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RESEARCH ARTICLE

MOLECULAR PROFILING IN EARLY ONSET GASTRIC MALIGNANCIES- A CASE SERIES

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ABSTRACT

We have encountered three cases of advanced gastric malignancies in persons in their early twenties. All three tumours were reported by histopathology to be poorly differentiated adenocarcinomas with signet cells and one among them also had a neuroendocrine component, proven by immunohistochemistry. They all had aggressive disease and were the first cases in their respective families. The presentation of these patients, molecular profile of the tumour and management will be discussed.

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INTRODUCTION

Gastric adenocarcinomas that occur prior to the sixth decade have increased in incidence worldwide. While sporadic or familial mutations may underlie most such cases, in keeping with the two hit theory of malignant transformation, changing trends in life style maybe a contributing factor.

Case Series

Patient 1 was a 22-year-old woman with a two month history of dyspepsia not responding to proton pump inhibitors. An upper GI endoscopy had been done at another hospital. It revealed a large antropyloric growth. The histopathological report showed that it was a poorly differentiated adenocarcinoma with signet ring cells. The immunohistochemistry report was that of an adenocarcinoma as well. Imaging studies revealed extensive liver metastasis and ascites. She deteriorated rapidly and succumbed to her disease following one cycle of chemotherapy.

Patient 2 was a 26-year-old woman who presented with marked weight loss and dyspepsia.

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Examination of the abdomen showed it to have a doughy consistency and TB Abdomen was suspected. However, upper GI endoscopy revealed an antropyloric growth. The biopsy report was that of a poorly differentiated adenocarcinoma with signet cells. Immunohistochemistry profile was that of an adenocarcinoma. As there was no evidence of metastasis on staging workup, she was posted for surgery. On laparotomy, the entire small bowel was encased in a mucinous matrix. Frozen section of the material showed it to be positive for malignancy. A distal gastrectomy with a roux-en-y bypass procedure was done. The membrane holding the bowel together was removed to the extent possible to free the small bowel. The patient has now completed eight cycles of chemotherapy and is well till date.

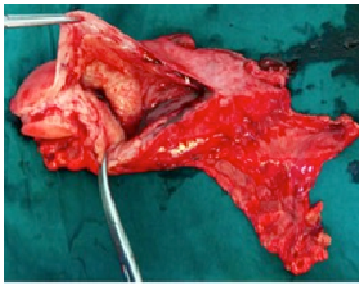


Fig. 1. Antropyloric Growth (Arrow)

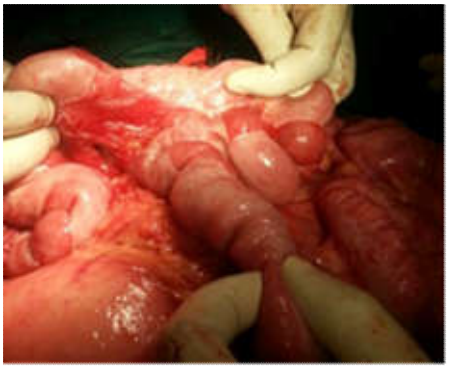


Fig 2. Small Bowel enclosed in a Mucinous Matrix (Arrow)

Patient 3 was a 25-year-old woman who presented with a mass in the epigastric region. A CT of the abdomen with oral and intravenous contrast showed that there was an extraluminal mass of probable gastric origin causing extraneous compression and gastric outlet obstruction. On upper GI endoscopy, as expected the stomach was distended and the duodenum could not be entered. There was a vague thickening in the pyloric region that was biopsied. The report, however, was inconclusive.

