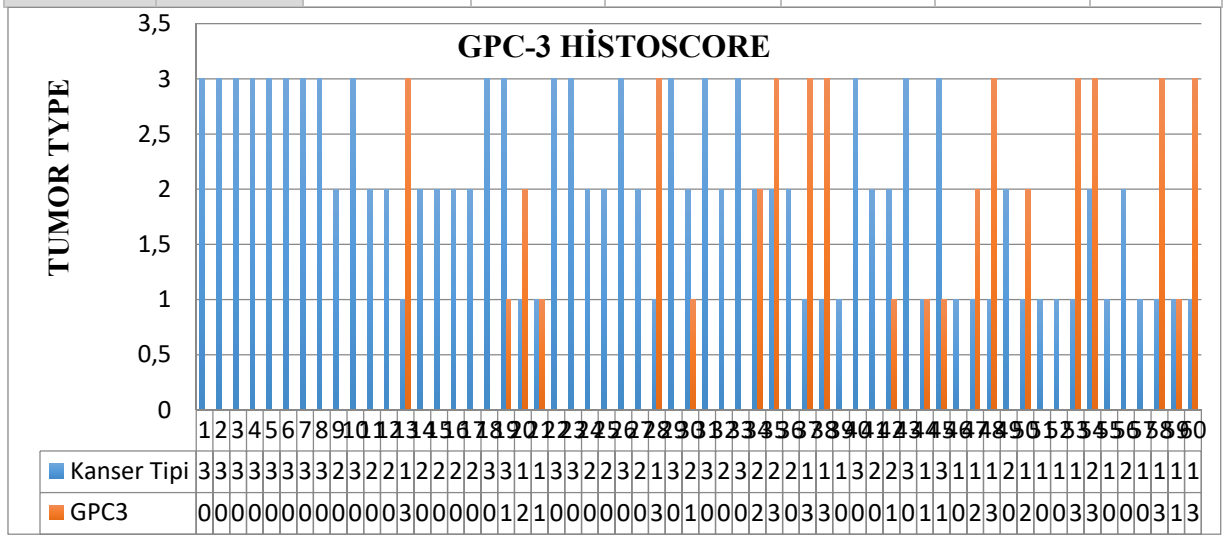
In the comparison between CK7 expression and cancer types, expression was detected in 14 (70%) cases with HCC, 17 (85%) cases with CCC, and 5 (25%) cases with colon adenocarcinoma; while no expression was observed in 6 (30%) cases with HCC, 3 (15%) cases with CCC, and 15 (75%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and CK7 (Pearson p:0.000, p<005).

In the comparison between CK20 expression and cancer types, expression was detected in 4 (20%) cases with HCC, in 1 (5%) case with CCC, and in 18 (90%) cases with colon adenocarcinoma; whereas expression was not observed in 16 (80%) cases with HCC, 19 (95%) cases with CCC, and 2 (10%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and CK20 (Pearson p:0.000, p<005).

In the comparison between CK19 expression and cancer types, expression was detected in 8 (40%) cases with HCC, 20 (100%) cases with CCC, and 18

