Study of EBV and Associated Gastric Malignancies

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Authors’ contributions

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

ABSTRACT

Introduction: Epstein Barr virus (EBV), is a member of the genus Lymphocryptoviridae and Herpesviridae subfamily. It is pervasive and infects more than 90% of the adult population worldwide. In childhood and early adulthood primary infection occurs through salivary contact. The majority of children are asymptomatic, but some adolescents and young adults can develop infectious mononucleosis with harmless clinical manifestations.

Objective: To analyze the expression of Epstein Barr Virus (EBV) Latent membrane protein – 1 (LMP-1) in various histological subtypes of gastric carcinoma.

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

Result: The most common presenting symptom associated with Gastric carcinoma was upper abdominal pain (epigastric pain). Both the LMP-1 IHC positive cases showed a low score when assessed using the LMP-1 immunohistochemistry scoring system (used in nasopharyngeal carcinomas). This may be due to the older age group of patients. For the effective control of viral cancer, there should be rapid, sensitive, specific, cost-effective diagnostics assays and...
**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.

**Keywords:** Epstein Barr virus; gastric carcinoma; latent membrane protein – 1; herpesviridae.

**LIST OF ABBREVIATIONS**

- Epstein Barr virus: EBV
- Latent membrane protein – 1: LMP-1
- Immunohistochemistry: IHC
- Gastrointestinal bleeding: GI bleed
- World Health Organization: WHO
- Hematoxylin and Eosin: H&E
- Gastric cancers: GC
- Hepatitis B virus: HBV
- Hepatitis C virus: HCV
- Epstein-Barr virus: EBV
- Human papillomavirus: HPV
- Epstein-Barr virus-associated gastric carcinoma: EBVaGC
- Lymphoepithelioma-like carcinomas: LLCs
- CD2 subset 1: CS1 antibody

**1. INTRODUCTION**

Epstein Barr Virus (EBV) belongs to the genus Lymphocryptoviridae, the gamma 1 subtype of the Subfamily Gamma herpesviridae and is one of the commonest viruses in humans [1]. It is pervasive in nature, infecting more than 95% of all individuals within forty years of life. In developing countries, infections occur at a very early age with general symptoms of acute viremia. In developed countries, the infections that occur in adolescent age causes infectious mononucleosis, a benign self-limiting lymphoproliferative disorder [2,3]. In acute stages the infection is benign and latent in the chronic stages. The virus is associated with many malignancies such as cancer. Initially, the association was found to be with endemic Burkitt’s lymphoma. Subsequently, other lymphomas (subtypes of Hodgkin’s and non-Hodgkin’s lymphomas) were also known to have an association with EBV. Epithelial malignancies such as lymphoepithelial carcinoma were also suspected to have an association with EBV. It is believed that tumours also arise as a result of genetic and epigenetic alterations produced by the virus, that transforms the normal cell into an immortalized proliferating cell [4,5,6].

Since Burke et al. [7] first detected EBV in undifferentiated lymphoepithelioma like gastric cancer in 1990, many types of research are undertaken to study the same [3,7,8,9]. EBV expresses latent membrane protein that can be detected immunohistochemically. Since viruses and cancer have been closely associated in many studies the present study is aimed at detecting the EBV expression in gastric carcinoma cells.

**1.1 Objective of the Study**

To analyze the expression of Epstein Barr Virus (EBV) Latent membrane protein – 1 (LMP-1) in various histological subtypes of gastric carcinoma.

**2. MATERIALS AND METHODS**

**2.1 Place and Duration of the Study**

This prospective study was carried out in the Department of Pathology, Sree Balaji Medical College and Hospital, with the help of the Department of General Surgery, Sree Balaji Medical College and Hospital, from October 2016 to September 2018.

**2.2 Sample Size**

A total of 43 cases suspected of gastric malignancy were taken for the study. Out of which, only 32 were proven to be malignant out of which only 30 had adequate clinical data. Finally, only these 30 cases were included in the study.

**2.2.1 Inclusion criteria**

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

**2.2.2 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 a second opinion) were excluded from the study.
2.3 Method of Data Collection

Out of the 43 cases, 32 cases were proven to be malignant, out of which 30 cases which 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 was done.

Viral diagnostic assays was done according to Ryan et al. [10].

