

# Pathology of Hepatocellular Adenoma: Subtypes and Rare Morphologic Features

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## ABSTRACT

**Background:** The molecular classification has been divided HCA into four main subgroups: hepatocyte-nuclear-factor-1a mutated (H-HCA), B-catenin type (HA-B), inflammatory type (HA-I) and unclassified type. Those subgroups were linked with risk factors, clinical behavior, histological features, imaging and malignant transformation. Subtyping is useful to predict HA's behavior and also to detect morphology which may have the potential to affect the prognosis. We aimed to review subtype features of our hepatocellular adenoma cases and discuss the importance of the rare morphologic features.

**Methods:** Fifteen Hepatocellular adenoma cases (10 resections, 3 explants and 2 biopsies) were included in this study. Hematoxylin and eosin (H&E) stained slides, Reticulin and Masson's trichrome stains, as well as immunohistochemical studies (IHC), were used to evaluate general morphologic and immunophenotypic features performed with B-catenin, SAA amyloid and Glutamine Synthetase(GS) using standard laboratory techniques in the Ventana Benchmark Ultra platform. CD34 immunohistochemical stains were performed on atypical cases to evaluate the presence of vascularization.

**Results:** By morphologic features and Immunohistochemistry, 3 HA-B (%20), 4 HA-I (%26.6), 4 HA-H (%26.6) and 4 HA-U (%26.6) cases were classified. Two HA cases had Dubin-Johnson pigment and Two of the beta-catenin mutated HAs had bone marrow metaplasia. In one of the cases, malignant transformation in the HA was present. The microscopic findings included hemorrhage, pigment formation, granuloma formation, presence of inflammation, presence and degree of steatosis, preserved or non-preserved reticulin network.

**Conclusions:** Besides the classic morphologic features; granuloma formation, pigmentation, bone marrow metaplasia can be seen in HAs. Although the prognostic significance of those is not known, they are considered to have a role in the development and progression of HA.

**Keywords:** Liver, hepatocellular adenoma, pathology

## HEPATOSELÜLER ADENOMA PATOLOJİSİ: ALT TİPLERİ VE NADİR GÖRÜLEN MORFOLOJİK ÖZELLİKLERİ

### ÖZET

**Amaç:** Hepatoselüler Adenoma moleküler sınıflandırmada, dört ana alt gruba ayrılmıştır: Mutasyona uğramış hepatosit-nükleer faktör-1a Hepatoselüler Adenoma (H-HCA), B-catenin tip Hepatoselüler Adenoma (HA-B), Enflamatuvar tip Hepatoselüler Adenoma (HA-I) ve sınıflandırılmayan tip. Bu alt gruplar risk faktörleri, klinik davranış, histolojik özellikler, görüntüleme ve malign transformasyon ile ilişkilendirilir. Hepatoselüler adenomayı sınıflandırmak, davranışını tahmin etmek ve ayrıca prognozu etkileme potansiyeli olabilecek morfolojiyi saptamak için yararlıdır. Hepatoselüler adenoma olgularımızın, subtıp özelliklerini gözden geçirmeyi ve nadir görülen morfolojik özelliklerin önemini tartışmayı amaçladık.

**Yöntem:** Onbeş Hepatoselüler adenoma olgusu (10 rezeksiyon, 3 eksplant ve 2 biyopsi) çalışmaya dahil edildi. Hepatoselüler adenomunun genel morfolojik ve immünofenotipik özelliklerini değerlendirmek için preparatlar, Hematoksilin-Eozin (H&E), Retikulin ve Masson trikrom boyaları ile boyandı; immünohistokimyasal olarak (IHK), B-catenin, SAA amiloid, Glutamin sentetaz antikorları, Ventana Benchmark Ultra platformunda standart laboratuvar teknikleri kullanarak uygulandı. Atipik vakalarda vaskülarizasyonun varlığını değerlendirmek için immünohistokimyasal CD34 yapıldı.