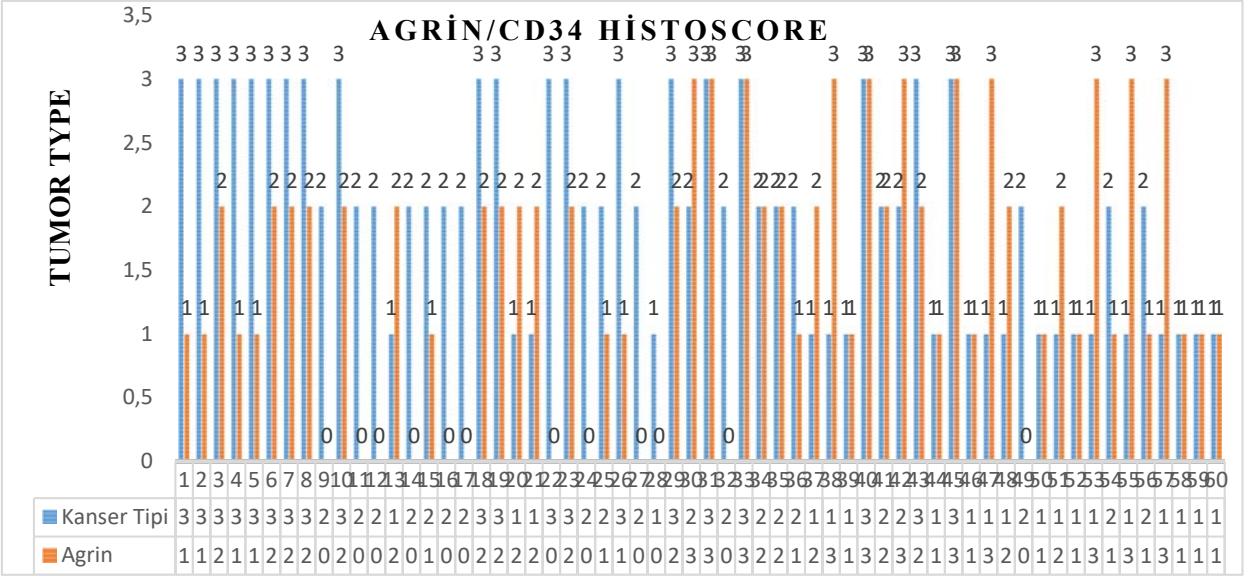
Table 1. Distribution of GPC-3 histoscore by tumor types, age and sex

		Number of cases (n=60)	GPC-3 Histoscore				P Value
			Negative	Weak	Middle	Strong	
Age (Year)	≥49	49 (%81,6)	33 (% 55)	6(%10)	4 (%6,6)	7 (%11,6)	0,062
	<49	11 (%18,3)	5 (%8,3)	1 (%0,16)	2 (%3,3)	2 (%3,3)	
Gender	Male	40 (%66.7)	23 (%38,3)	4 (%6,6)	4 (%6,6)	8 (%13,3)	0,156
	Female	20 (%33,3)	15 (%25)	3 (%5)	2 (%3,3)	1 (%1,6)	
Tumor types	HCC	20 (%33,3)	6 (%10)	3 (%5)	4 (%6,6)	8 (%13,3)	**0,003**
	CCC	20 (%33,3)	13 (%21,6)	2 (%3,3)	2 (%3,3)	1 (%1,6)	
	CAM	20 %33,3)	19 (%31,6)	2 (%3,3)	0 (%0)	0 (%0)	



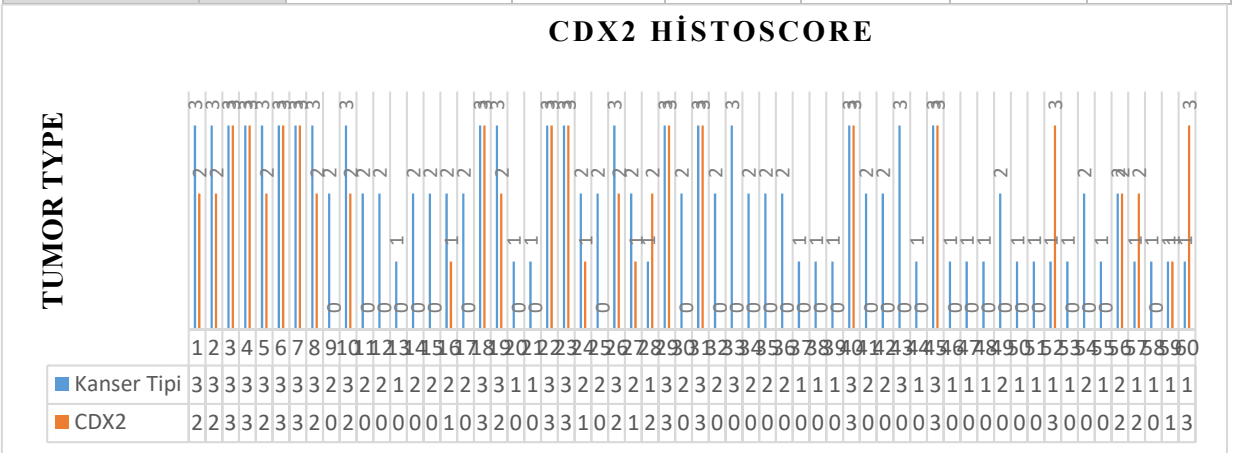
Tablo 2. Distribution of Agrin/CD34 histoscore by tumor types, age and sex

