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De novo germ-line mutation of *APC* gene in periampullary carcinoma with familial adenomatous polyps – A novel familial case report in South India

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Abstract

Periampullary carcinoma is a malignant tumour arising from the ampulla of vater. Adenomatous polyposis coli (*APC*) gene has a key role in stabilizing β-catenin pathway, in which hypermethylation in *APC* gene could lead to proteasome degradation of β-catenin. The aim of this case report is to identify the *APC* gene mutation and its influence on β-catenin pathway in patient with periampullary carcinoma. A 51-year-old woman was diagnosed with yellow discolouration of sclera, passing deep yellow coloured urine and pruritus. A family history of ovarian cancer had been reported in her mother. Her radiological, pathological and laboratory examination confirmed periampullary carcinoma. She underwent whipple's pancreaticoduodenectomy, and the histopathology of the resected specimen showed a well differentiated adenocarcinoma involving the ampulla of vater. Further, the tumour region was subjected to genetic screening by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP), cytogenetic analyses such as karyotyping and immunohistochemical techniques. These results showed non-sense mutation in *APC* gene at codon 1309, chromosomal alterations at 5q21 and irregular accumulation of β-catenin in nuclear membrane. The family history revealed a strong association of ovarian cancer (maternal) with a similar *APC* gene mutation. We conclude that periampullary carcinoma patient exhibit FAP due to *de novo* germ-line mutation of *APC* gene that engenders an inactivation of β-catenine/TCF mediated transcription function, which is linked with a family history of ovarian cancer.

Keywords: Periampullary carcinoma; Adenomatous polyposis coli; β-catenin; Pedigree; Offspring

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1. Introduction

Ampulla of vater carcinoma is a relatively uncommon neoplasm, which accounts for 6%–7% of periampullary tumours and 0.2% of gastrointestinal tract malignancies [1]. There is a noteworthy increased risk of periampullary cancer in patients with Familial Adenomatous Polyposis (FAP) [2]. FAP is an inherited autosomal dominant syndrome caused by the mutations in germ-line *APC* gene, located in the chromosome 5q21-22, which is typically categorized by the development of hundreds to thousands of adenomas in the rectum and colon during the second decade of the life [2]. The *APC* gene functions as a promoter for rapid deprivation of *CTNNB1* gene and also participates in Wnt signalling as a negative regulator.

β-catenin is an intracellular as well as a multifunctional protein which acts as an integral component of cell–cell adhesion and signal transduction for the Wnt [3]. The free cytoplasmic level of β-catenin is low as it is directed by the protein which destructs the ubiquitin proteasome system by *APC* gene with glycogen synthetase kinase − 3b (GSK-3b) [4]. The Wnt/β-catenin signalling pathway plays a critical role in the development and progression of ovarian cancer. In ovarian cancer, the components of Wnt/β-catenin/TCF/Lef signalling pathway have an up-regulating function [5]. In addition to these, inactivation of the *APC* gene in human leads to the deregulation of Wnt/β-catenin signalling and the formation of ovarian endometrioid adenocarcinoma [6]. Similarly, about 30% of APC mutations exist in periampullary carcinoma due to dysfunction of Wnt/β-catenin pathways [7]. Several factors, including genetic background, are known to increase the familial risk of ovarian cancer as well as periampullary cancer [8]. Previously, it was reported that a relatively high percentage of female FAP-CRC can metastasize to the ovary [9]; similarly, metastasis of carcinoma of ampulla of vater to the both ovaries has been reported [10]. In our report, the patient has been analysed for family history in which her mother was affected with ovarian cancer.

Thus the pedigree analysis of present case report suggests the inheritance of mutation from first generation to the second generation. The focal aim of the present case report was to analyse the alterations in *APC* gene which leads to the formation of FAP and dysfunction of β-catenin pathway in the periampullary cancer patient inherited from maternal lineage.

2. Case report

A 51 year old lady was hospitalized with the complaints of yellow discolouration of sclera, passing deep yellow coloured urine and pruritus for nearly two weeks. Her medical history revealed that she was suffering from hypertension and diabetes. There was no past medical history of chronic drug intake, jaundice, surgery or any endoscopic procedures. She suffered from loss of appetite and loss of weight for the past 2 months. Her detailed family history as well as pedigree was included with the paternal lineage along with 2nd and 3rd generations which revealed that the pro band's mother had abdominal distension with an ovarian tumour that was 12 cm in diameter (Fig. 1). Genetic analysis of the mother's ovarian cancer revealed similar mutation in the APC gene as that of the daughter with periampullary carcinoma. she was on regular follow-up for 17 months, when the disease relapsed. Immunohistochemically, the tumor cells were positive for CA125 and negative for CA19-9. The proband's mother died due to metastatic disease of ovarian cancer.

