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NUCLEAR TARGETING OF LATENT MEMBRANE PROTEIN 1 OF EPSTEIN BARR VIRUS IN TISSUES FROM PATIENTS WITH PANCREATIC CARCINOMA

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ABSTRACT : Many studies have implicated EBV in the pathogenesis as well as carcinogenesis of a relevant range of malignant squamous cell carcinomas.

The present retrospective study was designed to: (1) investigate the frequency of EBV infections associated with the pancreatic carcinoma, (2) explore the impact this virus on the expressed histopathological grades and the stages of those cancers; and (3) screen the expressedimmunohistochemical- EBV Latent Membrane Protein-1 reactions, as referred to signal scores and intensities of these reactions in the tested specimens.

The current retrospective study enrolled a number of 29 paraffinized pancreatic tissues from the archives of the period 2011 - 2017 of the major hospitals and many private histopathological laboratories in Baghdad. The research included 19 pancreatic carcinomatous tissues as well as 10 biopsies from an apparently normal pancreatic tissues. Each 4 mm – thick sections of the requested tissue block was stuck on a positive charged slide to be used for an immunohistochemical (IHC) technique for detecting EBV Latent Membrane Protein 1 using monoclonal primary antibodies against them.

Histopathological examination revealed that well, moderate and poor grades of pancreatic carcinomas constituted 21%, 63% and 16%, respectively. The EBV- LMP1 was detected in 42% (8 out of 19) of pancreatic cancerous tissues. Seven out of eight tissues with positive EBV-LMP1 reactions have moderate differentiated grade, while only one tissue (1 out of 8) have well differentiated grade and no expression of poor differentiation. It could possibly point that EBV infections in pancreatic carcinomas could have an initiating and/or cofactor roles, in collaboration with other important oncogenic factors or agents in the pancreatic carcinogenesis which could happened as an early- event along other molecular attacks in this process.

Key words : Epstein Bar Virus, Latent Membrane Protein 1, immunohistochemistry, pancreatic carcinoma.

INTRODUCTION

Pancreatic tumors which arethe 4th leading cause of cancer mortality in the United States, considered one of the less common tumors within the gastrointestinal tract (Zhang *et al*, 2007). According to the histological features, pancreatic carcinomaare divided into three categories: exocrine neoplasms, neuroendocrine tumors and mixed exocrine-endocrine tumors (Hannah and Peiguo, 2012).

Pancreatic cancer, as fourth leading cause of cancer-related death in Western countries has 5 year survival rate of 1–3% (Sung *et al*, 2009).

According to Iraqi Cancer Registry data 2009, pancreatic cancer represents the 8th commonest ten cancerin both genders (Iraqi Cancer Registry data, 2009).

Epstein-Barr virus is a member of the herpesvirus family and one of the most common human viruses. The EBV is a ubiquitous gamma herpes virus that infects more than 90% of the adult population worldwide, who were seropositive for EBV (Meytal and Asher, 2006; Ming *et al*, 2017).

Although, most EBV carriers do not display any clinical symptoms as the result of this viral infection, EBV is etiologically linked with up to 2% of all human malignancies (Ming *et al*, 2017). The genome of EBV can be detected in malignancies of both lymphoid and epithelial cell origin, such as Burkitt's lymphoma and nasopharyngeal carcinoma (Hisashi *et al*, 2012).

These facts made importance to investigate about the causatives of this cancer. Our objective is to detect the existence of EBV in a group of Iraqi patients with pancreatic carcinoma.

MATERIALS AND METHODS

Anti- Anti-EBV Latent Membrane Protein 1 antibody and EXPOSE Mouse and Rabbit specific HRP/DAB

detection IHC kit from ABCAM Company, United Kingdom were used in this study.