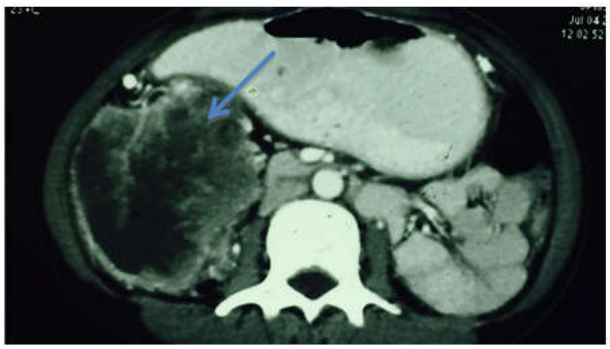


Fig 4: Fat planes with surrounding structures preserved

A core needle biopsy of the palpable mass was performed under ultrasound guidance. It was a poorly differentiated adenocarcinoma with neuroendocrine differentiation, as ascertained by immunohistochemistry.

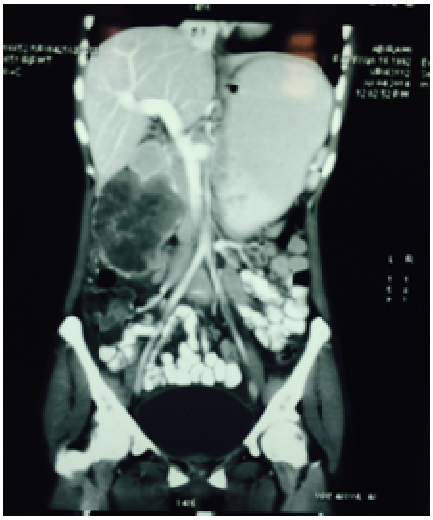


Fig 4: Fat planes with surrounding structures preserved

As there was no evidence of other metastasis and she had symptoms of gastric outlet obstruction, a laparotomy was done.

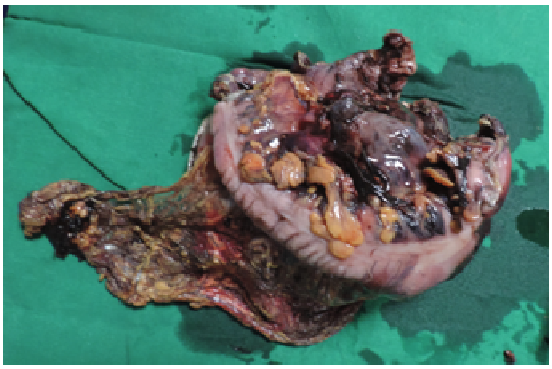
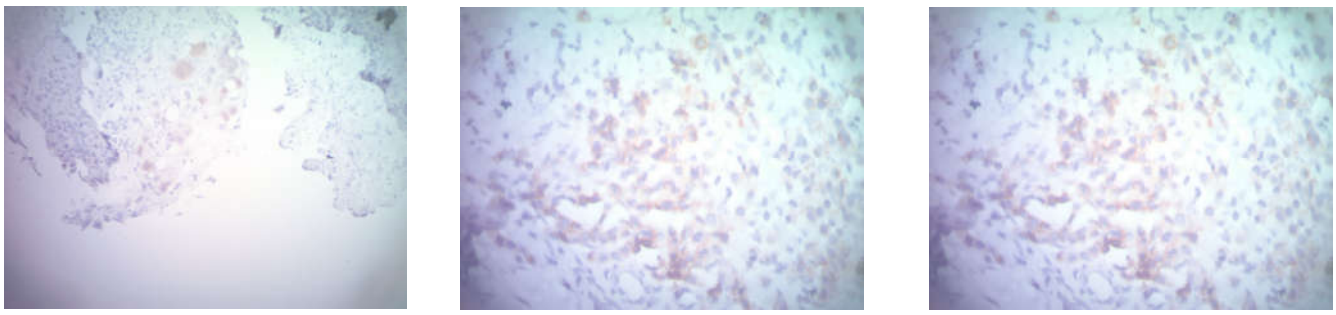


Fig. 5. The Resected Specimen (S: Subpyloric Node; T: Adherent Transverse Colon)

The palpable mass was determined to be an enlarged subpyloric node that was causing extrinsic compression of the stomach. The mid part of the transverse colon was found to be plastered to it, causing complete obliteration of the lumen. No growth was palpable within the stomach.