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

3. RESULTS

3.1 Clinical Features

A total of 30 gastric carcinoma cases diagnosed over a period from 2016-2018 were 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 was 29-80 years).

3.2 Age Distribution of Gastric Carcinoma

Most of the cases belong to the age group of 61-80 years.

<table>
<thead>
<tr>
<th>Years</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 20</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

3.3 Sex Distribution

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

3.4 Common Symptoms

3.5 Tumour Location

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

Fig. 1. Age distribution of gastric carcinoma
Fig. 2. Sex distribution

Table 2. Common symptoms

<table>
<thead>
<tr>
<th>Common Symptoms</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper abdominal pain (Epigastric pain)</td>
<td>59%</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>31%</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>5%</td>
</tr>
<tr>
<td>Loss of appetite (cachexia)</td>
<td>3%</td>
</tr>
<tr>
<td>GI Bleeding (Malena)</td>
<td>2%</td>
</tr>
</tbody>
</table>

Fig. 3. Common symptoms pathological features
3.6 Tumour Type WHO 2012 Classification

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

3.7 Tumour Type 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.

3.8 Tumour Grade

Out of 30 cases, 2 cases were well-differentiated type (G1), 10 were moderately differentiated (G2), 17 were poorly differentiated (G3) and 1 was of undifferentiated type (G4).

4. DISCUSSION

Gastric carcinoma is a serious public health problem worldwide with high rates of mortality. GLOBOCON statistical data states that 951,600 new gastric cancer cases was diagnosed in 2012 and in the same year deaths due to gastric carcinoma worldwide was around 720,000 [11]. The symptoms and signs of stomach cancer are often reported late when the disease is already in advanced stages. The 5-year survival rate is less than 30% in developed countries and around 20% in developing countries [12].

This indicates the need for early diagnosis and treatment strategies to improve survival. The present study assesses the age distribution, sex distribution, relationship between EBV and sporadic Indian GC and the role of latent membrane protein -1 in GC detection. Our health and wealth are highly affected due to various viruses. Recent emerging viruses like Dengue, Chikungunya, Nipah, Zika, and COVID-19 have raised a very serious health concern [13-17]. The non-emerging (prevalent) viruses are also not less dangerous. There are many viruses, which cause cancer in humans and animals. HBV, HCV, EBV and HPV are very famous viruses, responsible for large segments of human cancer. For early management of these viral agents, there should be effective and reliable diagnostic methods. Presently, viral disease can be diagnosed through electron microscopy, virus isolations, serological methods and molecular diagnosis. Virus isolation is the gold standard but has many problems with it [18]. Molecular diagnostic assays are comparatively better than other diagnostic methods for viral agents [19-29].
Table 3. Tumour location

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum/pylorus</td>
<td>18</td>
</tr>
<tr>
<td>Body of the stomach</td>
<td>4</td>
</tr>
<tr>
<td>Fundus/cardia of the stomach</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 4. Tumour type according to WHO 2010 classification

<table>
<thead>
<tr>
<th>Types</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular adenocarcinoma</td>
<td>17</td>
</tr>
<tr>
<td>Poorly cohesive carcinomas</td>
<td>08</td>
</tr>
<tr>
<td>Mixed adenocarcinomas</td>
<td>04</td>
</tr>
<tr>
<td>Carcinoma with lymphoid stroma</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 5. Tumour type according to WHO 2010 classification

Table 5. Tumour type according to Lauren classification

<table>
<thead>
<tr>
<th>Types</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal type carcinoma</td>
<td>23</td>
</tr>
<tr>
<td>Diffuse type carcinoma</td>
<td>04</td>
</tr>
<tr>
<td>Indeterminate type carcinoma</td>
<td>02</td>
</tr>
<tr>
<td>Lymphoepithelioma like carcinoma</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>
Fig. 6. Tumour type according to Lauren classification

Table 6. Tumour grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-differentiated (G1)</td>
<td>2</td>
</tr>
<tr>
<td>Moderately differentiated (G2)</td>
<td>10</td>
</tr>
<tr>
<td>Poorly differentiated (G3)</td>
<td>17</td>
</tr>
<tr>
<td>Undifferentiated (G4)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 7. Tumour grade
Fig. 8. Shows poorly cohesive type adenocarcinoma (H&E, 40x)

Fig. 9. Shows carcinoma with lymphoid stroma (H&E, 40x)

Fig. 10. LMP 1 immunostaining (moderate intensity) (LMP-1, 40x)
Fig. 11. LMP 1 immunostaining (weakly positive) (LMP-1, 40x)

Fig. 12. LMP 1 positive (intense staining) (LMP-1, 40x)

Fig. 13. LMP 1 positive (moderate staining) (LMP-1, 40x)
In general, our study showed male predominance accounting for 64% of all GC cases and the mean age was 58 years suggesting that gastric cancer appears more often in older individuals. These findings are in agreement with the literature which also has described the occurrence of gastric carcinomas in male and older patients [30,31].