After deparaffinizeation and rehydratetion by xylene and serially graded alcohol and then distill water, Incubation with 3% hydrogen peroxide for 10 minutes were used for blocking the activity of endogenous peroxidase activity. Slides were washed in phosphate-buffered saline. After treating with protein block by incubating at 37°C for 5 minutes then washing with PBS. Primary antibody was applied to cover tissue sections and incubated for 1 hours in humidity chamber at 37°C (Primary Antibody was prepared at dilution 1/100 for EBV LMP1). Slides were rinsed gently in PBS. The secondary antibody was added for 10 minutes at room temperature, followed by the addition of Streptavidine-HRP antibodies for 10 minutes at 37^0C. After washing, samples were stained with DAB for 15-45 minutes at room temperature. Slides were counterstained with hematoxylin for 30 second and washed well in running tap water, then dehydrated and mounting with permanent-mounting medium (DPX), examined under light microscope first at 10 then at 40 magnification was finally done (Basim, 2017).

Statistical analysis was done using Social Science Statistic (http://www.socscistatistics.com) and Excel application.

Inferential statistics: Fisher exact test was performed to find out the relation between each marker with the pancreatic cancer and normal groups. P value (<0.05) was considered statistically significant.

RESULTS

The immunohistochemical expression for EBV Latent Membrane Protein1 showed significant correlation with the pancreatic carcinoma and as shown in Table 1 and Fig. 1.

The immunohistochemical expressions for EBV LMP1 proteins in relation to age and gender are shown in Figs. 2 & 3. The majority of the positive EBV IHC expression were male with a ratio 3:1 of male to female and as shown in Fig. 2.

The EBV LMP1 expression according to the studied tumor grading illustrated in Fig. 3. The majority of the samples were moderately differentiated (36.8%; 7 out of 19) while just 5.2% (1 out of 19) presented aswell differentiated. The proportion / size of each grade in the studied samples of pancreatic cancers are shown in Fig. 4.

The TNM classification of the studied pancreatic cancers also showed that the majority of these cases have been staged as T3 followed by T2 stage while only 5% of the pancreatic cancers have been staged as T4 (Fig. 7). Regarding EBV LMP1 expression, there is no significant variation in the expression of EBV as shown in Fig. 6.

DISCUSSION

Regarding distribution of pancreatic cancers among Iraqi provinces, a rate of (1.86% of total) and (0.89/ 100.000 of population) was registered (THESS). Baghdad was the most affected one then followed by Babylon, Nineveh and Basrah. Moreover, this cancer in Iraq was representing the 8th among ten commonest cancer death in both genders (Iraqi Cancer Registry data, 2012).

In this research work, the male patients were 13, out of 19 (68.4%) while their female counterparts were 6, out of 19 (31.6%) constituting a ratio of 2/1 (Fig. 2).

Regarding Iraqi cancer registry (2012), the prevalence of newly registered pancreatic cancers cases in Iraq according to their genders was found to be 158 out of 283 (55.8%) in males compared to 125 out of 283 (44.2%) in female with a ratio of 1.5/1 (Iraqi Cancer Registry Data, 2012). These important facts have initiate our interest to investigate a possible role for EBV among the etiologies of these cancers.

According to the American Cancer Society, the EBV was mainly linked with development of nasopharyngeal cancers and certain lymphomas such as Burkitt lymphomas, whereas a link with Hodgkin lymphoma and some gastric cancer cases were well established. The gastrointestinal organs are among the most common systems affected by EBV (Thana *et al*, 2008).

It was proposed that EBV could spread from the nasopharynx to the stomach in such cases of lymphoepithelioma-like gastric carcinoma, based on the morphological similarities between undifferentiated nasopharyngeal carcinomas and these gastric carcinomas (Matthew and Razelle, 2004).

However and regarding the linkage of EBV with the pancreatic carcinomas, a scarcity of researches were faced.

In Iraq, up to our knowledge, this is the first attempted research to study a possible oncogenic role of EBV in a group of pancreatic carcinomas tissues obtained from Iraqi patients.

