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Comparison between Conventional Gastric Adenocarcinoma and EBV Associated Gastric Carcinoma

A. R. Arshad[1*], M. R. Rajashekar[1] and N. Srinivasan[1]

[1]Department of Orthopaedics, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Epstein Barr Virus (EBV or Human herpesvirus 4) belongs to the genus Lymphocryptoviridae, the gamma 1 subtype of the Subfamily Gamma herpes viridae and is one of the most common viruses in humans. It is present in all populations, infecting more than 95% of all individuals within the first four decades of life. In developing countries, infections occur very early in life with no specific characteristics other than the general symptoms of acute viremia. In developed countries however, the infection is usually delayed until adolescence or early childhood years where it causes infectious mononucleosis, a benign self-limiting lymphoproliferative disorder. Though the infection with EBV is benign in the acute stages and latent in the chronic phase in the vast majority of people, the virus has been demonstrated to be involved in the development of many malignancies with the list of such malignancies progressively increasing. The first association was with the endemic Burkitt's lymphoma. Subsequently, other lymphomas (subtypes of Hodgkin's and non-hodgkin's lymphomas) are also known to be associated with EBV infection. Epithelial malignancies such as lymphoepitheliomas of nasopharynx and stomach are included in the list of EBV associated tumors. Tumors arise as a result of genetic and epigenetic alterations produced by the virus, which transforms the normal cell into an immortalized proliferating cell. Since Burke et al first

**Corresponding author: E-mail: deanpublications@bharathuniv.ac.in;*

detected EBV in undifferentiated lymphoepithelioma like gastric cancer in 1990, many researches are undertaken to prove the same. EBV expresses latent membrane protein which can be detected immune histochemically. Our study is aimed at detecting the EBV expression in gastric carcinoma cells.

Keywords: Gastrology; carcinoma; immuno-histology; malignancy.

1. INTRODUCTION

Epstein Barr virus (EBV), also called Human Herpes Virus 4 (HHV-4), is a member of the Herpesviridae family. It is ubiquitous in nature and infects more than 90% of adult population worldwide. Primary infection normally occurs in childhood or early adulthood through salivary contact. Majority of children are asymptomatic, but some adolescents and young adults can develop infectious mononucleosis with harmless clinical manifestations [1]. EBV is composed of a linear double-stranded DNA genome surrounded by an icosahedral nucleocapsid which encodes about 85 genes. EBV is divided into 2 major types, EBV-1/EBV-A and EBV-2/EBV-B. Worldwide EBV-1/EBV-A is the most frequent type while EBV-2/EBV-B is more characteristic in Africa [2].

2. GASTRIC CARCINOMA

2.1 Epidemiology

Gastric cancer (GC) is the sixth most common malignancy in both sexes worldwide with nearly one million new cases estimated in 2012 (952.000 cases). The incidence rates are twice higher in males than in females. Nevertheless, age standardized rates (ASR) show that carcinoma of stomach is the fourth most frequent in men and the sixth in women. Despite a significant reduction of incidence and mortality rates over the past few decades, GC still remains the third leading cause of death by cancer. Approximately, 8% of cancer-related mortality in world is attributed to tumors of stomach. It is a major health problem with a distinct distribution according to geographical areas, socio-economic conditions and ethnic diversity. More than 70% of total cases occur in developing regions being the Eastern Asia (half cases in China), Eastern Europe and Latin America the areas with the highest age-standardized incidence rates. In contrast, the lowest incidence rates are observed in United States, Australia and some North European countries [3].

2.2 Classification

Gastric tumors are classified anatomically and histologically. Anatomically, GC is divided into proximal and distal tumors depending on their localization of stomach. Proximal tumors are found in cardia region whereas distal carcinomas are often located in the antrum/pyloric region. Histologically, tumors of stomach show high heterogeneity at both architectural and cytological level that makes it difficult for the establishment of well-defined classification system. Some classifications have been established to classify the histologic pattern of gastric adenocarcinomas: Ming, Carneiro and Goseki, but the most commonly used are those of World Health Organization (WHO) and Lauren [4].