**Bulgular:** Morfolojik özellikler ve immünohistokimyasal bulgulara göre 3 HA-B (%20), 4 HA-I (%26.6), 4 HA-H (%26.6) ve 4 HA-U (%26.6) olgular sınıflandırıldı. İki HA vakasında Dubin-Johnson pigmenti gözlemlendi, beta-catenin mutasyonuna uğramış HA'ların ikisinde kemik iliği metaplazisi vardı. Olguların birinde HA'da malign transformasyon gözlemlendi. Kanama, pigment oluşumu, granülom oluşumu, inflamasyon varlığı, steatoz varlığı ve derecesi, korunmuş veya korunmamış retikulin ağı gibi mikroskopik bulgular değerlendirildi.

**Sonuç:** HA'larda klasik morfolojik özelliklerin yanı sıra granülom oluşumu, pigmentasyon, ve kemik iliği metaplazisi görülebilmektedir. Bunların prognostik önemi bilinmemekle birlikte, HA'nın gelişimi ve progresyonunda rol oynayabileceği düşünülmektedir.

**Anahtar sözcükler:** Karaciğer, hepatoselüler adenoma, patoloji

**H**epatic adenomas (HAs) are benign liver tumors with epithelial origin (1). HAs are hormone-driven tumors, before the advent of oral contraceptives, they were very rare (1). Incidental detection of HAs was due to increased use of radiologic examinations and increased incidence and subset of HAs reported in men and women that had no history of oral contraceptive use (2). Large HAs can be symptomatic due to complications, such as rupture and hemorrhage (3,4,5). Malignant transformation risk (5%) also increases with the tumor size (6,7). Molecular-genetic pathways of oncogenesis have shown that HAs are monoclonal neoplasms. A molecular classification of HA was described in 2006, by French collaborative group (8). HAs have been divided into 4 subtypes, primarily based on molecular characteristics, strongly associated with risk factors, clinical features, and complications, as well as histologic, immunohistochemical and radiologic features. These 4 subtypes are (1) HAs with inactivating mutations of hepatocyte nuclear factor 1a (HNF1A; HA-H), (2) HAs with activating mutations of b-catenin gene (HA-B), (3) HAs without mutations of the HNF1A or b-catenin genes and inflammatory features (HA-I), and (4) unclassified HAs that have no specific gene mutations or unique morphologic features (HA-U) (8). HA-B subtype has an increased risk of malignant transformation in hepatocellular carcinoma (HCC) linked to telomerase reverse transcriptase (TERT) promoter mutations (7,9). In our study, we classified our cases due to known histologic and immunohistochemical features and demonstrated other uncommon histologic features and discussed their relations with the subtypes.

## Methods

HA cases diagnosed between 2012-2017 were retrieved from our pathology department archives. Fifteen cases were included in our study and were diagnosed as HA. From those, 10 were resection specimens, 3 of them were explants and 2 were tru-cut biopsies. For resection specimens, representative sections of the tumor and non-cancerous tumours of the liver were reviewed for histological features. For biopsies, only tumor tissue was available for review. Hematoxylin and eosin (H&E) stained slides, reticulin and Masson's trichrome stains as well as immunohistochemical studies (IHC) were used to evaluate general morphologic and immunophenotypic features. Tumor characteristics evaluated on routine H&E stained slides included: steatosis (mild=0–33%; moderate=33–66%; marked=>66% of the lesion), inflammation, sinusoidal dilatation (telangiectasia), nuclear atypia (nuclear pleomorphism, increased nuclear:cytoplasmic ratio) and architectural atypia (gland-like or acinar growth). Atypia was defined as the presence of any of the following: (1)

nuclear atypia, (2) any degree of architectural atypia, and/or (3) focal loss of reticulin staining.

