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Gastric Carcinoma with Lymphoid Stroma: Association with Epstein Virus Genome demonstrated by PCR

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Introduction

Gastric carcinoma with lymphoid stroma is a rare and well known entity. Histologically tumour is extremely cellular infiltrated by fairly large number of lymphocytes. The infiltrate can be so dense that a mistaken diagnosis of lymphoma may be made on endoscopic biopsy or even in resection specimen. The entity is known by various names, which include medullary carcinoma with lymphocytic infiltration of the stomach, blue cell cancer, lymphoepithelial carcinoma of stomach or gastric carcinoma with lymphoid stroma¹.

Association of this type of gastric carcinoma with Epstein Barr Virus is well known². In a study from Japan, EBV sequence was detected in 81 out of 99 cases. Whereas only 21 out of 42 ordinary adenocarcinoma showed this sequence³. We describe a case of gastric carcinoma with lymphoid stroma in a female. We were able to detect Epstein Barr Virus DNA by polymerase chain reaction in this case.

Case Report

A 55 years old female presented with signs and symptoms of gastric outlet obstruction. This was due to a pyloric growth for which a complete gastrectomy was performed. The specimen received was a stomach which on opening revealed an ulcerated tumour measuring 5 x 3 cms involving full thickness of the wall. Tumour was close to one of the excision margins i.e., within 0.2 cm. Sections were taken from the lesion. These 5 mm sections were stained with hematoxylin and eosin.

Histology revealed an infiltrative lesion formed predominately by lymphoid cells. Between these cells there was a scattered population of round to oval cells with scanty cytoplasm. In some areas there was a nesting pattern. Mitotic activity was variable. The stroma was non-desmoplastic. Tumour involved full thickness of the wall and three out of six recovered lymph nodes showed evidence of metastasis.