Lauren's classification is an essential system in gastric cancer history that over time have contributed to describe an association with several environmental factors, incidence trends and etiology. According to this classification, the two major histologic subtypes are intestinal and diffuse adenocarcinomas. The other types are classified to indeterminate type, when carcinoma is too undifferentiated and co-exist histological features, or uncommon variants. The relative frequencies are approximately 54% for intestinal type, 32% for the diffuse type and 15% for the indeterminate type. In 2010, WHO referred five subtypes that have been correlated with Lauren's classification as described in Table 1 [5].

Lymphoepithelioma-like carcinomas or medullary carcinomas are described by WHO as an uncommon subtype but are not represented in the Lauren's classification. This specific tumor, which is characterized by uniform proliferation of cancer cells throughout the lymphoid stroma, represents about 4% of all gastric carcinomas and more than approximately 80% of cases have EBV-infected cells.

3. MATERIALS AND METHODS

3.1 Source of Data

This prospective study was carried out in the Department of Pathology, Sree Balaji Medical College and Hospital, with the help of Department of Medical Gastroenterology, Sree Balaji Medical College and Hospital, during October 2016 to September 2018. A total of 43 cases suspected with gastric malignancy were taken for the study. Out of which, only 30 were proven to be malignant. So, only these 30 cases were included in the study.

Table 1. Comparison of gastric cancer classifications between WHO and Lauren's Classification systems

WHO (2010)	LAUREN (1965)
Papillary adenocarcinoma	Intestinal type
Tubular adenocarcinoma	
Mucinous adenocarcinoma	
Signet-ring cell carcinoma	Diffuse type
Poorly cohesive carcinoma	
Mixed carcinoma	Indeterminate
Uncommon variants	-

3.2 Inclusion Criteria

All cases of gastric malignancy detected by histopathology irrespective of age were included for study.

3.3 Exclusion Criteria

Those with poor clinical data were excluded from the study. Proven cases of gastric malignancy due to the non-availability of the blocks (blocks that were taken for treatment purpose and second opinion) were excluded from the study.

3.4 Method of Data Collection

Out of the 43 cases, 32 cases were proven to be malignant, out of which 30 cases had adequate clinical data were included in the study. Those materials were processed and sections were cut at 5 microns. Hematoxylin and eosin staining of sections was done. Histopathological examination of these sections were done.

LMP-1 immunohistochemical marker was used to demonstrate EBV in tissue sections.

3.5 IHC Markers Used

Latent membrane protein-1 (CS1-4 antibody) was used in all the gastric carcinoma cases. Section of nasopharyngeal carcinoma was taken as control for LMP-1. Immunostaining was scored on the basis of positive tumor cells and the relative immunostaining intensity. Five consecutive microscopic fields were analyzed.

4. RESULTS

This study was conducted in Sree Balaji medical college, Chennai, India. A total of 30 gastric carcinoma diagnosed over a period from 2016-2018 was selected. Out of which, 19 patients were male (63%), and 11 patients were female (37%) with a sex ratio of 2:1. The mean age of patients was 58 years (range 29 years to 80 years).

Majority of cases belong to age group 61-80 years.

4.1 Sex Distribution

Out of 30 cases 19 were male and 11 were female.

Table 2. Age distribution of gastric carcinoma

Years	No. of cases
10 - 20	0
21 - 30	1
31 - 40	1
41 - 50	7
51 - 60	9
61 - 80	12
Total	30

