CASE REPORT

Regressive liver adenomatosis following androgenic progestin therapy withdrawal: a case report with a 10-year follow-up and a molecular analysis

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Abstract

Objective: The relationship between sex hormones and hepatocellular adenoma development is well established. On the contrary, their contribution to liver adenomatosis (LA) development is still a debatable issue. Recently, inactivating mutations of hepatocyte nuclear factor-1 α (HNF-1 α) transcription factor gene or activating mutations of β -catenin have been demonstrated in some liver adenomas, and a possible link between HNF-1 α gene mutations and oral contraceptives has been suggested. Only two cases of regressive LA after hormone withdrawal therapy have been described so far but without any information concerning the molecular characteristics of the tumours.

Case: We report the case of a 48-year-old woman with LA, who had been taking an androgenic progestin therapy (lynestrenol) for 10 years. A major regression in the number and size of the lesions was observed 6 months after complete withdrawal of this therapy.

Methods: Hepatocellular adenomas were studied by immunohistochemistry for oestrogen, progesterone and androgen receptors (ER, PR and AR respectively), and for β -catenin. Direct sequencing of the HNF-1 α gene was also performed.

Results: For the first time, we demonstrate significant immunostaining of AR in the hepatocellular adenomas. This staining was negative in the partially regressive adenoma. Immunostainings for ER and PR were negative. HNF-1 α and the β -catenin pathways were not involved in tumour pathogenesis.

Conclusions: Our case suggests a role of androgenic progestin therapy in some cases of LA. Hormone therapy withdrawal may induce a significant regression in lesions.

European Journal of Endocrinology 156 617–621

Introduction

Hepatocellular adenomas are rare benign liver neoplasms occurring generally in three clinical settings: a) women in reproductive age taking oral contraceptives (1), b) men who are receiving anabolic androgenic steroid therapy (methyl testosterone derivatives; (2)) and c) patients with glycogen storage disease types I and III (3). The presence of more than ten hepatocellular adenomas defines a specific entity, liver adenomatosis (LA; (4)). Initially, LA was considered as a separate clinical entity distinct from isolated hepatocellular adenoma, affecting men and women equally and with no relation to oral contraceptive use (4). Recently, inactivating mutations of hepatocyte nuclear factor-1 α (HNF-1 α) transcription factor or activating mutations of β -catenin were identified in both LA and isolated hepatocellular adenomas, suggesting a possible overlap of these two entities (5, 6). Moreover,

additional data demonstrated that LA, like isolated hepatocellular adenomas, occurred mainly in women using oral contraceptives (5, 7–9). However, only two cases of regressive LA after hormone therapy withdrawal have been described (7, 10). The importance of hormonal contraceptive use in LA is thus questionable. Moreover, in these two regressive cases there is no information concerning the molecular characteristics of the tumours, whereas a possible link between HNF-1 α mutations, cellular proliferation and the use of oral contraceptives has recently been suggested (5).

Case report

In April 1990, a 48-year-old woman was admitted, suffering from asthenia, weight loss and pain in the right upper abdominal quadrant. She was 156 cm tall

and weighed 46 kg. Her alcohol intake was 50 g/day. After two uneventful pregnancies, she had been taking a synthetic progestogen (lynestrenol (Orgamétil) 5 mg/day) for 10 years. There was no family history of liver tumours, glycogenosis or androgenic steroid intake. Clinical examination showed liver enlargement. Liver function tests were as follows: serum aspartate aminotransferase 43 IU/l (normal <35 IU/l) and γ -glutamyl transferase 123 IU/l (normal <33 IU/l). Serum alanine aminotransferase, alkaline phosphatase and serum bilirubin were within normal ranges. Hepatitis B surface antigen (HbsAg) and hepatitis B virus antibodies (anti-HBs and anti-HBc) were negative. Serum tumour markers (carcinoembryonic antigen and α -fetoprotein) were within normal range. Blood glucose and fasting morning blood glucose levels were normal. Ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) demonstrated ten liver lesions measuring from <1 up to 6 cm, with no sign of intra-abdominal haemorrhage (Fig. 1A). Laparoscopic liver biopsy was performed on four lesions. Three lesions, including a 6 cm liver lesion, were hepatocellular adenomas composed of steatotic hepatocytes without cellular atypia, arranged in two to three cell thick plates separated by sinusoids intermingled with numerous thin-walled vessels, without portal triad and ductules. There was neither inflammatory infiltrate nor sinusoid dilatation. One lesion was a focal nodular hyperplasia (FNH) composed of thickened hepatocyte plates and fibrous septa, containing thick arterial structures with inflammatory cells and ductular reaction. Histology of non-tumoural liver tissue showed numerous steatotic hepatocyte foci without any preferential acinar distribution. There were no perivenular or periportal fibrosis, Mallory bodies, or neutrophil polymorph infiltrate.