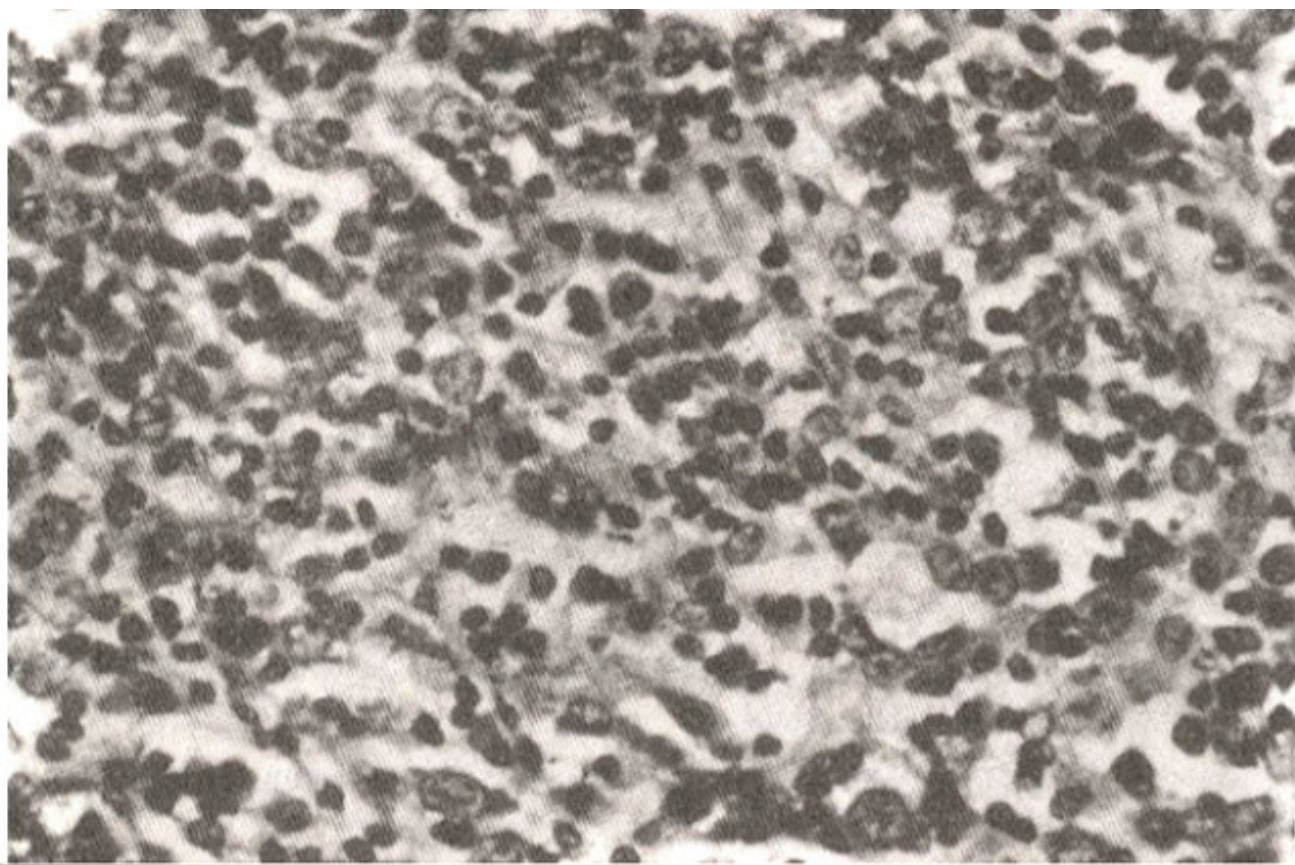


Figure 1. Section shows a dense infiltrate of mononuclear cells with prominent nucleoli. These are obscured by accompanying lymphocytes and plasma cells (hematoxylin and eosin stains x 400).

The differential diagnosis of this tumour was a gastric carcinoma with lymphoid stroma and a non-Hodgkin's lymphoma. Immunohistochemistry using peroxidase antiperoxidase method was attempted. There was a dense mixed population of B and T lymphocytes. Majority of these were of T-phenotype, although lymphoid follicles contained B cells.