Immunohistochemistry for  $\beta$ -catenin (BD Bioscience, San Jose, CA, 1:50 dilution), SAA amyloid (Biocare Medical, CA, 1:50 dilution) and GS (Millipore, Billerica, MA, 1:2000) were performed in all cases using standard laboratory techniques in the Ventana Benchmark Ultra platform (Tucson, AZ, USA). GS IHC was scored as 0 (negative, or weak perivascular staining in <10% of the tumor), 1+ (weak, or strong <50% of the tumor), and 2+ (focal strong staining, or >50% of the tumor).  $\beta$ -catenin IHC was graded as 0 (membranous staining) or 1 (nuclear staining in any percentage of tumor cells). SAA stains were scored from 0 to 2+ (Score of 0=negative or <10% staining, 1+=10–50% staining, and 2+ = >50% positive staining). In most cases, we used adjacent non-tumoral liver as internal negative controls, including negative SAA staining, membranous  $\beta$ -catenin pattern, and normal centrilobular GS positivity. CD34 immunohistochemical stains (DAKO, Carpinteria, CA, 1:200) were performed on atypical cases to evaluate for the presence of sinusoidal capillarization.

## Results

Clinical characteristics of a total of 15 HA cases (10 resections, 3 explants and 2 biopsies) were included in this study (male=5, female=10, 2-50, mean age 34 years). One patient had multiple adenomas. The average size of HA was 9,9 cm (range: 1,5-23 cm). Detailed clinical history was available in 13 resection cases. Risk factors for the development of HA at the time of resection (e.g. use of oral contraceptives, glycogen storage disease, obesity) were identified. Among the female patients, the use of oral contraceptives (OCP) was identified in 7 out of 10 (70%) cases. One of 5 male patients (20%) reported anabolic steroid use. One case had glycogen storage disease (A8). The patients' demographics are summarized in Table 1.

The histopathological and immunohistochemical analysis was done first, we attempted to classify each adenoma based on IHC pattern, as previously described (10). HA with strong and diffuse GS staining (score 2+) and/or  $\beta$ -catenin nuclear staining, regardless of the SAA staining status, were categorized as HA-B. The remaining HA with SAA positivity (scores 1+ to 2+) were classified as HA-I. By applying the above criteria, we identified 3 HA-B (20%), 4 HA-I (26.6%), 4 HA-H (26.6%), and 4 HA-U (26.6%).

The microscopic findings included hemorrhage, pigment formation, granuloma formation, presence of inflammation, presence and degree of steatosis, preserved or non-preserved reticulin network (Table 2).

**Table 1.** The patient's demographic features

	<i>Age</i>	<i>Gender</i>	<i>Tumor diameter-Cm</i>	<i>localisation</i>	<i>Number of adenoma</i>	<i>OC use</i>	<i>Liver parenchyma</i>	<i>Radiologic diagnosis</i>
A1	32	M	16	Right lobe	solitary	-	N	Adenoma?
A2	19	M	22	Right lobe	solitary	-	N	Adenoma?
A3	25	F	5,2	Right lobe	solitary	+	N	Adenoma?
A4	20	M	1,5	Right lobe	solitary	-	N	met?
A5	46	M	8,5	Right lobe	solitary	-	N	Adenoma
A6	23	F	9	Right lobe	solitary	-	steatosis	Adenoma?
A7	28	F	5	Right lobe	solitary	-	N	Adenoma?
A8	2	F	1,6-2	Right-left lobes	adenomatosis	-	steatosis	Adenoma?
A9	40	M	23	Right lobe	solitary	-	steatosis	Adenoma?
A10	32	F	3,5	Right lobe	solitary	+	N	Adenoma?
A11	33	F	9,5	Right lobe	solitary	+	N	FNH?
A12	35	F	4,2	Right lobe	solitary	+	N	Adenoma?
A13	44	F	2,2	Right lobe	solitary	+	N	met?
A14	50	F	18	Right lobe	solitary	-	N	cystadenoma
A15	37	F	4,5	Right lobe	solitary	+	N	Adenoma? no data