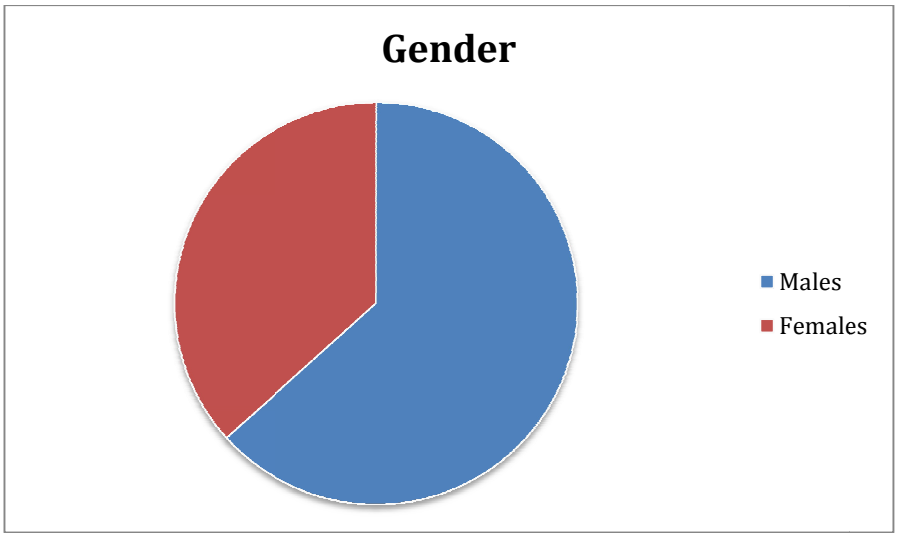

Fig. 1. Sex distribution

Common symptoms:

Table 3. Common symptoms

Common symptoms	Percentage
Upper abdominal pain (Epigastric pain)	59%
Loss of weight	31%
Nausea and vomiting	5%
Loss of appetite (cachexia)	3%
GI Bleeding (Malena)	2%

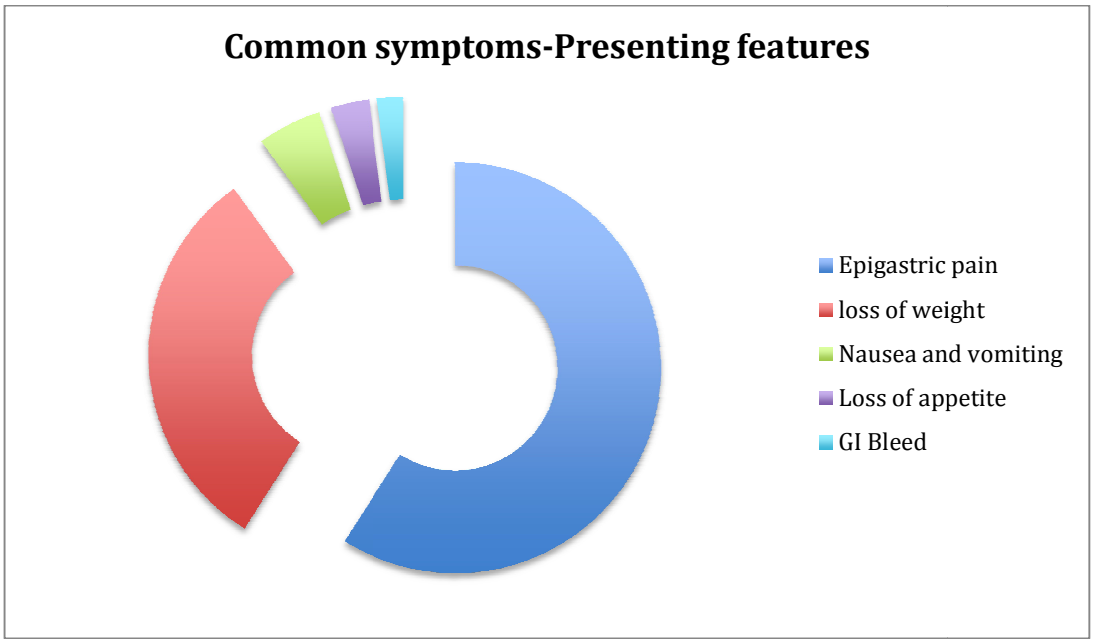

Fig. 2. Common symptoms

4.2 Pathological Features

4.2.1 Tumour location

In 18 (60%) cases, the tumor was located in antrum/pylorus. In 4 (13%) cases the tumor was found in body of the stomach, and in 8 (27%) cases in the fundus/corpus of the stomach.

Table 4. Tumor location

Location	No. of cases
Antrum/pylorus	18
Body of the stomach	4
Fundus/corpus of the stomach	8
Total	30

4.2.2 Tumour type

WHO 2012 classification: According to the WHO classification, of the 30 cases of gastric carcinomas, 17 were classified as tubular adenocarcinoma, 8 cases as poorly cohesive carcinoma, 5 were mixed adenocarcinoma and 1 case was diagnosed to be carcinoma with lymphoid stroma.