As shown in Table 1, the expression of EBV in pancreatic cancer tissues was found to be 8 out of 19 (42%) with a significant correlation (P=0.0265). These significant results in line with that interpretation proposing the pancreatic infection with EBV might be attributed to

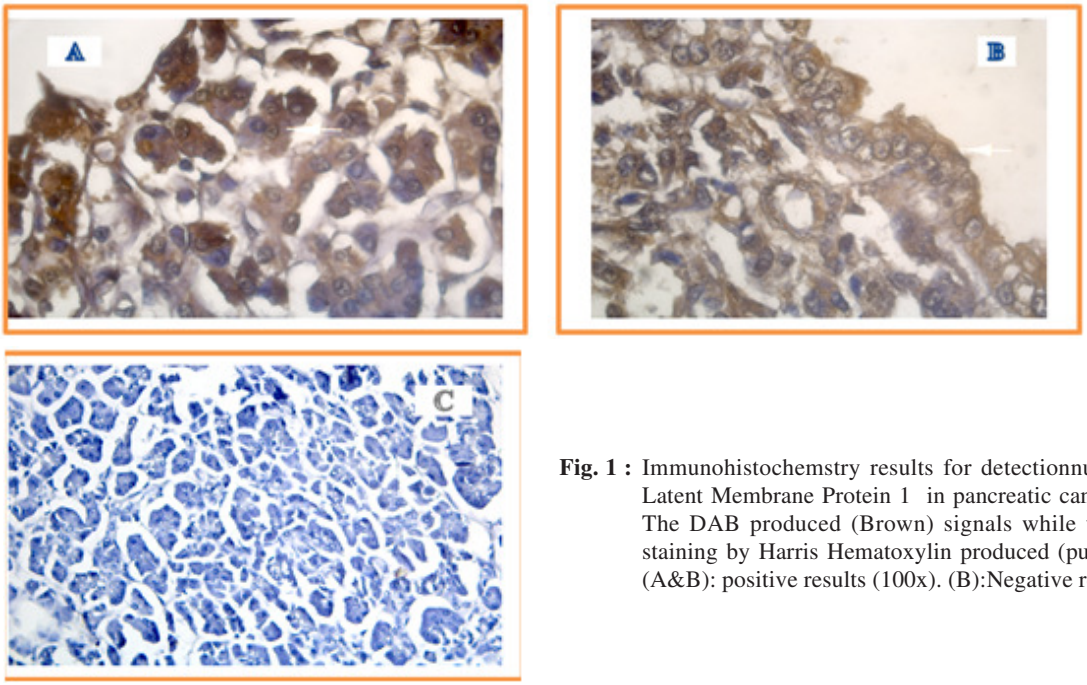

Fig. 1 : Immunohistochemstry results for detectionnuclear EBV Latent Membrane Protein 1 in pancreatic cancer tissues. The DAB produced (Brown) signals while the counter staining by Harris Hematoxylin produced (purple) color; (A&B): positive results (100x). (B):Negative result.

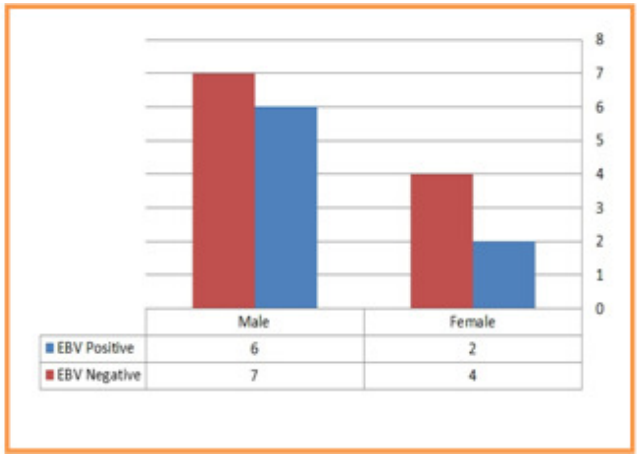

Fig. 2 : The EBV-IHC expression according to the Gender.