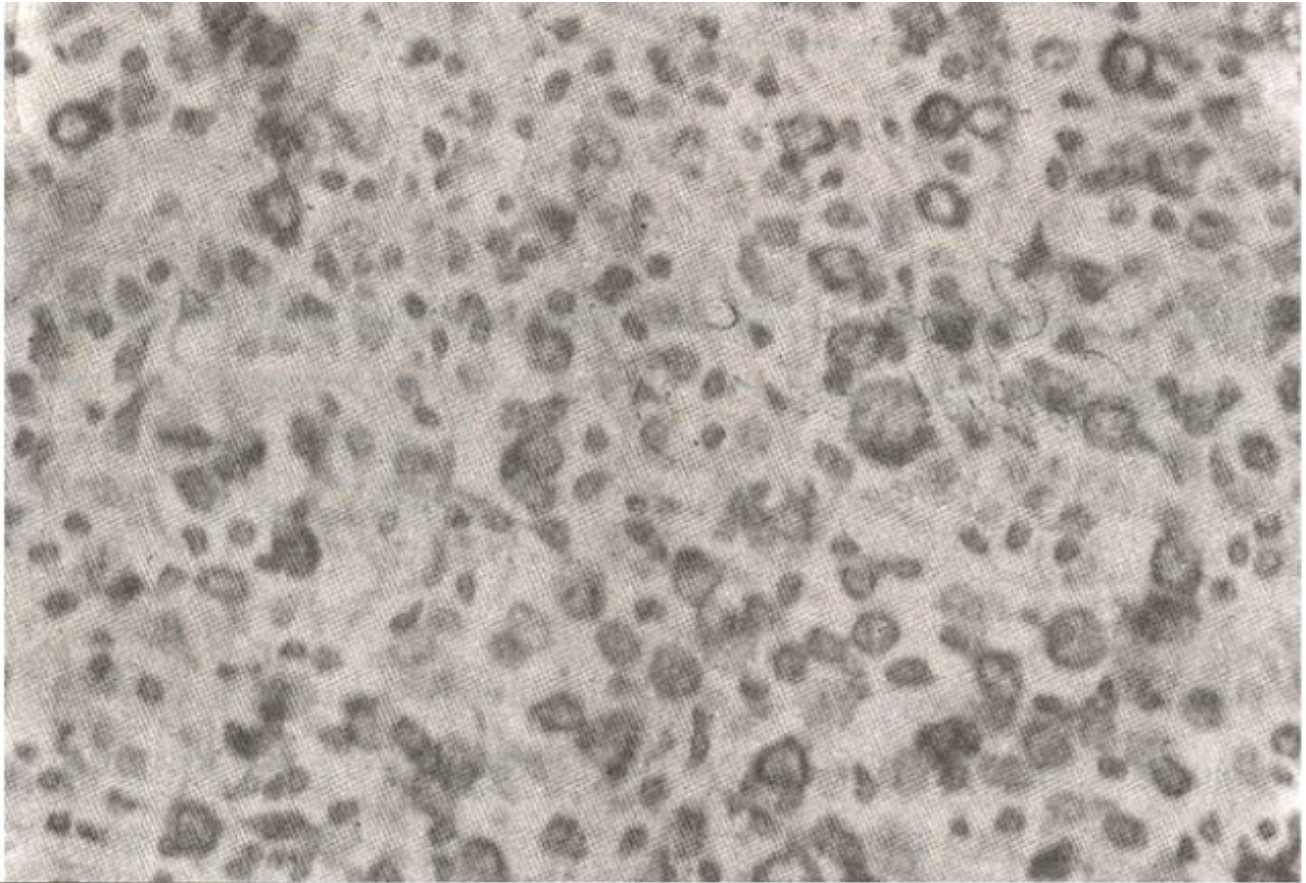


Figure 2. Tumor cell cytoplasm is strongly decorated by cytokeratin marker AE1/AE3. Unstained cells were stained by leukocyte common antigen L26 (Pan B marker) and UCHL1 (Pan T marker). Peroxidase anti peroxidase method x 400.

However, there were a number of tumour cells morphologically similar to lymphoid cells, which were reactive for both cytokeratin antibodies. Mucin stains PAS and alcian blue were equivocal. On the basis of cytokeratin positivity a diagnosis of gastric carcinoma with lymphoid stroma was made. In view of well known association of this tumour with Epstein Barr Virus, we explored this lesion further using polymerase chain reaction method.

Paraffin embedded tissue sections of 5 mm were deparaffinized and dried. DNA was extracted by Nucleon DNA Extraction Kit (Nucleon Biosciences, USA). PCR assay was carried out according to the method described⁴. Primer pairs specific for gp220 region of EBV genome were synthesized by using an Applied Biosystems DNA synthesizer.