Table 5. Tumor type according to WHO 2010 classification

Types	No. of cases
Tubular adenocarcinoma	17
Poorly cohesive carcinomas	08
Mixed adenocarcinomas	04
Carcinoma with lymphoid stroma	01
Total	30

4.3 According to Lauren Classification

Based on Lauren classification of gastric carcinoma, 23 were intestinal type carcinomas, 4 were diffuse type carcinomas, 2 were indeterminate type and 1 was lymphoepithelioma like carcinoma.

4.4 EBV Immunohistochemistry Results

Out of 30 gastric carcinoma cases, 02 showed EBV expression. Both these patients were male and aged above 65 years.

Latent membrane protein-1 expression in tumor cells, studied using anti LMP-1 antibody immunohistochemistry was assessed using a scoring system based on the percentage of positive cells and the intensity of staining (commonly used in nasopharyngeal carcinomas).

Table 6. Tumor type according to lauren classification

Types	No. of cases
Intestinal type carcinoma	23
Diffuse type carcinoma	04
Indeterminate type carcinoma	02
Lymphoepithelioma like carcinoma	01
Total	30

Table 7. EBV expression in different histological types (Based on WHO 2010 Classification)

Histological types	EBV expression
Tubular adenocarcinoma type	0
Poorly cohesive adenocarcinoma type	01
Mixed adenocarcinoma type	0
Carcinoma with lymphoid stroma	01
Total	**02**

Table 8. Based on Lauren classification

Histological type	EBV expression
Intestinal type adenocarcinoma	01
Diffuse type adenocarcinoma	0
Indeterminate type adenocarcinoma	0
Lymphoepithelioma like carcinoma	01
Total	**02**

Scoring Method

- 0 - none seen in the section
- 1 - presence of rare positive cells but not exceeding 25%
- 2 -26 to 50% positive cells
- 3 -51 to 75% positive cells
- 4 -76 to 100% positive cells

Immunostaining Intensity

- 0 -none
- 1 -weak
- 2 -moderate
- 3 -intense

Table 9. Details of LMP-1 positive cases

S. no.	Age/Sex	HPE (WHO)	HPE (LAUREN)	SITE	EBV (LMP-1)
1.	80/M	Carcinoma with lymphoid stroma	Lymphoepithelioma like carcinoma	Fundus/Corpus	Positive
2.	77/M	Poorly cohesive adenocarcinoma	Intestinal type carcinoma	Fundus/ Corpus	Positive

Table 10. LMP-1 immunohistochemistry scoring

Age/Sex	Tumor grade	Staining intensity (A)	Percentage of LMP-1 Positive cells (B)	Total score (A + B)
80/M	Undifferentiated carcinoma	weak to moderate (2)	Positive cells not exceeding 25% (1)	2 + 1 (3)
77/M	Poorly differentiated carcinoma	Moderate (2)	Positive cells not exceeding 25% (1)	2 + 1 (3)

Both the LMP-1 positive cases in our study were observed to have a score of 3.