Table 1. Immunohistochemistry Profile (E Cadherin Normal Expression: +++)

	Cytokeratin 7 (CK 7)	Cytokeratin 20 (CK 20)	Synaptophysin	Ki-67	Her 2neu	E Cadherin
Patient 1	+	Focal +	-	50 – 60%	3+	+
Patient 2	+	-	-	>80%	-	++
Patient 3	+	-	+	50 – 60%	-	++



Patient 1: + **Patient 2: ++** **Patient 3: ++**

Fig. 6. E Cadherin Expression (Normal Expression: +++)

A gastrotomy was done, a biopsy was taken from a suspicious region and frozen section came back positive for malignancy. A distal gastrectomy with anterior gastrojejunostomy with resection of involved transverse colon and reestablishment of bowel continuity with side to side colo-colicanastomosis was done. Considering the age group of these patients, additional immunohistochemistry for epidermal growth factor receptor status and the expression of E Cadherin was done. In the first patient, E Cadherin expression was markedly reduced and there was increased Her 2 neu expression. The other two showed some decrease in E cadherin expression in comparison to normal gastric mucosa with no Her 2 neu expression.

DISCUSSION

Carcinoma stomach is implicated third among the leading causes of cancer-related death worldwide (Jemal *et al*., 2011). It is predicted that deaths from gastric cancer will rise from the 15th to the 10th cause of mortality from all causes globally by 2030 (Mathers and Loncar, 2006). Thus, new modalities of treatment are required at the earliest. The TNM stage,establishedby the depth of invasion of gastric wall (T), the involvement of lymph nodes (N) and the presence of distant metastasis (M), is the most important prognostic factor for gastric cancer. Prognosis, however, varies among patients in the same stage. Therefore, additional classification parameters need to be defined in addition to the TNM and the classic pathologic characteristics of the tumor in order to better identify the biologic subsets of this disease. According to the World Health Organization (WHO) and the Laurén classifications, there are two histological types, the diffuse gastric cancer and intestinal gastric cancer (Peleteiro *et al*., 2011). Intestinal gastric cancer is more associated with environmental factors such as infection of *H. pylori*, high salty diet, smoking, and obesity (Figueiredo *et al*., 2002 and Peleteiro *et al*., 2011), while diffuse gastric cancer is characterized by poorly differentiated cells and is more commonly observed in younger patients, with an obvious hereditary form. It has been reported that around 10% of the gastric cancer cases are familial clustering (Fléjou *et al*., 2011). The criteria for the diagnosis of hereditary diffuse gastric cancer (HDGC), proposed by the International Gastric Cancer Linkage Consortium (IGCLC):

- Two or more documented cases of diffuse gastric cancer in first/second degree relatives, with at least one diagnosed before the age of 50 or
- Three or more cases of documented diffuse gastric cancer in first/second degree relatives, independently of age (Paredes, 2012).

All three of our patients did not have family history of gastric cancer, and were thus the index cases in their families. Biological prognostic factors are often derived from the genetic process, which is thought to represent a crucial step to gastric cancer (HER2, E-cadherin, EGFR, DNA copy number changes, microsatellite instability, and changes in expression of several factors including thymidilate synthase, beta-catenin, mucin antigen, p53, COX-2, matrix metalloproteinases, and vascular endothelial growth factor receptor), (Correa, 1996). Some of these potential prognostic factors can also be predictive of response to therapy as they are a molecular target either to chemotherapeutics or to biologic/targeted therapies (Correa, 1996). Cytokeratin 7 (CK7) and Cytokeratin 20 (CK20) expression, tested by immunohistochemistry, is variable in the intestinal type, while 70% of hereditary diffuse gastric cancer show positive staining for CK7 and variable staining for CK20. Ki 67 is a proliferative index and has now become a prognostic indicator in most malignancies. This was also ascertained by immunohistochemistry. In our case, testing for Synaptophysin, a marker of neuroendocrine cells was only undertaken as the preliminary histopathological examination showed cells suspicious of such differentiation. This was done by immunohistochemistry as well.