		Number of Cases (n=60)	Agrin/CD34 Histoscore				P Value
			Negative	Weak	Middle	Strong	
Age (Year)	≥49	49 (%81,6)	11 (% 18,3)	15 (%25)	16 (%26,6)	9 (%15)	**0,040**
	<49	11 (%18,3)	1 (%1,6)	3 (%5)	2 (%3,3)	3 (%5)	
Gender	Male	40 (%66,7)	8 (%13,3)	13 (%21,6)	12 (%20)	7 (%11,6)	**0,050**
	Female	20 (%33,3)	4 (%6,6)	5 (%8,3)	7 (%11,6)	4 (%6,6)	
Tumor types	HCC	20 (%33,3)	1 (%1,6)	7 (%11,6)	5 (%8,3)	4 (%6,6)	**0,004**
	CCC	20 (%33,3)	10 (%16,6)	5 (%8,3)	7 (%11,6)	2 (%3,3)	
	CAM	20 %33,3)	1 (%1,6)	4 (%6,6)	10 (%16,6)	4 (%6,6)	



Tablo 3. Distribution of CDX2 histoscore by tumor types, age and sex

		Number of Cases (n=60)	CDX2 histoscore				
			Negative	Weak	Middle	Strong	P Value
Age (Year)	≥49	49 (%51,25)	29 (% 48,3)	4 (%6,6)	8 (%13,3)	10 (%16,6)	**0,012**
	<49	11 (%48,75)	4 (%6,6)	0 (%0)	2 (%3,3)	3 (%5)	
Gender	Male	40 (%66.7)	24 (%40)	4 (%6,6)	8 (%13,3)	5 (%8,3)	**0,036**
	Female	20 (%33,3)	9 (%15)	0 (%0)	2 (%3,3)	8 (%13,3)	
Tumor types	HCC	20 (%33,3)	15 (%25)	1 (%1,6)	2 (%3,3)	2 (%3,3)	**0,000**
	CCC	20 (%33,3)	16 (%26,6)	3 (%5)	0 (%0)	0 (%0)	
	CAM	20 %33,3)	2 (%3,3)	0 (0)	8 (%13,3)	11 (%18,3)	



(90%) cases with colon adenocarcinoma; whilst expression was not observed in 12 (60%) cases with HCC and in 2 (10%) cases with colon adenocarcinoma. These findings showed a statistically significant relationship between cancer type and CK19 (Pearson p:0.000, p<005).

In the comparison between CK8/18 expression and cancer types, expression was detected in 20 (100%) cases with HCC, 3 (15%) cases with CCC, and 1 (5%) case with colon adenocarcinoma; while no expression was observed in 17 (85%) patients with CCC and 19 (95%) patients with colon adenocarcinoma. These

findings showed a statistically significant relationship between cancer type and CK8 (Pearson p:0.000, p<005).

4. DISCUSSION

HCC is one of the most common primary malignancies and ranks fourth in the world for cancer-related deaths. It constitutes 5% of all cancers and 70-80% of liver cancers. In the 2000s, 564,000 new cases were described in the world. Its prevalence varies according to geographical localizations. Its incidence increases with age (65 years and older). It is rare in North America and Western Europe before the age of 50 (1-2,32).