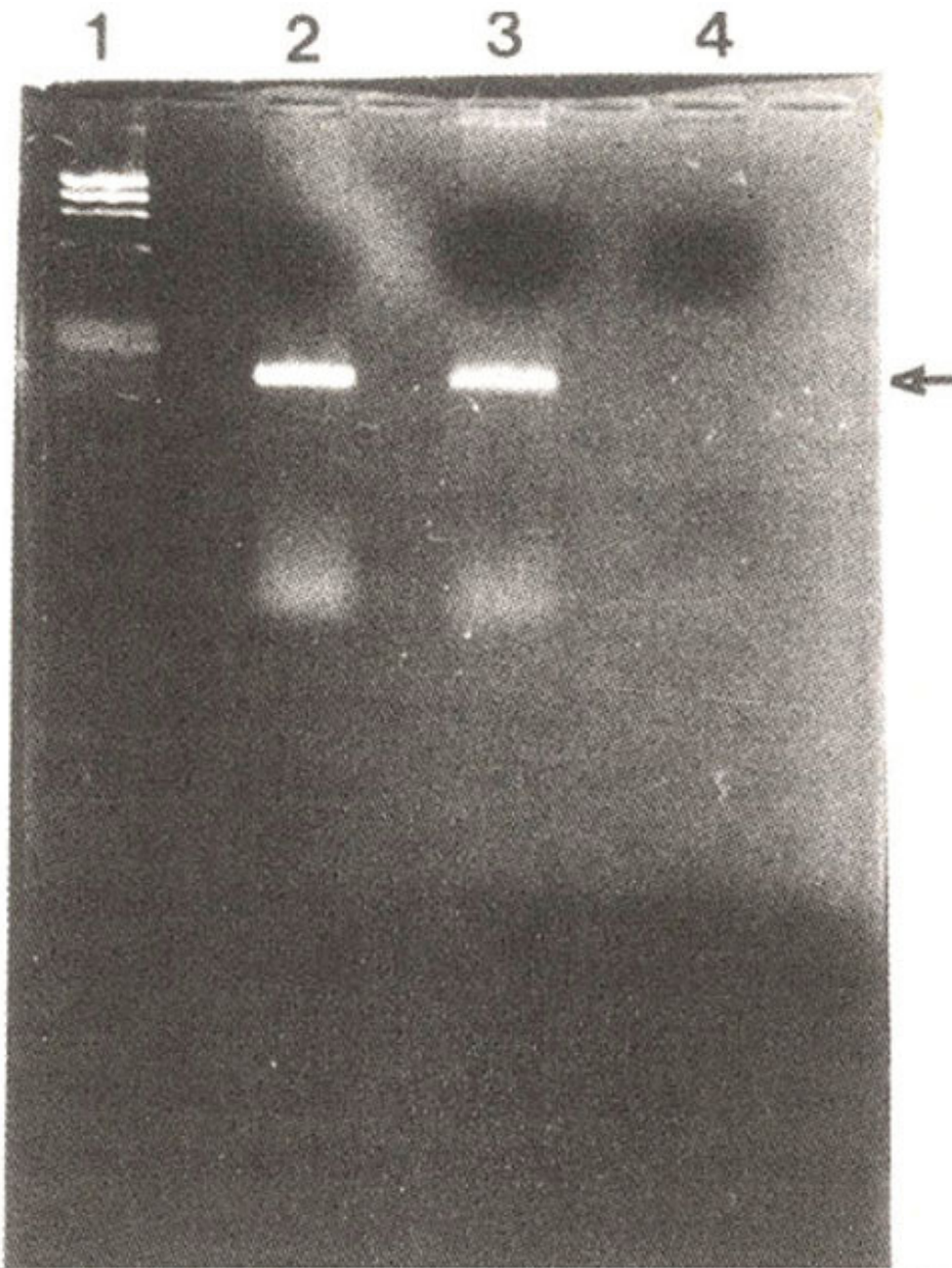


Figure 3. A representative photograph of an agarose gel stained with ethidium bromide showing PCR amplified products of Epstein Barr Virus DNA with gp220 specific primers. Lane 1: DNA markers (fX 174 DNA digested with Hae III), Lane 2: DNA from gastric carcinoma, Lane 3: DNA of EBV positive Raji cell line, Lane 4: Negative control DNA. The arrow points to 239 base pair product.

The PCR was performed in a Perkin Elmer 9600 thermocycler. DNA was added to the PCR mixture which contained, reaction buffer (10mM Tris pH 7.4, 50mM KCl and 1.5 mM MgCl), 200 mM each of dNTPs, 0.5 U Taq polymerase, 200 ng of each primer in a final volume of 100 ml. After initial denaturation at 94°C, 45 cycles of amplification were performed at 94°C for 1.30 min, 53°C for 1.30 min and extension at 72°C for 1.30 min. The amplified product was subjected to electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. An amplified product of 239 bp was suggestive of EBV DNA. Both positive and negative controls were used with each assay. Immunocytochemistry was done by three step peroxidase antiperoxidase method. Primary antibody

was laid for 90 minutes, followed by secondary antibody and PAP complex for 45 minutes each. Color was developed by diaminobenzidine. Monoclonal antibodies used included cytokeratin CAM 5.2, AE1/AE3 and lymphoid markers LCA (CD45), Pan B (L26) and Pan T (UCHLI).

Discussion

Gastric carcinoma with lymphoid stroma is a relatively rare entity. In a series of 867 cases only 27 cases were found¹. In another study of 8457 gastric carcinoma there were 132 cases³. Watanabe H⁵ et al described 49 cases in a series of 1041 cases. Significantly favourable prognosis of gastric carcinoma with lymphoid stroma in comparison to ordinary carcinoma requires accurate classification and may not be possible without immunocytochemistry in some cases³. It is thought that infiltrating lymphocytes play a role in immune surveillance against invading tumour cells and hence long term survival. In this case though EBV DNA was detected by polymerase chain reaction, but are being carried out to determine the location of EBV DNA by in-situ hybridization.

The Epstein Barr Virus is associated with a variety of lymphoproliferative disorders such as Burkitt's lymphoma, Hodgkin disease, T cell lymphoma alongwith epithelial malignancies including nasopharyngeal carcinoma, lymphoepithelioma as carcinoma of the salivary glands, lung and thymus. Both ordinary gastric adenocarcinomas and carcinomas with lymphoid stroma are known to have EBV sequences. However, this association remains significant in the latter category in stomach. Oda et al⁶ showed EBV DNA and RNA within the nuclei of carcinoma cells by in-situ hybridization and polymerase chain reaction respectively. Infiltrating lymphocytes and normal epithelium adjacent to tumour were EBV negative. Southern Blot analysis indicated clonal proliferation of tumour cells and episomal form of EBV. The conclusion was that EBV infection occurs before transformation and may be related to oncogenesis of EBV associated gastric carcinoma.

The mechanism of an abundant lymphocytic infiltration in GCLS could be a direct response either to the virus or to the virally induced antigens expressed in the neoplastic cells⁷. Immunohistochemical studies as in our case have confirmed presence of both T-cell and B-cell populations as shown by Minamoto et al¹. T-cell distribution was even throughout the tumour particularly adjacent to carcinoma cell nests. B-cells however, were sparsely scattered in the tumour. However, the main cellular component of lymphoid follicles were B-cells.

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