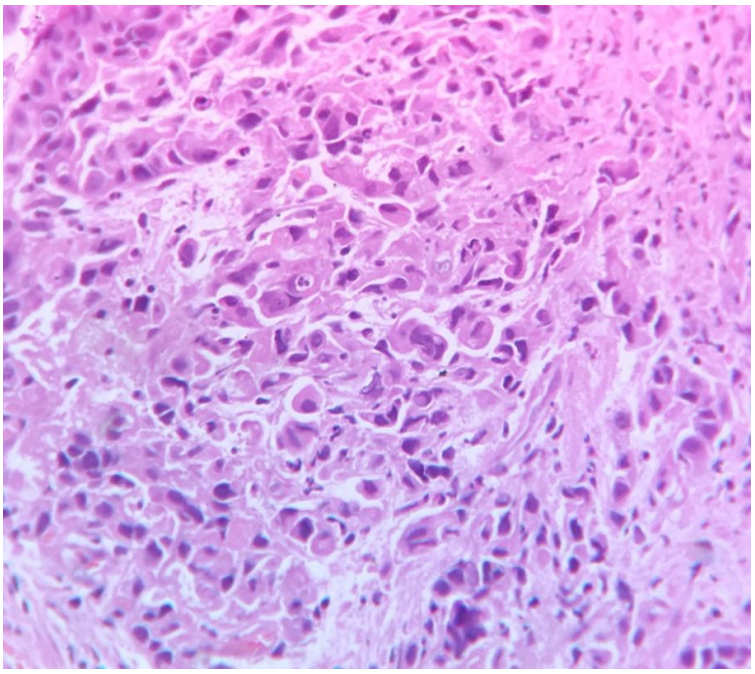

Fig. 3. Shows poorly cohesive type adenocarcinoma

5. DISCUSSION

Gastric carcinoma is a serious public health problem worldwide with high rates of mortality. GLOBOCON 2012 statistical data revealed about 951,600 newly diagnosed gastric cancer cases. In 2012 deaths due to gastric carcinoma worldwide was around 720,000 [6]. The symptoms and sign of the stomach cancer are often reported late when the disease is already in advanced stages and 5-year survival is less than 30% in developed countries and around 20% in developing countries. This indicates the need for an early diagnosis and treatment strategies to improve the survival. The present study assess the age distribution, sex distribution, relationship between EBV and sporadic Indian GC and the role of latent membrane protein -1 in GC detection.