Major risk factors are cirrhosis, chronic hepatitis B, chronic hepatitis C, toxic (alcohol and aflatoxins) and metabolic causes (diabetes mellitus, nonalcoholic fatty liver disease (NASCH), hereditary hemochromatosis, primary biliary cirrhosis, autoimmune hepatitis) (1-3).

Catching the disease at an early stage is important for the treatment, recurrence and presentation of the tumor. Only 25% of patients are diagnosed early to receive appropriate curative treatment. The surveillance of inoperable patients is approximately 5 months. The 5-year surveillance is less than 10-15% (33).

Although HCC constitutes approximately 10-15% of liver tumors, it varies according to geographical regions. Its incidence is increasing worldwide. Parasites such as Clonorchis sinensis and opisthorchis viverrini, nitrosamines and aflatoxin are in the first place in its etiology (32).

Metastatic carcinomas of the liver are the most common malignant tumors of the liver. It has the highest ranking among malignancies developing from

FIGURE 1. Colon Adenocarcinoma metastasing of liver (GPC3 NEGATİVE)

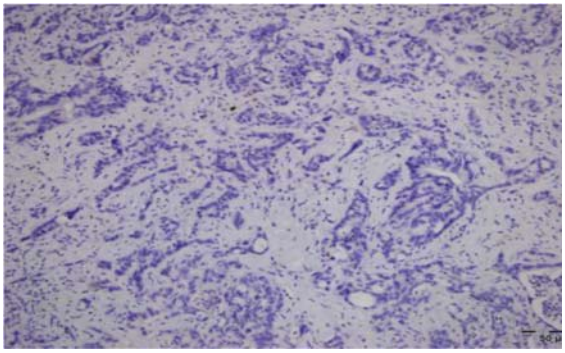


FIGURE 2. HCC (GPC3 +1 POSİTİVE)

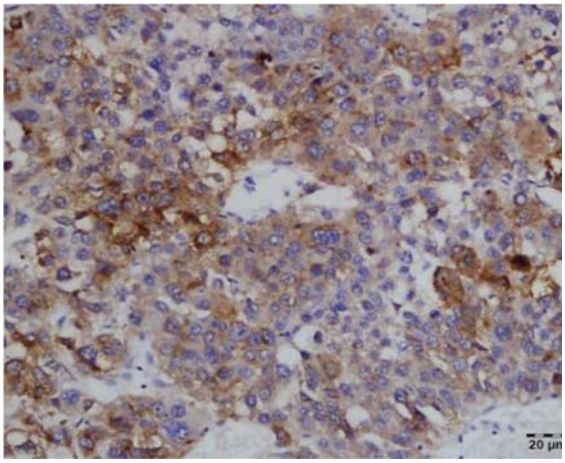


FIGURE 3. HCC (GPC3 +3 POSİTİVE)

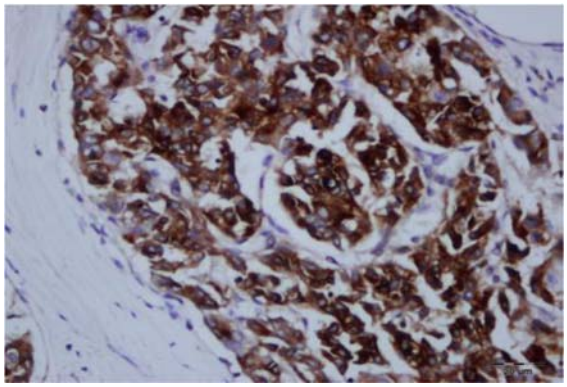


FIGURE 4. HCC (AGRİN/CD34 +2 POSİTİVE)