The patient follow-up included US and CT once a year. In July 1993, lynestrenol was changed for a postmenopausal hormonal treatment consisting of percutaneous oestrogens and oral progesterone (1.5 mg 17- β -oestradiol and 100 mg/pill daily respectively). Radiological follow-up performed within a 6-month post-study period showed no changes in hepatic lesions. In January 1994, hormonal therapy was completely withdrawn, and 6 months later there

was a major significant decrease in the number and size of the lesions; US showed only one liver lesion, the largest lesion in the right lobe. This lesion regularly shrunk. In October 1996, it measured 3 cm in diameter. In January 2000, US and MRI showed this same liver lesion without any change in size (Fig. 1B). At this time, a cystic mass was also discovered at the head of the pancreas, measuring 6.5 cm in diameter, along with atheroma of the abdominal aorta. An endoscopic US-guided fine needle aspiration of the pancreas revealed a poorly differentiated carcinoma. Preoperative arteriography demonstrated an occlusion of the celiac trunk and the superior mesenteric artery, which contraindicated pancreaticoduodenal resection (Whipple procedure). The patient died in June 2000 of a paraneoplastic hypercalcaemia, related to the pancreatic cancer.

Pathologic post-mortem examination revealed that the pancreatic cancer corresponded to an anaplastic carcinoma. The liver measured 29 $\times$ 26 $\times$ 9 cm and weighed 1360 g. Macroscopic and microscopic examinations of the liver showed one hepatocellular adenoma in the right lobe measuring 3 cm with focal fatty changes, large areas of necrosis, haemorrhage and fibrosis within the lesion (Fig. 2A). A second nodule of 2 mm in the right liver corresponded to an FNH. A few small nodular steatotic hepatocyte foci without any preferential acinar distribution were also present in the macroscopically normal liver (Fig. 2B). No metastasis was seen.

Genomic DNA was extracted from the formalin-fixed, paraffin-embedded laparoscopic liver biopsies (hepatocellular adenoma and normal liver tissue), because post-mortem tissue did not provide material of suitable quality for DNA amplification.

Direct sequencing of exon-intron boundaries of the *HNF-1 α* gene was performed after genomic DNA amplification, as described previously (5). Neither somatic nor germline inactivating mutation was found in the hepatocellular adenoma and the normal liver tissue.

An immunohistochemical study of β -catenin (E-5, Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:250 dilution) oestrogen receptors' (ER; clone 1D5, Dakocytomation, Trappes, France, 1:200 dilution), progesterone

Figure 1 Magnetic resonance imaging (T2 weighed). (A) In 1990, multiple high signal intensity lesions in the liver – the largest located in the right lobe measured 6 cm; (B) in 2000, only one high signal intensity lesion in the liver located in the right lobe measured 3 cm.

Figure 2 In 2000, liver histology (haematoxylin-eosin-saffron staining, $\times 25$, scale bar: 500 μm). (A) The 3 cm lesion in the right lobe corresponded to a hepatocellular adenoma with areas of necrosis, haemorrhage and fibrosis (normal liver in the right part of the figure, star); (B) hepatocellular steatotic foci (arrowheads) within the non-tumoural liver tissue.

receptors' (PR; MU-328-UC, Biogenex, San Ramon, CA, USA, 1:100 dilution) and androgen receptors' (AR; clone AR441, Dakocytomation, 1:50 dilution) protein expression was performed on the formalin-fixed, paraffin-embedded partially regressive hepatocellular adenoma and three adenomas biopsied in 1990. A result showing 5% or more of immunopositive nuclei was considered positive. β -catenin staining was observed on the hepatocyte plasma membrane in the adenoma and in the normal liver tissue, with no overexpression or nuclear reactivity in the adenoma. Immunostainings for ER and PR were negative. In contrast, immunostaining for AR was positive in adenomas biopsied in 1990, with a patchy distribution, and negative in the partially regressive hepatocellular adenoma and in the normal liver tissue.

Discussion

LA was arbitrarily defined as the presence of more than ten hepatocellular adenomas in the liver (4). However, our case corresponds to a genuine LA even though less than ten liver lesions corresponded to hepatocellular adenomas. Indeed, ten liver lesions were identified by imaging techniques and, amongst them, one lesion was an FNH. Among the nine remaining lesions, three were histologically proved to be adenomas and the six other lesions had imaging criteria characteristic of hepatocellular adenoma. Indeed, some authors define LA as from six or even four liver adenomas (9, 11). Moreover, histological analysis of the laparoscopic biopsies in 1990 and of the whole liver parenchyma in 2000 showed numerous microscopic hepatocellular steatotic foci. The steatotic foci were probably not related to alcohol intake as they had no preferential acinar distribution and were still present in January 2000, although alcohol intake had been stopped since January 1998. These steatotic foci could correspond to adenoma precursor and are proposed as strong evidence of adenomatosis (9). The coexistence of hepatocellular adenomas with FNH and vascular tumour has been described elsewhere in the literature (8, 9). FNH lesions associated with hepatocellular

adenomas may be secondary to systemic and local vascular disturbances induced by oral contraceptives, tumour-induced growth factors, thrombosis and local arteriovenous shunting (12).