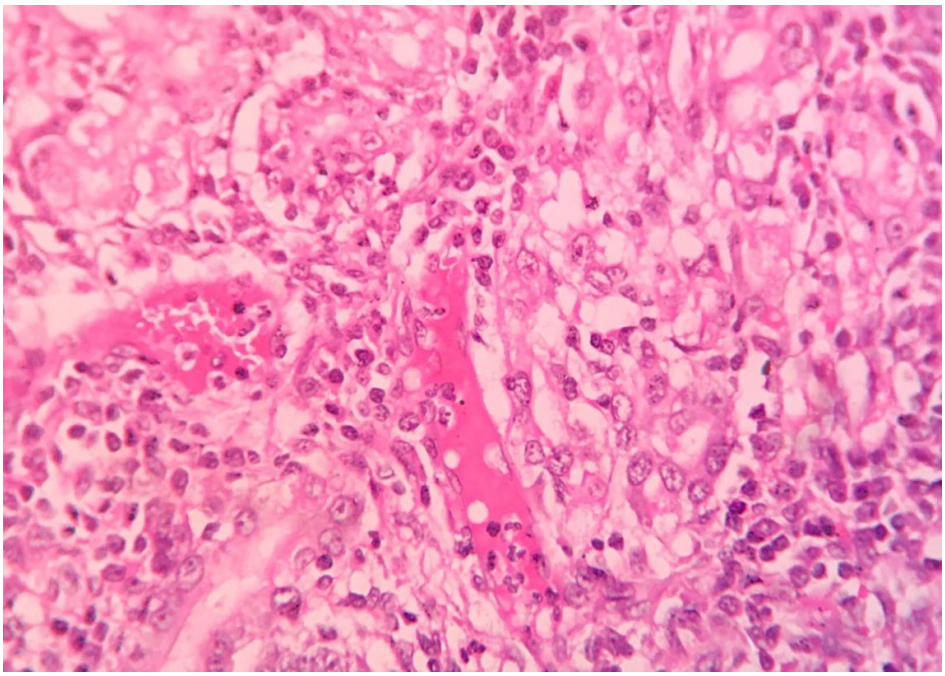

Fig. 4. Shows carcinoma with lymphoid stroma

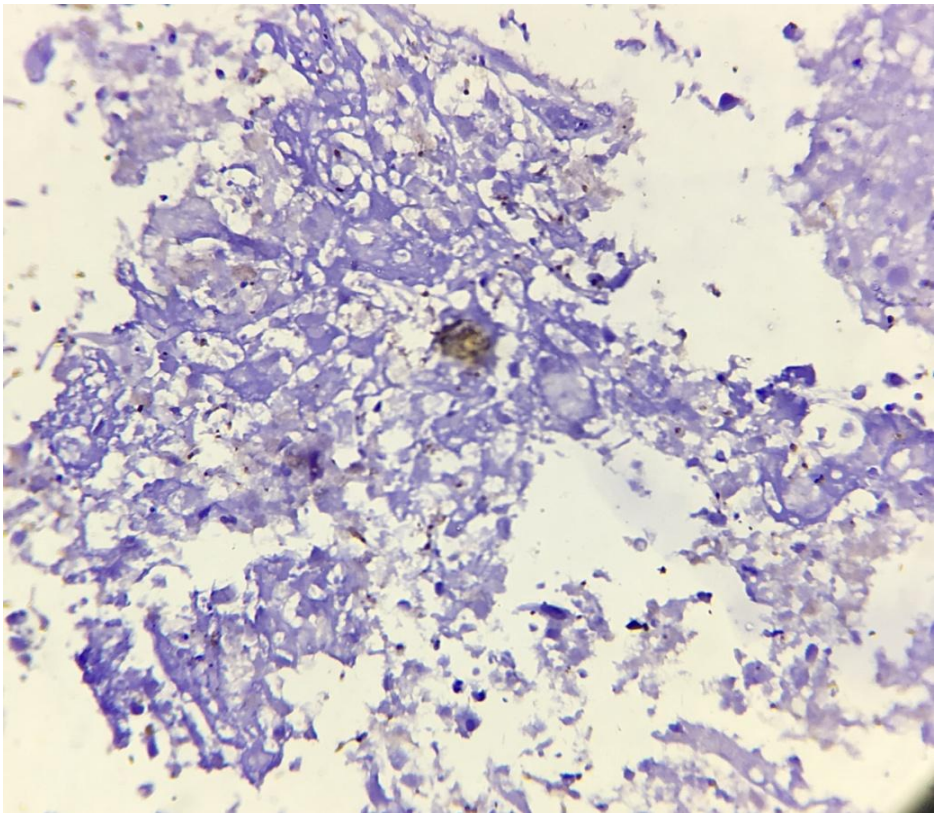

Fig. 5. LMP 1 immunostaining (moderate Intensity)

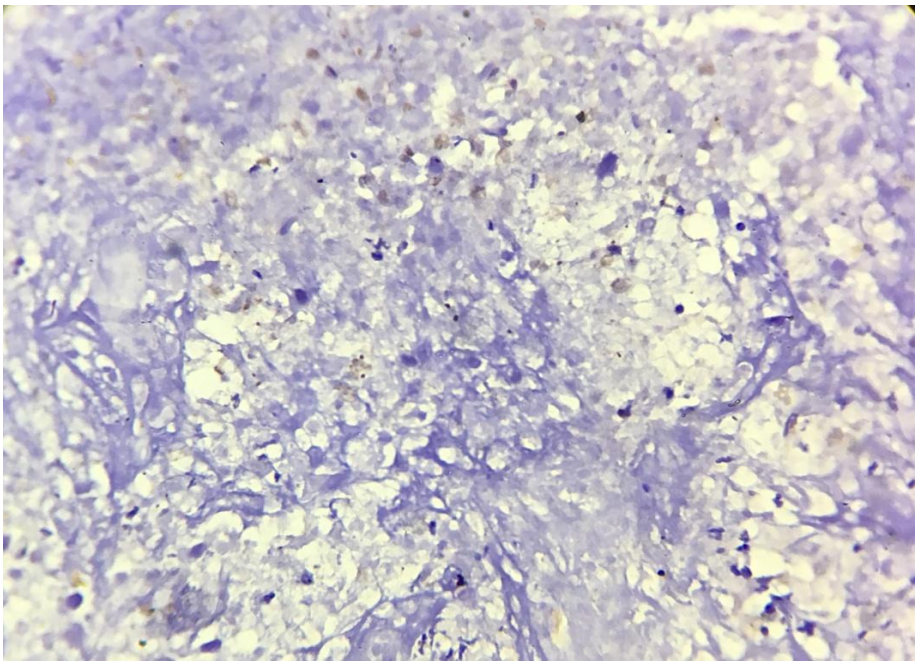

Fig. 6. LMP 1 immunostaining (weakly positive)