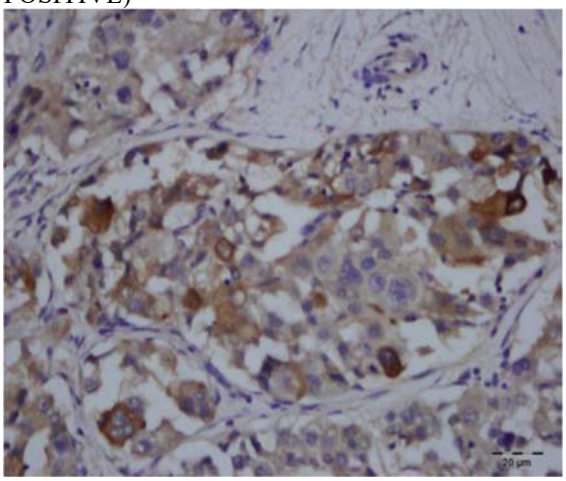


FIGURE 5. HCC (AGRİN/CD34 +3 POSİTİVE)

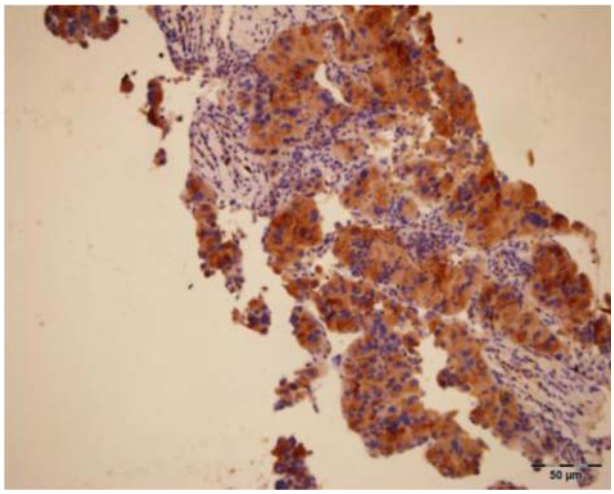


FIGURE 6. Colon Adenocarcinoma metastasing of liver (CDX2 +3 POSİTİVE)

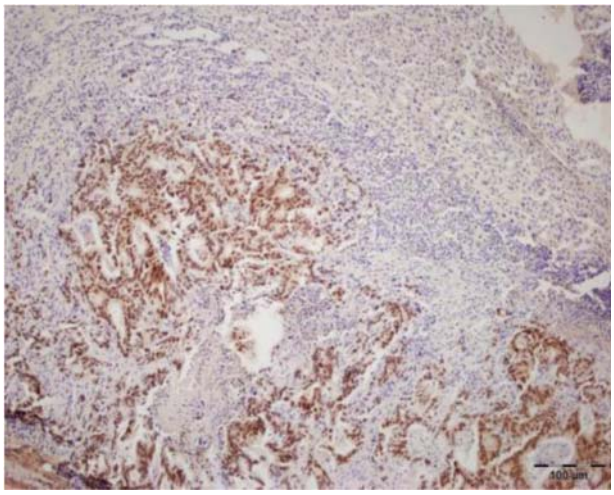


FIGURE 7. HCC (HepPar-1 +3POSİTİVE)

