RESEARCH ARTICLE

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# The High Presence of HCMV pp71 Proteins, Correlate with P63 Expression in Pancreatic Cancer Tumor Tissue

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## Abstract

**Objective:** The purpose of this retrospective investigation was (1) to screen the existence of HCMV in pancreatic cancer tissues in relation to the histopathological grading system of such tumor tissues. (2) To evaluate the expression of the (P63) tumor suppressor gene in these tissues. (3) To find out the impact of the coexistence of (HCMV) along with the p63 on the occurring histopathological alterations. **Methods:** The current retrospective cohort study included 35 paraffinized pancreatic tissues from the archives of major hospitals and numerous private histopathological laboratories from 2015 to 2020. (Twenty-five pancreatic carcinomatous tissues and 10 biopsies from seemingly normal pancreatic tissues were examined). Tissue slices from the desired tissue blocks were subjected to the immunohistochemistry (IHC) technique to detect Human Cytomegalovirus pp71 and tumor suppressor P63 proteins with aid of monoclonal primary antibodies. **Results:** The HCMV pp71 proteins were found in 92% (23 out of 25) of pancreatic tumor tissues, while it was in two (20%) of healthy pancreatic tissues. in comparison, the p63 proteins were found in 76% (19 out of 25) of tumor tissues and in four (40%) of their correlative healthy tissues. **Conclusion:** The increased expression of HCMV in malignant pancreatic tissue may indicate its primary or secondary role in the emergence of this type of cancer, whereupon HCMV inactivation may be useful in the treatment of this type of cancer. On the other hand, p63's high levels of expression in malignant pancreatic tumors reflect either an oppressive function or an unfortunate mutation that prevents it from functioning.

**Keywords:** Pancreatic cancer- P63- Human cytomegalovirus- immunohistochemistry.

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## Introduction

Pancreatic carcinomas (PC) is a cancerous tumor that develops from the cells of the pancreas, a glandular organ. Pancreatic malignancies can be classified as endocrine or non-endocrine. Based on histological features, pancreatic malignancies include Ductal adenocarcinoma, Cystadenocarcinoma, and others (Pappalardo et al., 2021). The PC incidence and fatality rates have been rising year after year across the world. In (2015), 367,411 new cases were reported worldwide, with 359,335 fatalities (Hu et al., 2021). Pancreatic carcinomas are responsible for roughly 4% of all cancer fatalities. Furthermore, it is a very aggressive malignancy, with 80% of patients having locally progressed or metastasized at the time of diagnosis (Puckett et al., 2023). According to The GLOBOCAN (Global Cancer Observatory), pancreatic cancer accounts for 2.7 percent of all cancer cases recorded in Iraq (Castanon et al., 2019). Human cytomegalovirus (HCMV) is a herpes virus that is found all over the world. In immunocompromised persons, such as organ transplant recipients and AIDS patients, human cytomegalovirus causes severe and frequently fatal infections. It reactivates in healthy viral carriers on a regular basis, but the host immune response normally controls this (Kiros et al., 2021). Monocytes may serve as a reservoir for latent HCMV, which can be reactivated by cellular differentiation or inflammation. HCMV infection and cancer have been studied for decades. HCMV infection may cause numerous human malignancies, according on seroepidemiologic and tumor tissue viral DNA, mRNA, and/or antigens (De Groof et al., 2021; Elder et al., 2019).

Some researchers employ the term "oncomodulation" to explain HCMV's role in malignancies, even though human CMV is not an oncogenic virus. The fact that HCMV is frequently found in tumor tissues could be explained by the fact that it promotes tumor growth while not being an oncogenic virus (Kwon et al., 2021). Oncomodulation occurs when HCMV infects tumor cells and alters their aggressiveness (Touma et al., 2021).

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Virus regulatory proteins and noncoding RNA affect tumor cell features such as proliferation, survival, invasion, angiogenic factor production, and immunogenicity, causing human cytomegalovirus-induced oncomodulation. Consequently, HCMV infection may induce tumor cells to change phenotype and become more malignant, as well as tumor progression (Adamson et al., 2020).

The TP63 gene, located on chromosome 3q28, encodes tumor protein 63 (p63), a transcription factor in the p53 gene family. Cell cycle-regulating genes and basal layer keratins are only two examples of the many genes whose expression is controlled by p63 throughout the development of the ectoderm and its offspring. The diagnostic value of sustained elevated p63 expression in the selected cell and tissue types is based on their use as biomarkers. It is a widespread practice in diagnostic pathology to employ p63 immunohistochemistry (IHC) to detect cancer-relevant cell types, such as basal cells in the prostate and breast glands (Nasiri et al., 2021; Steurer et al., 2021) .