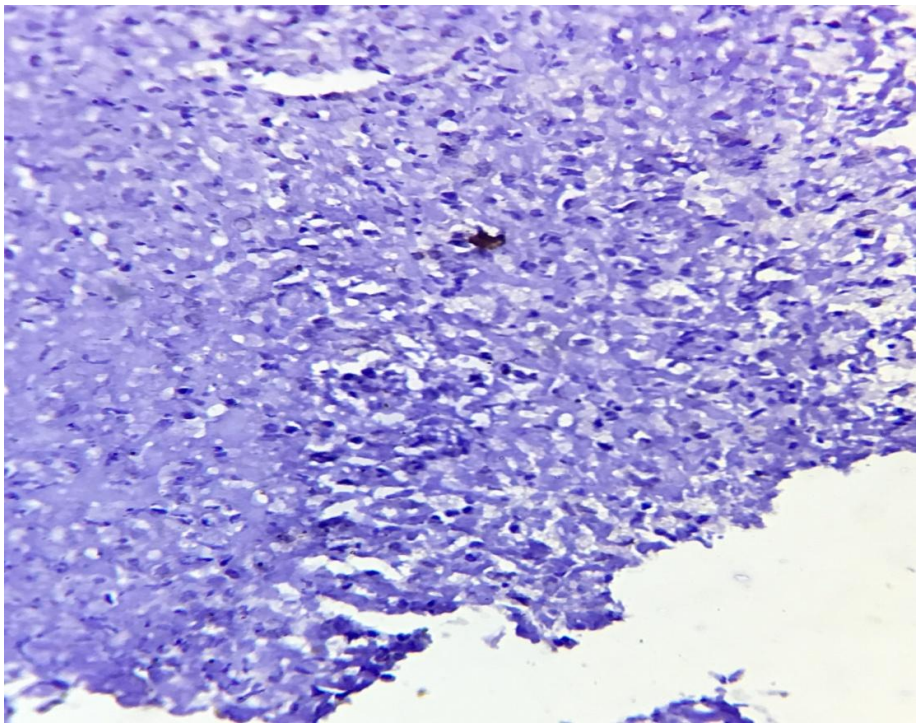

Fig. 7. LMP 1 positive (Intense Staining)

In general, our study showed male predominance accounting 64% of all GC cases and the mean age was of 58 years old suggesting that gastric cancer appear more often in older individuals. These finding are in agreement with literature which also has

described the occurrence of gastric carcinomas in male and older patients. Most of the patients in this study, presented with upper abdominal pain (62.5%) and loss of weight (30%). Others (7.5%) presented with nausea, vomiting and upper GI bleed [7].

Out of 30 cases, 17 were tubular adenocarcinoma, 8 were poorly differentiated carcinoma, 5 were mixed carcinoma and 1 was carcinoma with lymphoid stroma (according to WHO classification).23 were intestinal type carcinoma, 04 were diffuse type carcinoma, 02 were indeterminate type carcinoma and 1 was lympho epithelioma like carcinoma (according to Lauren's classification).

Over past the 30 years, there have been described a new subset of gastric cancer, EBVaGC. In fact, about 10% of all GC have been associated with EBV infection however, the role of EBV in gastric carcinogenesis remains unclear. Recently, two studies suggested a new classification based on molecular features of gastric tumors and in these classifications arise a new four subtypes of gastric cancers: tumors positive for Epstein–Barr Virus, microsatellite unstable tumours, genomically stable tumors and tumors with chromosomal instability.

To confirm, the association of EBV in a given tumor, the virus must be detected with in the tumor cells. As per literature LMP-1 potentiates a variety of signaling pathways including the nuclear factor kb, Mitogen activated protein kinase, and phosphotidylinositol 3 –Kinase Alt pathways and involved is angiogenesis which is a key step in tumor growth, invasion and metastasis .

So the presence of EBV in gastric carcinoma cells can be confirmed by the presence of LMP-1 staining. Immunohistochemistry was done with latent membrane protein- 1 and it was found that LMP-1 was positive in 2 out of 30 gastric carcinoma cases.

This study showed that the prevalence of EBV in gastric tumors is of 6.6%. These findings are in agreement with previous studies. Studies have demonstrated that high EBV-positive rate has been found in low-incidence area and low EBV-positive rate has been found in a high gastric-cancer incidence area. Sousa, et al. (2008) in a systematic review demonstrated that North of America (region with low prevalence in GC) has shown an association between EBV and GC of 12.9%; conversely, in regions with a high risk for GC (Asia), it was demonstrated that EBVaGCs accounted only 7.99% of all gastric cancers [6].