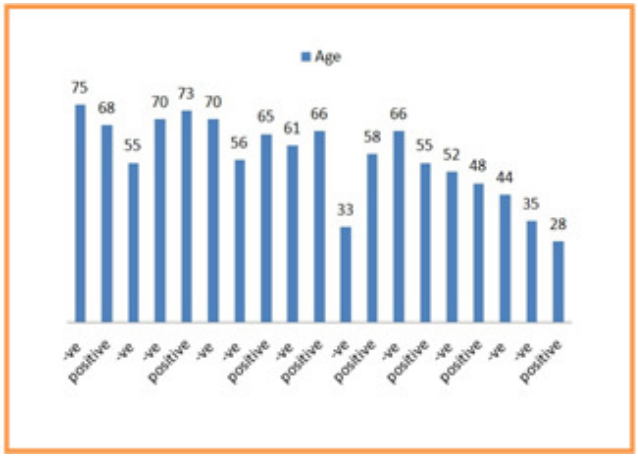

Fig. 3 : The EBV-IHC expression according to the age.

the approximation of that organ to the stomach which lies posteriorly to the stomach (Peiguo *et al*, 2000). The importance of these results coincide, by analogy, with the importance of those obtained by Mohammed Ali *et al* (2017), who found that (32.4%) of their studied breast cancers tissues were infected with EBV.

Due to the nature of pancreatic carcinoma , the disease does not cause symptoms until the later stages, this is supported by the Zhang *et al* (2007), who found that less than 10% of pancreatic cancers are detected at a stage where cure is possible. The overall survival for this group of cancers is only about 5% (Hannah and Peiguo, 2012). In addition to these findings, almost 10% of the gastric carcinomas throughout the world are EBV-carrying tumor cells (Iraqi Cancer Registry Center, 2012). A characteristic feature of EBV-associated gastric carcinomas is lymphoepithelioma-like carcinoma of diffused-type and lymphoid infiltration. The reflux of bile and pancreatic juice is considered to cause regenerative atypia and cell proliferation in epithelial cells (Hisashi *et al*, 2012).

Table 1 : The immunohistochemical expression of CMV pp65 antigen I tissues with pancreatic carcinoma.

IHC expression of EBV	Pancreatic cancer	Normal pancreatic tissue	Marginal Row Totals
EBV positive	8	0	8
EBV negative	11	10	21
Marginal column Totals	19	10	29 (Grand total)

The Fisher exact test statistic value is 0.0265. The result is significant at $p < 0.05$.

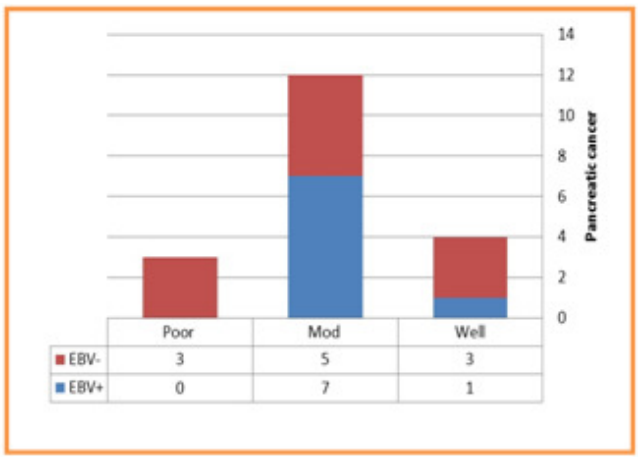

Fig. 4 : The EBV-IHC expression according to the cancer grading.

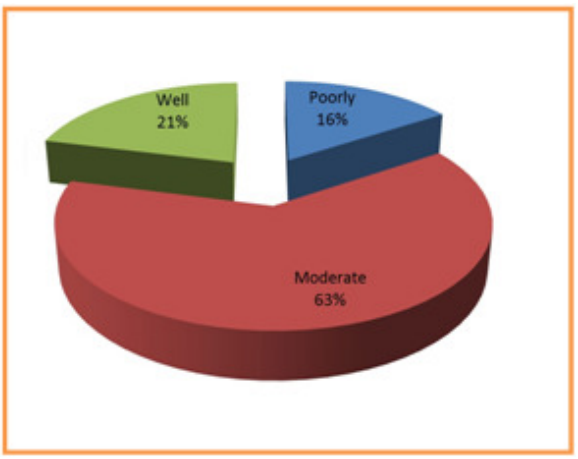

Fig. 5 : Proportion of each grade of the studied pancreatic cancers.