**Gender of the patients:** 5 Male (33%), 10 Female (66%). **FNH:** Focal nodular hyperplasia  
**N:** nonspecific parenchymal changes

**Table 2.** Microscopic features of adenomas

	<i>Steatosis</i>	<i>Inflammation</i>	<i>Hemorrhage</i>	<i>Atypia</i>	<i>Other</i>	<i>Material</i>	<i>beta-c</i>	<i>GS</i>	<i>AA</i>	<i>HA-type</i>
A1	(-)	present	present	absent	Pigment, BM metaplasia, peliosis	Transplant hepatectomy	(-)	(-)	(-)	HA-U
A2	focal/mild	present	present	absent	pigment, BM metaplasia, peliosis	Transplant hepatectomy	(+)	(+)	(-)	HA-B
A3	focal/mild	absent	absent	absent		res	(-)	(-)	(-)	HA-U
A4	(-)	absent	absent	absent		biopsy	(-)	(+)	(-)	HA-B
A5	(-)	absent	present	present	osseous and BM metaplasia, cystic degen.	resection	(+)	(+)	(-)	HA-B
A6	focal/mild	present	absent	absent		resection	(-)	(-)	(+)	HA-I
A7	diffuse	absent	absent	absent		resection	(-)	(-)	(-)	HA-H
A8	(-)	absent	absent	absent		Transplant hepatectomy	(-)	(-)	(-)	HA-U
A9	focal/mild	present	absent	absent		biopsy	(-)	(-)	(+)	HA-I
A10	focal/mild	present	absent	absent		wedge	(-)	(-)	(+)	HA-I
A11	diffuse	present	absent	absent	non-necrotizing granuloma	resection	(-)	(-)	(-)	HA-H
A12	diffuse	absent	absent	absent		resection	(-)	(-)	(+)	HA-H
A13	diffuse	absent	absent	absent		resection	(-)	(+)	(+)	HA-H
A14	(-)	present	present	absent	hemorrhagic necrosis	resection	(-)	(+)	(-)	HA-U
A15	focal/mild	absent	present	absent	hemorrhagic necrosis	resection	(-)	(-)	(-)	HA-I