Biochemical data of the daughter with periampullary carcinoma showed hyperbilirubinemia (total bilirubin of 14.8 mg/dL with a direct bilirubin of 12.9 mg/dL) and the liver chemistry tests revealed Aspartate Aminotransferase (AST) of 32 U/L, Alanine Aminotransferase (ALT) of 30 U/L, Alkaline phosphatase (ALP) of 788 U/L with Internationalised Normal Ratio (INR) of 0.91. Reports were negative for HBsAg, Anti HCV and HIV. On evaluation, ultrasonogram and contrast enhanced computerized tomogram (CECT) revealed distended gall bladder with dilated intra and extra hepatic biliary radicals (IHBR and EHBR) with asymmetric thickening of lower end of common bile duct (CBD) suggestive of periampullary carcinoma (Fig. 2). There was no liver metastasis and ascites. X-Ray of the chest was normal. Upper gastrointestinal endoscopy showed ulcerated lesion in ampulla with adjacent polyps. Biopsy of the lesion revealed adenocarcinoma. On colonoscopy there were multiple polyps in the colon and few polyps in the rectum (Fig. 3). Patient underwent Whipple's pancreaticduodenectomy and the specimen showed an ulcerated periampullary tumour of about 2 × 2 cm size (Fig. 4). Histologically, the resected specimen showed a well differentiated adenocarcinoma involving the ampulla of vater. Nodes were negative for malignancy. Margins were free of tumour without any lymphovascular invasion. The pathological stage of the tumor was pT2N0Mx. On microscopic examination with H&E staining (Fig. 5), the neoplasm composed of well-formed glands lined

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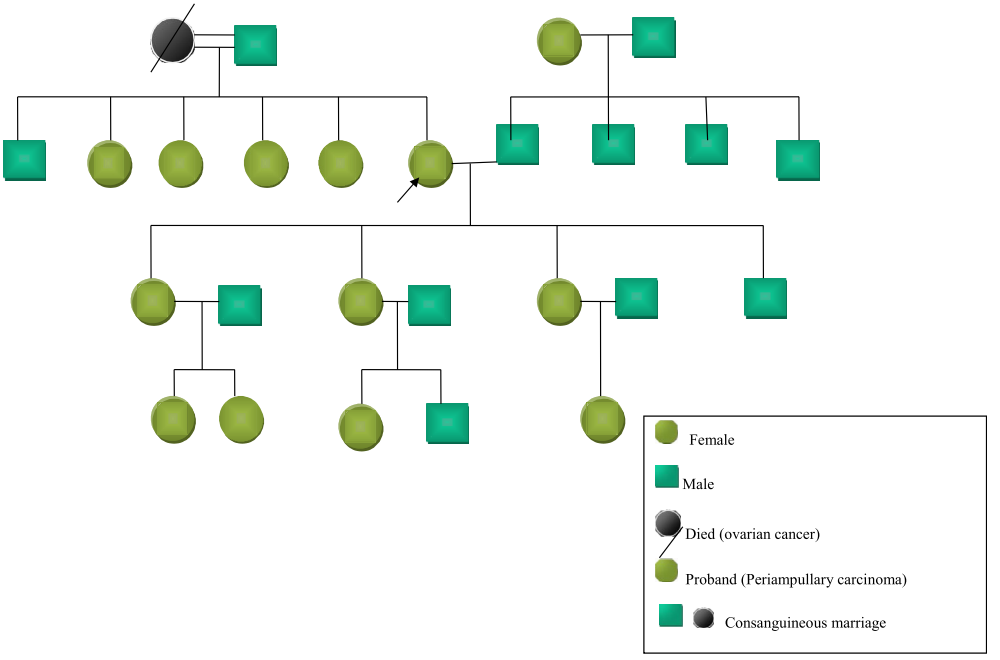

Fig. 1. **Pedigree Analysis of Ampullary carcinoma Patient**. The pedigree analysis revealed that the proband's mother had abdominal distension with ovarian tumour which was the reason for the proband to receive periampullary cancer thus showing that the disease has been transmitted through maternal lineages.