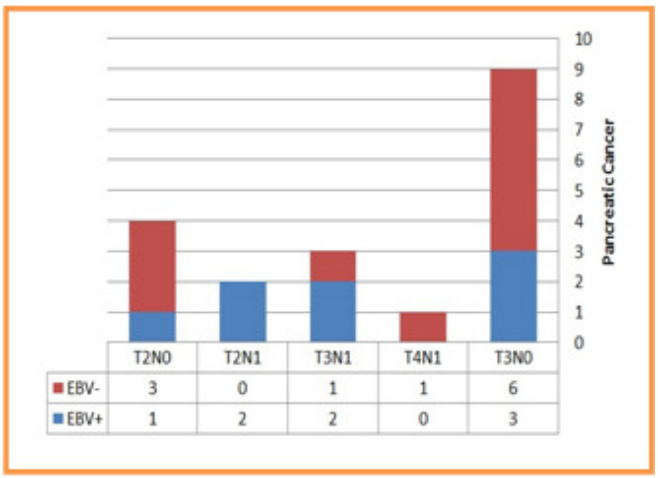

Fig. 6 : The EBV-IHC expression according to the pancreatic cancer staging.

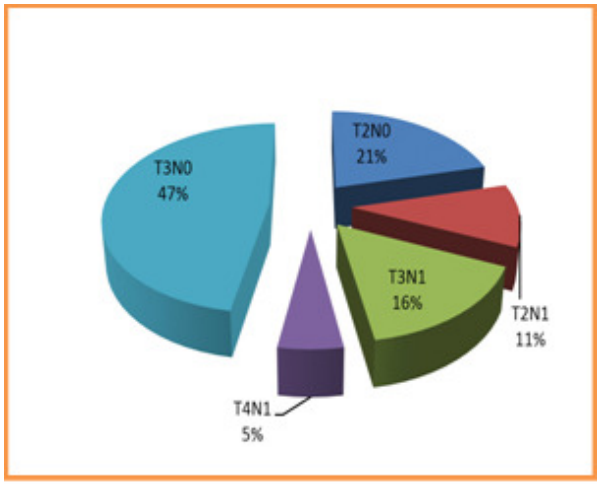

Fig. 7 : Proportion of each TNM stage of the studied pancreatic cancers.

Moreover, the hypothesis that EBV-related pancreatic lymphoepithelial carcinomas as a second primary tumors was favored, based on the considerable delay of manifestation of these tumors, their unusual sites, their pathologic presentations, the exclusive peri-pancreatic nodal spread and the different histological manifestations of these lesions (Kekis *et al*, 2004).

Epstein-Barr virus infection was considered in the differential diagnosis of patients with acute hepatitis combined with pancreatitis (Seok *et al*, 2013). There may be a multifactorial link between HBV an EBV infections, yet, the correlative mechanism of pancreatitis development in association to infectious hepatitis is unknown. The proposed hypotheses are direct destruction and inflammation of pancreatic acinar cells by the viruses. (Shimoda *et al*, 1981); development of edema of the ampulla of vater leading to obstruction of the pancreatic fluid outflow (Bhagat *et al*, 2008) or a biliary sludge induced by diet change during the state in course of viral hepatitis resulting in obstruction of the pancreatic fluid outflow tract (Basaranoglu *et al*, 2006).

On reviewing (Fig. 4), the high rates of human EBV infections as well as their correlation to the differentiation of the studied pancreatic cancers could point for a role for EBV infections, as a molecular event, among other important carcinogenic agents and / or events in these cancers, which could also probably happened as an early event in pancreatic carcinogenesis.

In the other way, it could also be concluded that these EBV infections might not be associated in the initiation of pancreatic cancers, yet could increase their risk in this process.

Larger studies are necessary to identify the risk of EBV infections or their exact roles as an initiating or cofactor roles in pancreatic carcinogenesis remain to be further determined. Further research is also needed to investigate the involvement of other pathogenic infections in pancreatic carcinogenesis.

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