The same relationship is verified in our study since we observed a low prevalence of EBVaGC (6.6%), a considered country with high incidence of GC. Considering other risk factors for GC development, we found that male predominance is also a strong characteristic of EBVaGC. Regarding age distribution of patients with EBVaGC, it is yet little understood. In present study EBV-positive cases were observed in patients over than 65 years. Regarding the tumor location, we observed that in our study there was a high predominance of gastric tumors in the distal region (60%). Curiously, this is the anatomic location with lower prevalence of EBV. As previously reported, the presence of EBV has been mostly associated to body, and cardia region of stomach. Hence, our results which also showed a higher prevalence of EBVaGc in proximal regions, may explain the lower prevalence of EBVaGC in our study.

Histology-specific analysis of EBVaGC using Lauren's classification has shown controversial data. Chang et al. (2001) and Corvalan et al. (2001) demonstrated a strong EBV association with diffuse types, however Yoshiwara et al. (2005) described an equal proportion between intestinal and diffuse types. In our study, EBVaGC was only found in intestinal-types and lymphoepithelioma like carcinoma types without any case reported to diffuse-type [8].

Regarding the lymphoepithelioma-like carcinomas (LLCs) it was observed that all samples showed positivity for EBV. These findings are in agreement with literature which has described that more than 80% of LLCs are associated with EBV infection. Despite the low frequency of LLCs (about 4% of all gastric carcinomas), the pathologists should distinguish this subset of gastric cancer because it has been demonstrated that patients have a better prognosis when compared with other types of gastric cancer.

Available literature on EBV positive gastric carcinomas have not used the scoring system, generally employed in nasopharyngeal carcinomas. However, we attempted to use the scoring system and it was observed, that the scores were low, which is in consistent with the literature studies on nasopharyngeal carcinomas,

which have also shown low scores in older age group and high scores in younger age group.

Abdel Majiid Khabir et al. observed in his study that no biopsy is completely devoid of LMP-1 positive cells and he also suggested the use of S12 antibody which is more sensitive in staining tissue section than CS1-4 antibody. In this present study we used CS1-4 antibody for detecting the presence of EBV in tissue sections.

Despite the limitations of LMP-1, its simplicity, applicability to paraffin sections and its use as an indicator of progressiveness of the tumor has made it an attractive ancillary method for early diagnosis of EBV associated gastric carcinoma.

6. CONCLUSION

Hence our study justifies the role of EBV in the oncogenesis of gastric carcinoma. More elaborate and extensive studies are warranted to further emphasize this theory. Days would not be far off for a targeted therapy and an effective vaccine for EBV that would prevent primary infection or modulate its course leading to the reduction in the incidence of EBV associated gastric carcinoma, similar to the reduction of cervical cancer and hepatoma after HPV vaccine and hepatitis vaccine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Henle G, Henle W, Diehl V. Relation of Burkitt's tumor-associated herpes-ytpe virus to infectious mononucleosis. Proceedings of the National Academy of Sciences. 1968;59(1):94-101.
2. Butel JS. Viral carcinogenesis: Revelation of molecular mechanisms and etiology of human disease. Carcinogenesis. 2000; 21(3):405-26.
3. Burke AP, Yen TS, Shekitka KM, Sobin LH. Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. Modern pathology: An official journal of the United States and Canadian Academy of Pathology, Inc. 1990;3(3): 377-80.
4. Grotto I, Mimouni D, Huerta M, Mimouni M, Cohen D, Robin G, Pitlik S, Green MS. Clinical and laboratory presentation of EBV positive infectious mononucleosis in young adults. Epidemiology & Infection. 2003; 131(1):683-9.
5. Roy P, Piard F, Dusserre-Guion L, Martin L, Michiels-Marzais D, Faivre J. Prognostic comparison of the pathological classifications of gastric cancer: A population-based study. Histopathology. 1998;33(4):304-10.
6. Hu B, El Hajj N, Sittler S, Lammert N, Barnes R, Meloni-Ehrig A. Gastric cancer: Classification, histology and application of molecular pathology. Journal of Gastrointestinal Oncology. 2012;3(3):251.
7. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 2015; 136(5):E359-86.
8. Bittar Z, Fend F, Quintanilla-Martinez L. Lymphoepithelioma-like carcinoma of the stomach: A case report and review of the literature. Diagnostic Pathology. 2013; 8(1):184.

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