There is mounting evidence of the role of increased HER2 expression in patients with gastric cancer, and it has been consistently correlated to poor outcomes and a more aggressive disease. A higher rate of HER2 expression in intestinal histologic type than in diffuse type has consistently been reported. Its expression is seen more in gastroesophageal junction tumours. Trastuzumab suppresses the growth of human gastric cancer with HER2 overexpression in vitro and in vivo. Patient 1 expressed Her 2 neu. The addition of Trastuzumab may have stemmed the aggressive nature of her disease. However, this testing was done posthumously. Studies suggest that germline mutations of *CDH1* gene and subsequent inactivation of the second allele of E-cadherin triggered by methylation, mutation, or loss of heterozygosity (LOH) leads to HDGC (Fléjou, 2011 and Gall and Frampton, 2013). The former is seen approximately 30% of HDGC. The *CDH1* gene locates in the human chromosome 16q22.1 and comprises 16 exons transcribed into a 4.5 Kb mRNA and encodes for E-cadherin (Takeichi *et al*., 1995). E-cadherin is a calcium-dependent cell-cell adhesion molecule playing a crucial role in establishing epithelial architecture and maintaining cell polarity and differentiation (Oliveira *et al*., 2009 and Valastyan *et al*., 2011). Aberrant splice variants or abnormal maturation of E-cadherin leads to downregulation of E-cadherin and contributes to human hereditary diffuse gastric cancer (HDGC). E-cadherin is pivotal in maintaining the epithelial architecture and cell polarity, while dysregulation of E-cadherin contributes to tumor invasion and progression (Vleminckx *et al*., 1991). In addition to its role in cell-cell adhesion, E-cadherin and the cadherin-catenin complex could modulate various signaling pathways in epithelial cells.

Although still at the preliminary phase, it has been pointed out that targeting alternative pre-mRNA splicing such as the aberrant splice variants or their resulting products are potential therapeutic targets for HDGC (Yagi and Takeichi, 2000). This will make personalized therapies possible. Studies have shown that high levels of soluble E-cadherin in serum 3 to 6 months after curative surgery could predict recurrence of gastric carcinoma (Richards *et al*., 1999). Individuals with familial diffuse gastric cancer should take *CDH1* genetic screening. Individuals without *CDH1* mutation should take clinical surveillance by upper GI endoscopy, while the ones with *CDH1* high risk missense mutations or truncating mutations was strongly recommended to take prophylactic gastrectomy and under close follow-up (Brooks-Wilson *et al*., 2004 and Barber, 2008). *H. pylori* infection is involved in promoter

hypermethylation of genes associated with the initiation and progression of gastric carcinogenesis (Nasri, *et al*., 2008). Methylation of *CDH1* has been reported to be regulated by *H. pylori* infection in chronic gastritis and intestinal metaplasia patients, indicating that E-cadherin plays an important role in gastric cancer initiation (David *et al*., 2012 and Rajasekaran *et al*., 2012). Importantly, eradication of *H. pylori* infection is able to reverse the hypermethylation status of *CDH1*, thus delaying or reversing *H. pylori* induced gastric carcinogenesis (Gofuku *et al*., 1998). In our patients, Her 2 neu and E cadherin expression were determined by immunohistochemistry. CDH1 mutation testing was not performed. While the first patient had an aggressive course and died within a month of diagnosis, the other two remain well and on regular follow-up. The variations in prognosis within the same AJCC stage make it clear that such staging is inadequate to determine treatment and predict prognosis. Since Her2 neu testing is readily available, and we have a drug that has been proven to be effective in the ToGA trials, it should be made a part of routine initial work up of all gastric cancer patients. E cadherin testing, also can similarly be used to predict prognosis and pinpoint individuals who require CDH 1 mutation testing and screening for family members.

Conclusion

When young patients present with advanced gastric malignancies, we have to go one step beyond the traditional workup. A thorough understanding of the demographic profile of these individuals along with possible family history will help better understand the context of this disease. Hence molecular profiling is vital, both in aiding treatment, for predicting amenability to chemotherapy and radiation and for prognostication. Further, counseling of other family members regarding their risk for developing the same or associated malignancies becomes possible.

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