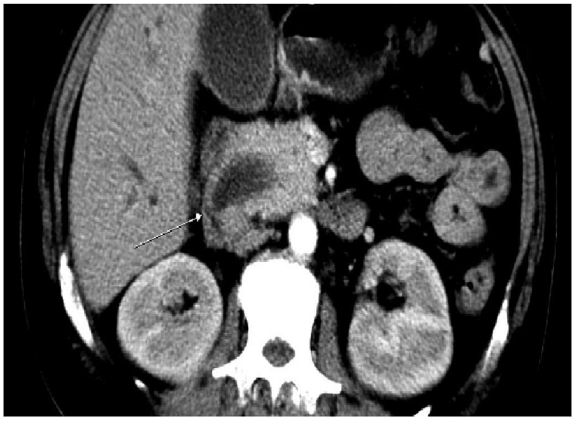

Fig. 2. **Contrast enhanced computerised tomogram**. CT scan showing asymmetric thickening of lower end of the common bile duct with distended gall bladder and dilated intra and extra hepatic biliary radical, indicative of periampullary carcinoma.

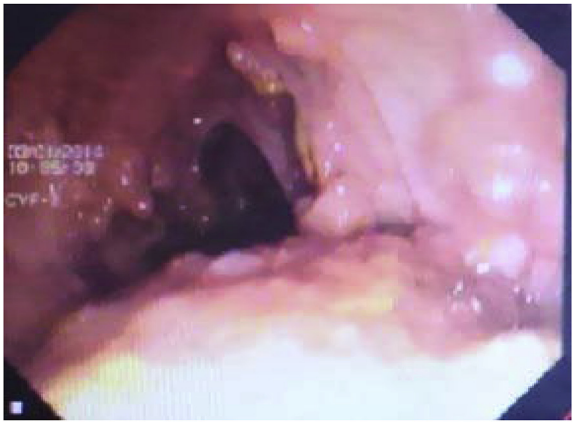

Fig. 3. **Colonoscopy**. Colonoscopy revealed multiple polyps in colon and a few polyps in rectum.

by columnar cells with vesicular nucleus and very little cytoplasm. The glands are arranged in back to back fashion in one focus.

The pedigree chart of the proband illustrated an exact evidence for the inheritance of the periampullary cancer through her mother's lineage affected with ovarian cancer. We observed deletion of 5q21 chromosomal alteration in periampullary carcinoma patient and a non-sense germ-line mutation in *APC* gene with premature truncated protein at codon 1309 with 5 bp deletion (AAAGA) in exon 15 adjacent to inflammatory zone. Previous records in the database showed that her mother was affected with *APC* gene mutation which lead to ovarian cancer. This depicts that *APC* gene has a pertinent role in the early stages of periampullary carcinogenesis, and the allelic loss of chromosome 5q21 is an important prognostic marker. By using diaminobenzidine as the chromogen in an

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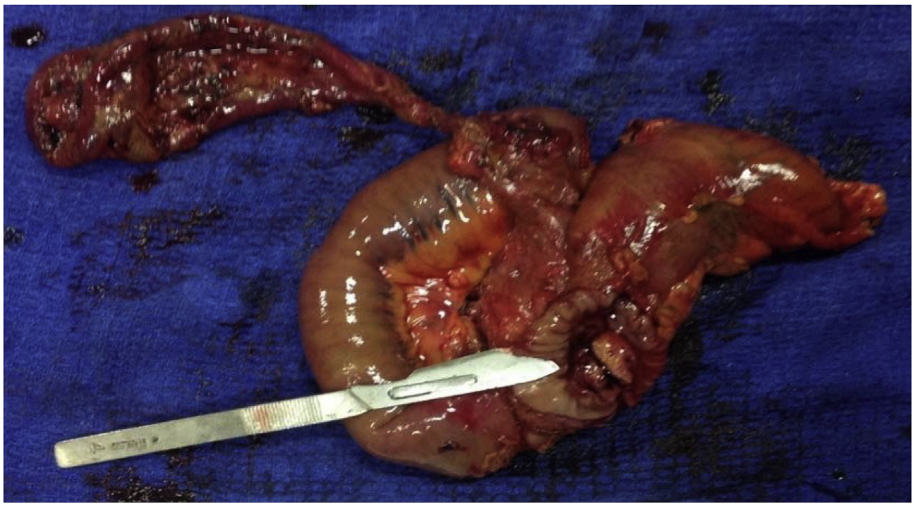

Fig. 4. **Whipple's pancreaticduodenectomy specimen**. The Whipple's pancreaticduodenectomy specimen showing ulceration with periampullary tumour of 2 × 2 cm size.