It is well known that isolated hepatocellular adenoma development and progression are related to oral contraceptive use, with an estimated annual incidence of 0.3 to 4 per 100 000 women per year (13, 14). Although not initially recognised, there is also an association between oral contraceptive use and LA. It is admitted that tumour development is related to the ethinyl oestradiol component in a dose-dependent manner (15, 16). However, the majority of oral contraceptives associate ethinyl estradiol and an androgenic progestin (e.g. levonorgestrel).

Oestrogens and their receptors have been shown to be associated with hepatocyte proliferation *in vivo* and *in vitro* (14). The role of androgens remains poorly studied.

The role of progestational hormones in LA and isolated adenomas is not well defined, but three cases of hepatocellular adenomas have already been reported in women receiving progestins (17, 18).

Progestins are a large class of compounds characterised by their affinity for the two PR isoforms, but with various binding capacities for ARs and glucocorticoid receptors (19). 19-nortestosterone derivatives (to which lynestrenol belongs) have a potent antigonadotrophic effect. They bind with a high affinity to progesterone and ARs. Interestingly, the three cases of hepatocellular adenomas observed in women receiving a progestational agent used progestins belonging to 19-nortestosterone derivatives (norethisterone (17) and s.c. levonorgestrel implant (18)).

A temporal relationship between progestin therapy and the induction of LA cannot be clearly demonstrated in our case because the presence of hepatocellular adenomas has not been excluded before starting androgenic therapy. In contrast, our case, with a 10-year follow-up clearly demonstrates for the first time a temporal relationship between progestin therapy and an almost complete regression after hormone therapy withdrawal. Several cases of complete regression of hepatocellular adenomas have been

reported after discontinuation of oral contraceptive use (10). To our knowledge, only two cases of regressive LA have been described after hormonal therapy withdrawal (7, 10). In both these cases, the female patients had been taking oral oestrogenic contraceptive drugs.

In our case, as well in the case of the woman with LA complicating progestational implant (18), we found no expression of PRs, assessed by immunohistochemistry, in the adenomas (biopsies of three adenomas in 1990 and the only adenoma left in 2000) or in the normal liver tissue. In contrast, we could demonstrate a significant staining of ARs, suggesting that tumour development could be related to the androgenic action of progestin therapy.

We performed genetic and immunohistochemical analyses to precisely identify the type of LA. Two genetic pathways have been identified in LA so far. In nearly half of the cases, there is a biallelic mutation of the *TCF-1* gene coding for HNF-1 α transcription factor (6). A possible link between HNF-1 α mutations, cellular proliferation and oral contraceptive use was recently suggested (5). In a few cases, there is a β -catenin activation with or without β -catenin mutation (6, 20). No other recurrent genetic alteration has been identified so far (21). Our case corresponds to a non-familial LA without HNF-1 α mutation or β -catenin activation. Indeed, we found no HNF-1 α mutations in the hepatocellular adenoma and the non-neoplastic liver tissue by direct sequencing. β -catenin immunostaining showed no β -catenin overexpression or nuclear staining, and thus no activation of the Wnt/ β -catenin pathway. We did not analyse the β -catenin gene for mutations because the immunohistochemical study of β -catenin is a sensitive tool for the detection of activation in the Wnt-signalling pathway (20). Recently, a classification of hepatocellular adenomas based on a genotype–phenotype correlation has been proposed (6). In our case, hepatocellular adenomas can be classified as ‘non-mutated and non-inflammatory’.

Androgenic progestin therapy may play a role in some cases of LA, although probably in rare cases. Consequently, it may be relevant to search for pre-existing liver pathological condition before starting a long-lasting treatment, in particular with a US examination. Occasional execution of US examination may be reasonable in these patients. Nevertheless, further reports of similar cases will provide more information regarding the opportunity of a surveillance programme of these patients.

Because of the bleeding risk with i.p. haemorrhage, which is the main complication of LA, preventative surgical resections are proposed (22). The number and size of lesions, the presence of symptoms and the surgical risk guide the treatment. In this case report, we show that a significant regression of liver adenomas in the setting of LA can be seen after hormone withdrawal. Thus, hormonal therapy withdrawal should be tried and this may help avoid surgery.

Acknowledgements

This work was supported by the Inserm, ‘Réseaux de recherche clinique et réseaux de recherche en santé des populations’. Emmanuelle Jeannot was supported by an ARC (Association pour la Recherche sur le Cancer) doctoral fellowship.

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Received 13 January 2007

Accepted 13 March 2007