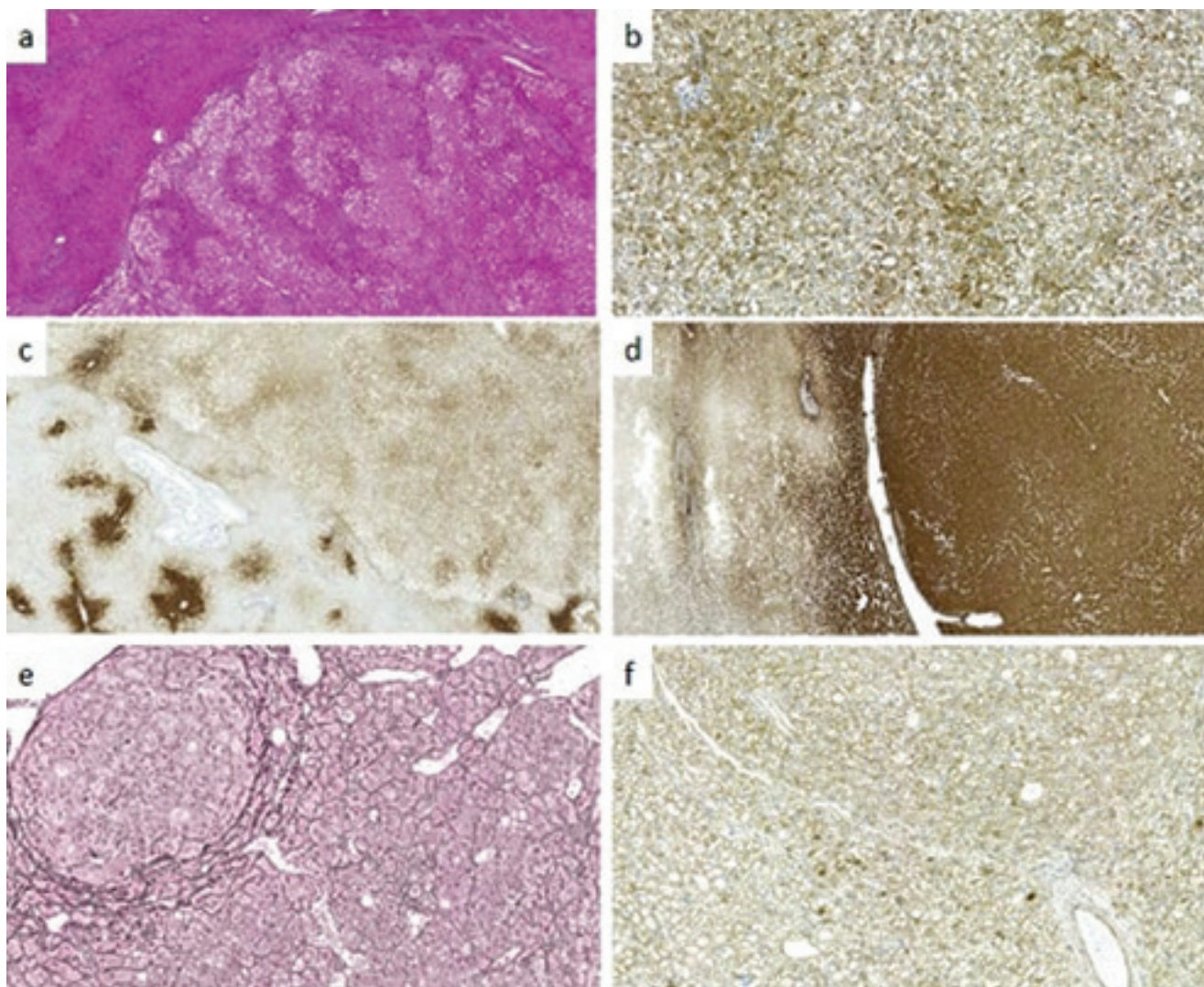
**HA:** hepatocellular adenoma, **beta-C:** beta-catenin, **GS:** glutamine synthetase, **AA:** Amyloid A