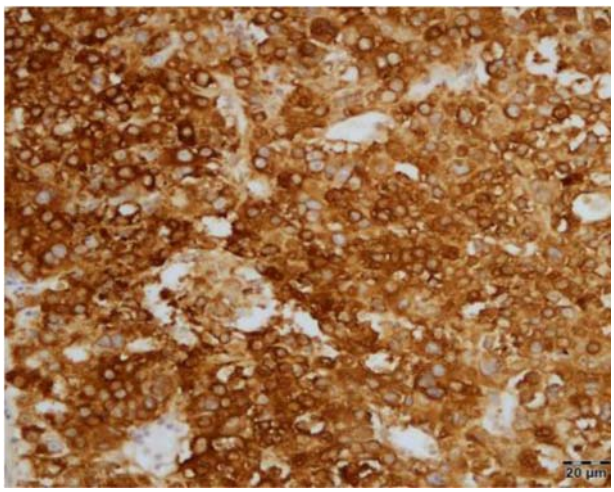
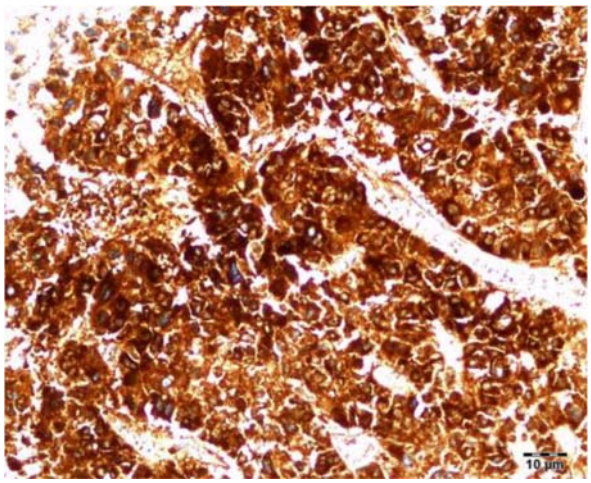


FIGURE 8: HCC (AFP +3POSİTİVE)



noncirrhotic liver in all developed western countries (32).

Identification of new molecular targets and markers is an urgent need for the development of new treatment approaches in liver malignancies. The fact that these markers guide us in early diagnosis will have a more effective sensitivity and specificity, as well as increase in the success of patients in curative treatments and prolongation of their surveys will lead to positive results that closely concern and affect the patient population (1,34).

The most important of these markers today is Glypican-3 (GPC-3). Its structure, function and biology are important. GPC-3 is an antibody whose routine use has started relatively, but has not become widespread, and its efficiency has been proven in studies.

Differentiating HCC from CCA, especially ICC (intrahepatic cholangiocarcinoma), poses an important problem. In the study of Hsu et al. in 1997, GPC-3 was not expressed in ICC, gallbladder adenocarcinoma and colon adenocarcinoma; however, it was expressed in 75% of HCC and 10% of gastric adenocarcinoma (14). Yamauchi et al. tested GPC-3 expression in CCC in a small series of 16 subjects and results were negative. (36). Daniel Baumhoer et al. found that using GPC-3 was limited in the differential diagnosis of CCC and some other metastatic tumors (35). In our case, 17/20 (85%) of the cases with HCC and 3/20 of the cases with CCC had GPC-3 positivity, and in accordance with the literature, it was established that GPC-3 is very important in the diagnosis to differentiate HCC from CCC.

Mitchell Ho et al. observed that the expression of GPC-3 was mostly in the cytoplasmic pattern, while Shirakowa et al. previously observed that there was a mixed pattern (cytoplasmic and membranous) in about half of the HCC cases, and a cytoplasmic pattern in the other half (34). In our study, we observed that the most of the cases (15 out of 17

positive cases) had a cytoplasmic staining pattern. In the other 2 cases, we detected a stronger cytoplasmic pattern with a weak membranous staining. Contrary to the literature, we observed that our findings were in the direction of cytoplasmic staining.

Naoko Yamauchi et al. observed in their study the immunohistochemical reactivity of GPC-3 in the differential diagnosis of benign and malignant hepatocellular lesions by using these monoclonal antibodies in paraffin tissues. While expression was observed in fetal liver, expression could not been detected in normal adult liver, cirrhosis and hepatitis. They found positive results in 84% (47/56) of the cases with HCC and hepatoblastoma. When comparing AFP and GPC-3 immunohistochemically, they found that GPC-3 was much more sensitive and specific in HCC. They observed that GPC-3 is an important oncofetal protein in the differential diagnosis. Naoko Yamauchi et al. found that GPC-3 was superior to AFP in HCC cases (36). In our study, positivity was 85% with GPC-3 and 75% (15/20) with AFP. These data were evaluated as similar with the literature.