Most of the patients in this study, presented with upper abdominal pain (62.5%) and loss of weight (30%). Others (7.5%) reported nausea, vomiting and upper GI bleed. 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 lymphoepithelioma like carcinoma (according to Lauren’s classification).

Over the past 30 years, a new subset of gastric cancer, EBVaGC has emerged. About 10% of all GC have been associated with EBV infection however, the role of EBV in gastric carcinogenesis remains unclear [32]. Recently, two studies suggested a new classification based on molecular features of gastric tumours and in these classifications arise four new subtypes of gastric cancers: tumours positive for Epstein–Barr Virus, microsatellite unstable tumours, genomically stable tumours and tumours with chromosomal instability.

To confirm, the association of EBV in a given tumour, the virus must be detected within the tumour cells. As per the literature, LMP-1 potentiates a variety of signalling pathways including the nuclear factor kb, Mitoigen-activated protein kinase, and phosphatidylinositol 3 - Kinase Alt pathways and involved in angiogenesis which is a key step in tumour growth, invasion and metastasis [33]. So the presence of EBV in gastric carcinoma cells can be confirmed by the presence of LMP-1 staining. In the present study 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 tumours is 6.6%. These findings are in agreement with previous studies. Studies have demonstrated that a high EBV-positive rate has been found in the low-incidence area of gastric cancer and a low EBV-positive rate has been found in a high gastric- cancer incidence area. Sousa, et al. [34] in a systematic review demonstrated that North of America (the 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. The same relationship is verified in our study since we observed a low prevalence of EBVaGC (6.6%) in a country considered to have a high incidence of GC. Considering other risk factors for GC development, we found that male predominance is also a strong characteristic of EBVaGC. The age distribution of patients with EBVaGC is yet to be explored.

In the present study, EBV-positive cases were observed in patients over 65 years. Regarding the tumour location, we observed that there was a high predominance of gastric tumours in the distal region (60%). Curiously, this is the anatomic location with a lower prevalence of EBV. As previously reported, the presence of EBV has been mostly associated with the body and cardia region of the stomach [35]. Hence, our results, which also showed a higher prevalence of EBVaGc in proximal regions, may explain the lower prevalence of EBVaGC in the present study.

Histology-specific analysis of EBVaGC using Lauren’s classification has shown controversial data. Chang et al. [30] and Corvalan et al. demonstrated a strong EBV association with diffuse types, however, Yoshiwara et al. [36] 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 in diffuse-type.

Regarding the lymphoepithelioma-like carcinomas (LLCs), it was observed that all samples showed positivity for EBV. These findings are in agreement with the 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), 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 [37].
The 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 inconsistent with the literature studies on nasopharyngeal carcinomas, which have also shown low scores in the older age group and high scores in the younger age group. Abdel Majiid Khabir et al. [38] observed in their 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 the 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 tumour has made it attractive. It serves as an ancillary method for early diagnosis of EBV related or associated gastric carcinoma. To effectively manage it vaccines or antivirals should be administered. In the absence of vaccines or antivirals, ethnomedicine is gaining interest because of its safety and broad-spectrum activity over synthetic drugs. Medicinal plants play a significant role in controlling various viral infections of Herpes Simplex Viruses, Dengue, Chikungunya [39-44]. In-Silico based screening approach provided more rational metabolites with high and specific potential and reduced futile exercise as compared to conventional in-vitro methods

5. 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. HPV vaccine and hepatitis vaccine was found for cervical cancer and hepatoma. Similarly, it wont be long before targeted therapy and an effective vaccine for EBV preventing primary infection or modulating its course leading to the reduction in EBV associated gastric carcinoma, is found.

CONSENT

As per international standard or university standard, patient's consent has been collected and preserved by the authors.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

ACKNOWLEDGMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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