## Discussion

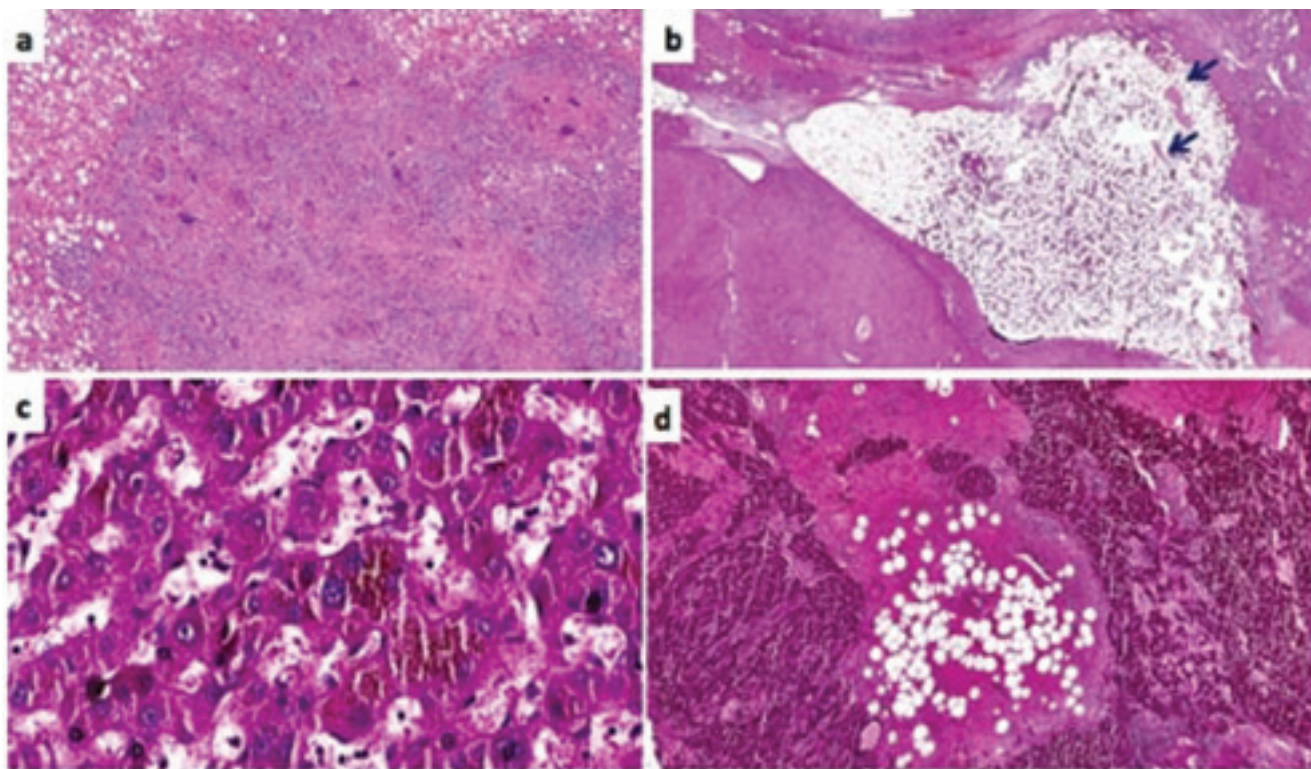
Hepatocellular adenoma is a rarely encountered lesion with a low tendency of malignant transformation (6,7). HAs are monoclonal neoplasms with unique molecular changes that involve oncogenic signaling pathways. In 2006, molecular-pathogenic classification was proposed dividing HAs into 4 groups. Most can be subclassified on the basis of molecular changes with varying degrees of malignant potential. HNF1alpha- Inactivated HCA (HA-H), Beta-catenin activated HA (HA-B), Inflammatory HCA (HA-I) characterised by increased expression of serum amyloid A (SAA), Unclassified HCA (HA-U) (8).

This classification is clinically relevant because the co-existence of Beta-catenin activation and HNF1 alpha

mutation increases the risk of malignancy, this subtype of HA-B and some of the HA-I have increased the potential for malignant transformation (2). Morphologic and immunohistochemical features also correlate with the subtypes (10). Different degrees of telangiectasia, inflammation, steatosis, ductular proliferation and atypia can be seen in HAs. Immunohistochemistry results of glutamine synthetase (GS), serum amyloid A (SAA), liver fatty acid-binding protein (LFABP), beta-catenin also correlate with the mutations (10). Due to morphologic and immunohistochemical features, we classified our cases. Besides the expected results, we have obtained 5 unusual findings among 15 cases which are 2 bone marrow metaplasias, 1 granuloma formation and 2 pigmented adenoma. These findings are not in the diagnostic criteria of hepatocellular adenoma.



**Figure 1.** (A) Ademo (H&E). Left side is the liver parenchyma, right side represents the adenoma, there is no fibrous capsule around the adenoma; (B) SAA immune stain positive adenoma; (C) GS immune stain, mild positive staining at the adenoma (right side), at the normal parenchyma GS positivity present around the central vein (left side); (D) Strong GS positivity, present at the B-catenin mutated adenoma; (E) Histochemistry, reticulin stain. Adenoma with focal atypia and reticulin loss; (F) focal and scarce B-catenin nuclear positivity at the B-catenin mutated adenoma.