Saverio Ligato et al. observed GPC-3 to be positive in 16 of 17 patients with metastatic tumors (3 colorectal carcinoma, 3 pancreatic carcinomas, 2 breast carcinoma, 2 gastric carcinoma, 1 adenoid cystic carcinoma, 3 non-small cell carcinoma lung carcinoma, 1 lung small cell carcinoma. carcinoma, 1 high grade sarcoma, 1 neuroendocrine especially anaplastic carcinoma) (37). Naoko Yamauchi et al. found positive in 1 of 23 colorectal metastatic tumors in their study (94). In our study, all metastatic cases were colorectal carcinoma. Of these, we obtained positivity in 1 (5%) of 20 cases. Our findings were evaluated as to be coherent with the literature.

Nafis Shafizadeh et al. compared GPC3 with HepPar1 in 80 resection materials with HCC. GPC3 was expressed in 79% (46/80) of poorly differentiated cases and 6.4% (7/80) of HCC fibrolamellar variant. Of 46 HCC cases, 56% were well differentiated, 83% moderately differentiated, and 89% poorly differentiated. Reticulin loss and focal abnormalities that closely resemble hepatic adenomas were noted in 10 of 16 well-differentiated cases. GPC3 expression was observed in 50% of this group. GPC3 was found to be more highly positive in cirrhotic liver. While it was negative in all hepatic adenomas and macroregenerative nodules, it was positive in 3 high-grade dysplastic nodules. Focal staining was observed in the patient with 4 cirrhotic regenerative nodules. Compared with HepPar1, GPC3 was more sensitive in poorly differentiated HCC cases (GPC3 89%, HepPar1 63%). The difference was more obvious when only diffuse positive staining was considered. In conclusion, Nafis Shafizadeh et al. showed that GPC3 has a high sensitivity for the diagnosis of HCC, and it is less sensitive in well-differentiated and fibrolamellar variant HCC, while its superiority over HepPar1 is obvious in poorly differentiated cases (38). In our study, GPC3 expression was observed in 85% (17/20) cases with HCC, while its expression was observed in 95% (19/20) with HepPar1. It was thought that the reason why the sensitivity of GPC3 was lower than HepPar1 in our cases was due to the fact that the cases included better differentiated HCCs.

Dina Kandil et al. accepted Grade 0 (no staining) and Grade 1 (weak staining) as a negative result in their immunocytochemical study with GPC-3 in the cytological materials of 20 patients with HCC, 20 with metastatic tumors and 20 with benign lesions. Grade 2 (moderate cytoplasmic staining) and Grade 3 (strong cytoplasmic staining) were evaluated as positive. While 90% (18/20) staining was obtained in the HCC group, they found negative in 100% (20/20) of metastatic tumors and 100% (20/20) of benign lesions. Strong and moderate expressions were not shown in any case. In HCC, the sensitivity of immunocytochemical staining was 90% and the specificity was 100%. Immunocytochemical staining was found to be superior to immunohistochemical staining in 25% of cases with HCC (39).

Peter Tatrai et al. detected small amounts of agrin in the basement membranes of the bile ducts and blood vessels in their study. They observed a significant, dramatic increase in the amount of agrin with the increase in the blood vessels and bile duct structures of the newly formed septal structures in liver cirrhosis, as in the areas of neovascularization in HCC. In this study, which was conducted in 29 liver cases with HCC and cirrhosis, a strong positivity for agrin was observed in the basement membranes of blood vessels and bile ducts, while negativity was always detected in regenerative nodules in cirrhosis (18).