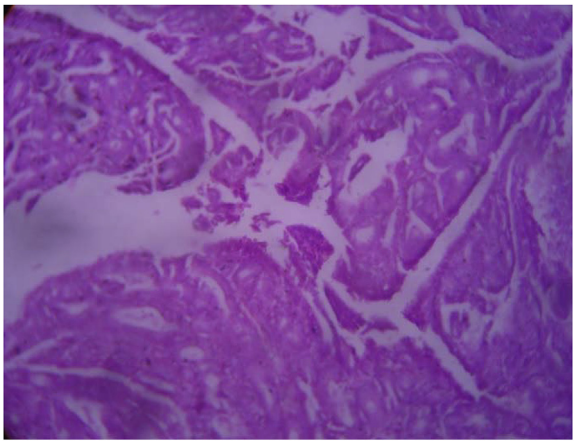

Fig. 5. **Microscopic examination on H&E staining**. H&E staining showing neoplasm composed of well-formed glands lined by columnar cells with vesicular nucleus and scant cytoplasm. The glands are arranged in back to back fashion in one focus.

automated staining system, immunohistochemical staining of β-catenine was carried out, where the degree of positive immunoreactivity was graded according to the percentage of stained cells. The resulted staining was found to be between 70 and 100% in the nuclear region which confirmed an abnormal localization of the β-catenine pathway.

3. Discussion

Periampullary carcinomas are cancers arising in and around the ampulla of vater, presenting as obstructive jaundice [11]. The genetic mutation in periampullary carcinoma has not been widely reported in the literature. Schildkraut et al. [12] reported that the degree of familial inheritance of cancer was based on a score derived from a kinship coefficient. The present case study reports that on screening of the patient with periampullary carcinoma with a family history of ovarian cancer, a *de novo* germ-line mutation of *APC* gene and abnormal expression of β-catenin in nuclear membrane is detected. Interestingly, pedigree analysis also suggested a tenacious family history of ovarian cancer (maternal) with an analogous *APC* gene mutation.

Earlier study had reported that the progression of periampullary polyps i.e. adenomas to carcinoma was 5% over a period of 10 years [13]. In the present study, the patient had more number of polyps in the colon, with few polyps in the rectum. The germ-line mutation of *APC* gene in human leads to a condition called FAP which progresses to adenocarcinoma in the colorectum and in the duodenum [16]. To our knowledge, this is the first report of a case in which *APC* gene mutation is found both in the mother with ovarian cancer and in the proband with periampullary cancer. To support our findings, Crobach et al. [14] hypothesized that ovarian metastases was more common in female FAP patients compared to sporadic patients with CRC. In addition, national wide database reported that, ovarian metastases occurred in at least 15% of female FAP CRC cases. Similar to the lines of Dihlmann et al. [15], we observed a non sense germ-line mutation of *APC* gene in polyposis coli at exon 15 codon 1309 with 5 bp deletion of AAAGA. Bjorn et al. [16] reported a germ-

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line mutation in *APC* gene from downstream of codon 1051 with FAP which was associated with stage IV periampullary adenomas as well as a malignant course of the periampullary adenomatosis. Our observation has also revealed mutations in germ-line codons in periampullary carcinoma, further confirming the maternal lineage as a carrier of *APC* gene mutation.

Najjia et al. [17] had reported that the initiation for duodenal tumour was due to excess β-catenin expression in the duodenal cells. Also, Morin et al. [18] reported that mutation in *APC* gene increases the accumulation of β-catenin in cytoplasmic region and this would be an oncoprotein through constitutive β − catenin − Tcf − regulated transcription. Mostly, the mutations or polymorphisms in *CTNNB1* or *APC* gene leads to irregular activation of Wnt/β-catenin signalling at the onset of various types of malignancies in ovarian cancer [19]. In line with this study, we also observed in this patient with periampullary carcinoma, *APC* gene mutation which leads to accumulation of β-catenin in nuclear membrane along with ovarian cancer found in the maternal lineage.

4. Conclusion

In conclusion, periampullary carcinoma due to a *de novo* germline mutation in the codon 1309 of the *APC* gene, chromosomal alteration in the 521 region and irregular accumulation of β-catenin is reported in this case study, with a maternal history of ovarian cancer from pedigree analysis. This case report unfolds the concept that genetic alteration in the first degree relatives has a high probability of transmission to the progeny for rare malignancies.

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