**Figure 2.** Unusual morphologic features. (A) Granuloma formation; (B) Bone marrow metaplasia; arrows for the bone fragments; (C) Pigment at the hepatocyte; (D) Fat metaplasia.

The presence of bone marrow metaplasia was an unusual characteristic of hepatic adenoma. In literature, four HA cases were presented having bone marrow metaplasia (11,12,13,14), two of them were discovered in a glycogen-storage disease-associated hepatic adenoma (12,13), none of them were associated with oral contraceptive use. All were big masses and two of them were giant hepatic adenomas (11,14). Two of the case had hepatocellular carcinoma arising in the hepatic adenoma (12,14). Our cases were beta-catenin mutated HAs. In one of our cases, malignant transformation in the HA was present. Both of them were big masses; one of them due to giant mass that caused liver function problems, treated with liver transplantation. The presence of bone marrow metaplasia could be explained by the effort of the liver to regenerate damaged hepatic tissue (15). It has already been demonstrated that marrow-derived stem cells could be attracted by the damaged liver tissue upon the release of cytokines and migration factors. At that point, stem cells could differentiate into hepatic progenitor cells and then into mature hepatocytes. Hepatic progenitor cells have also a role in human liver tumor development (11). Bone marrow metaplasia was reported in some of the hepatocellular carcinomas as well (16).

Another uncommon morphologic feature is the granulomatous reaction in the hepatic adenoma. In literature, granuloma formation was seen in a few cases, some of them associated with oral contraceptive use, the others were present in inflammatory type HA (17, 18). It was proposed that the hepatic granulomas in these cases are a response to persistent inflammation caused by (inflammatory) HA, a local reaction to a neoplasm, chronic use of OCs, or a combination of these factors. In our HA case, the patient has OC use history and HA has typical morphologic features of inflammatory subtype (17).

Iron (19) or other pigments such as lipofuscin granules, Dubin Johnson pigment (20,21,22,23) are occasionally observed in HA. Two of our cases have Dubin Johnson pigment and they were diagnosed as pigmented hepatic adenomas; one of them was HA-B type and the other one was unclassified type. In literature, several reports suggested that pigmented hepatocellular adenomas have increased risk of atypia and malignancy, especially in men (20).

## Conclusion

The molecular-pathogenic classification determined 4 subtypes that correlate with morphologic and

immunohistochemical features. Besides the classical morphologic features; granuloma formation, pigmentation and bone marrow metaplasia can be seen occasionally in HAs. Although the prognostic significance of those is

not known, they can correlate with the development and progression of HAs and can be related to increased risk of malignancy. So in the pathologic reports, the unusual morphologic features should also be mentioned.