Considering the selectivity of agrin in tumor microvascularization, it has been observed that it may help in recognizing malignant transformation in cirrhotic liver. As a result of these studies, it was thought that agrin was probably originated from active myofibroblasts, vascular smooth muscle cells and biliary epithelial cells. When agrin, which is dependent on various growth factors, is examined, it has been reported that it has a supportive role in bile duct proliferation and a stimulating role in tumor vascularization, as in integrins (18-19,22). Agrin is an immunohistochemical marker that detects malignant nodules in the liver.

Peter Tatrai, in his study of 38 benign (8 large regenerative nodules, 23 low-grade dysplastic nodules, 7 high-grade dysplastic nodules) and 29 malignant liver lesions, determined that agrin, like

CD34, was 93.1% sensitive and 92.6% specific. (20).

E.Batmunkh et al. studied agrin in 80 liver resection materials immunohistochemically. They were observed in the portal areas around the bile ducts and vascular structures in the normal liver, while they were observed in the basement membrane of the newly formed vessels in HCC. They found that basement membrane-specific tumor antigens are also present in well-differentiated HCC areas and may play a role in tumor progression (22).

When the literature was examined, no staining with Agrin/CD34 was observed in colorectal metastatic carcinomas, while we had 1 positive case in our study. It was thought that this might be caused by antibodies.

Christopher A et al. found in their work that while CDX2 staining was not observed in any of the HCC cases, positive staining was observed in all 60 colorectal carcinoma cases. While positivity was obtained in 9 of 17 cases with CCA, no staining was detected in 8 (40).

Alexandre Shariley et al. performed immunocytochemical studies to overcome the difficulties of cytological diagnosis in fine needle aspiration biopsies of the liver. In this study, they established a large panel of monoclonal antibodies, including CDX2, in the differential diagnosis of regenerative nodules and metastatic carcinomas from HCC. While a strong positivity was observed with CDX2 in most cases with colon carcinoma metastasis, they evaluated CDX2 as negative in cases with HCC and CCA (41).

A. Jinewath et al. investigated the relationship between the intestinal phenotype of the CDX2 gene with gastric and gallbladder carcinoma. They studied at CCA to demonstrate the value of immunohistochemical staining to compare the expression of this protein with clinicopathological factors of CDX2, whose role in cholangiocarcinogenesis is unknown. They found that it was expressed in 29/59 cases (49.2%). Its expression was found to be slightly higher in papillary type CCA than tubular type CCA (37.3%). They showed that papillary type CCA is more associated with intestinal type phenotype and can be used in the differential diagnosis from tubular type CCA (42).

In our study, immunoreactivity with CDX2 was observed in 18/20 (90%) cases with colon adenocarcinoma metastasis, while expression was not observed in 2/20 (10%) cases. These 2 cases were observed to be poorly differentiated.

In cases with CCA, we found a lower reactivity compared to higher values in the literature, with CDX2 positivity in 1/19 (5%) cases. We thought that this low reactivity was due to the tubular type (90%) of our cases. While no expression was observed in the literature in cases with HCC, we found CDX2 positivity in 5/20 (25%) cases in our study.

In conclusion, Glypican-3, Agrin, CDX2 were studied in 3 different types of liver tumors (Hepatocellular carcinoma, cholangiocellular carcinoma, colon adenocarcinoma metastasis) by immunohistochemical method. The antibodies studied were evaluated together with previously studied HepPar1, AFP, CEA, CD10, CK7, CK20, CK19, CK8, CK18. Expression of Glypican-3 was 85% in HCC and 15% in CCC. In HCC, 95% (19/20) expression was observed with HepPar1. In cases with HCC, positivity was 85% with GPC-3 and 75% (15/20) with AFP. With these data, it was determined that GPC3 is a superior oncofetal antigen compared to AFP. Expression of Agrin/CD342 in HCC was 95%. The expression of CDX2 in patients with colon adenocarcinoma metastases was 95%. With these findings, we concluded that GPC-3 and CDX2 are markers can be used in routine studies, and Agrin/CD34 can be put into routine practice as a result of larger series studies.

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