## References

- Nault JC, Bioulac-Sage P, Zucman-Rossi J. Hepatocellular benign tumors—from molecular classification to personalized clinical care. *Gastroenterology* 2013;144:888–902. [CrossRef]
- Dhingra S, Fiel MI. Update on the New Classification of Hepatic Adenomas Clinical, Molecular, and Pathologic Characteristics. *Arch Pathol Lab Med*. 2014;138:1090–97 [CrossRef]
- Bioulac-Sage P, Laumonier H, Couchy G, et al. Hepatocellular adenoma management and phenotypic classification: the Bordeaux experience. *Hepatology* 2009;50:481–9. [CrossRef]
- Dokmak S, Paradis V, Vilgrain V, et al. A single-center surgical experience of 122 patients with single and multiple hepatocellular adenomas. *Gastroenterology* 2009;137:1698–705. [CrossRef]
- van Aalten SM, de Man RA, JN IJ, et al. Systematic review of haemorrhage and rupture of hepatocellular adenomas. *British J Surg* 2012, 99:911–6. [CrossRef] <https://doi.org/10.1002/bjs.8762>
- EASL Clinical Practice Guidelines on the management of benign liver tumours. *J Hepatol* 2016;65:386–398. [CrossRef]
- Nault JC, Couchy G, Balabaud C et al. Molecular classification of hepatocellular adenoma associates with risk factors, bleeding, and malignant transformation. *Gastroenterology* 2017, 152: 880–94. [CrossRef]
- Zucman-Rossi J, Jeannot E, Nhieu JT, et al. Genotype-phenotype correlation in hepatic adenoma: new classification and relationship with HCC. *Hepatology*. 2006;43:515–24. [CrossRef]
- Farges O, Dokmak S. Malignant transformation of liver adenoma: an analysis of the literature. *Dig Surg*. 2010;27:32–8. [CrossRef]
- Margolske E, Bao F, Gonzales AK et al. Hepatocellular adenoma classification: a comparative evaluation of immunohistochemistry and targeted mutational analysis. *Diagn Pathol*. 2016;11:27. [CrossRef]
- Ramacciato G, Nigri GR, Aurello P et al Giant hepatic adenoma with bone marrow metaplasia not associated with oral contraceptive intake. *World J Surg Oncol*. 2006; 4: 58. [CrossRef]
- Iguchi T, Yamagata M, Sonoda T et al. Malignant transformation of hepatocellular adenoma with bone marrow metaplasia arising in glycogen storage disease type I: A case report. *Mol Clin Oncol*. 2016 Nov; 5: 599–603. [CrossRef]
- Moriura S, Kuroda M, Kimura A, Iwatsuka Y, Ikeda S, Sakai T, Usui A. Case report: hepatic adenoma with bone marrow metaplasia in a patient with glycogen storage disease type 1a. *J Gastroenterol Hepatol*. 1996 Jun;11:556–9. [CrossRef]
- Kang HJ, Jeon HJ, Kim SW et al. Hepatocellular Carcinoma Arising in a Huge Hepatocellular Adenoma with Bone Marrow Metaplasia. *J Pathology Translational Medicine*. Published online: December 27, 2017. [CrossRef]
- Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. *Blood*. 2003;102:3483–93. [CrossRef]
- Copin P, Ronot M, Vilgrain V. Hepatocellular Carcinoma With Osseous Metaplasia and Bone Marrow Elements. *Clinical Gastroenterology and Hepatology* March 2015;13: 26–7. [CrossRef]
- Bieze M, Bioulac-Sage P, Verheij J et al. Hepatocellular Adenomas Associated with Hepatic Granulomas: Experience in Five Cases. *Case Rep Gastroenterol*. 2012 Sep-Dec; 6: 677–83. [CrossRef]
- Malatjalian DA, Graham CH. Liver adenoma with granulomas. The appearance of granulomas in oral contraceptive-related hepatocellular adenoma and in the surrounding nontumorous liver. *Arch Pathol Lab Med*. 1982 May;106:244–6.
- Weinstein DA, Roy CN, Fleming MD et al. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood*, 2002, 100: 3776–81. [CrossRef]
- Mounajjed T, Yasir S, Aleff PA, Torbenson MS. Pigmented hepatocellular adenomas have a high risk of atypia and malignancy. *Mod Pathol*. 2015 Sep;28:1265–74. [CrossRef]
- Hechtman JF, Raoufi M, Fiel MI et al., Hepatocellular carcinoma arising in a pigmented telangiectatic adenoma with nuclear  $\beta$ -catenin and glutamine synthetase positivity: case report and review of the literature. *The American Journal of Surgical Pathology*, 2011 35:927–32. [CrossRef]
- Masuda T, Beppu T, Ikeda K et al. Pigmented hepatocellular adenoma: report of a case. *Surgery Today*, 2011, 41: 881–3. [CrossRef]
- Hasan N, Coutts M, Portmann B. Pigmented liver cell adenoma in two male patients. *The American Journal of Surgical Pathology*, 2000, 24:1429–32. [CrossRef]