Homologs of the tumor suppressor gene p53, called p63, produce proteins with or without a transactivating domain (TAp63) at their amino termini (Np63). Furthermore, various isoforms emerge from alternative splicing. When overproduced, TAp63 isoforms can cause apoptosis, whereas Np63 isoforms inhibit apoptosis. The relevance of isoform profiles in different cancers has been explored by several researchers (Bartas et al., 2019).

The prognostic significance of p63 expression and its level of expression has been shown to vary widely amongst tumor types. Head and neck squamous cell carcinomas have been associated with increased p63 staining, and p63 may be a marker for metaplastic breast carcinoma. Loss of p63 expression, on the other hand, has been linked to tumor development and reduced survival in lung and urothelial malignancies. P63 was expressed nearly exclusively in endometrioid carcinomas, and its lack was linked to myometrial infiltration (Sinha et al., 2015; Zaha, 2014).

## Materials and Methods

The study aims to explore whether or not HCMV expression is linked to oncomodulatory or transformative functions in pancreatic cancer, as well as to extend more to probable involvement of the p63 tumor suppressor protein in pancreatic carcinogenesis in Iraqi patients.

Material and methods: Thirty-five formalin-fixed paraffin-embedded archival tissues representing years from 2018 to 2021, collected from private shistopathology laboratories, were used in this investigation (25 samples from carcinomatous pancreatic tissues and 10 biopsies from seemingly normal pancreatic tissues taken from cancer-negative samples). Tissue sections about 4-5 mm thick were put on a positively charged slide for an immunohistochemical (IHC) technique to look for HCMV pp71 and p63 expression proteins. This was done using Anti- pp71 HCMV and Anti-p63 antibodies from the Abcam company in the UK, according to the manufacturer's instructions. The samples were then looked at under a microscope at 10 and 40 times magnification (Areej et al., 2013).

*Statistical analysis*

The data were analyzed using the SPSS software tool, version 24. Pearson chi-square were employed to accept or reject statistical hypotheses. As well as, Spearman's rho to find out the relationships between markers. P value ($P \leq 0.05$) was regarded as statistically significant (Wang, 2003).

## Results

*Results of Immunohistochemical Detection of Human Cytomegalovirus pp71 among Studied Tissues*

Table (1) reveal the HCMV-IHC expression and scoring signals in PC and control tissues. Out of 25 PC sections, 23(92%) showed positive HCMV-IHC reactions as in the following grade distribution; well-differentiated (4.35%), moderately differentiated (13.04) poorly differentiated (17.39%), and undifferentiated (65. 2%). In comparison 2(20%) out of the ten healthy sections showed positive reactions. There were significant differences (P=0.05) between PC sections and healthy ones on one side and (P<0.05) between the different grades of PC sections themselves on the other side.

*Results of the Expression of p63 -IHC and Signal Scoring among Study Groups*

As shown in table (2), p63 proteins were identified in 76% (19 of 25) of malignant tissues and 40% (4 of 8) of their corresponding healthy tissues. In the pancreatic cancers group, it was expressed in well-differentiated tissues in a ratio of (5.26%), in moderately differentiated (10.53%), in poorly differentiated (26.32%) and in undifferentiated grade, it was expressed in a ratio of (57.89%). Statistically, Significant differences were found among the four grades (well, moderate, poorly, and undifferentiated) at (P<0.05) level. While there was a Non-Significant difference (P>0.05) between cancerous and control tissues.

*Correlations between HCMV and p63 Signal Scores in Pancreatic Carcinomas*

Table (3) shows the relationship between the expression of HCMV and p63 signal scores in pancreatic carcinomatous tissues. The correlation was positive (0.009 **) and highly significant at $P < 0.05$ level by using Spearman's rho correlations.

## Discussion

Pathologic evidence suggests that HCMV infections may be involved in the aetiology of numerous human cancers (Geisler et al., 2019). Infecting the majority of human organs, including the blood, uterine cervix, brain, breast, colon, eyes, kidneys, liver, lungs, and gingival sulcus fluid, the virus has a broad tropism in many healthy individuals (Jassim and Ali, 2019; Scrivano et al., 2011). However, there is a scarcity of evidence on the involvement of CMV (reactivation and/or disease-causing) in individuals with solid tumors (Schlick et al., 2015).

Table 1. Human Cytomegalovirus-pp71 IHC Expression in Relation to Histopathological Pancreas Cancer Grading

| Studied Groups | Diagnosis | | HCMV-pp71 | | Score | | Pearson Chi-Square |
|---|---|---|---|---|---|---|---|
| | | | Positive | Negative | | | (P-value) |
| Pancreatic Carcinomas | Well | N | 1 | 2 | + | 1 | P=0.03 |
| | differentiated | % | 4.35% | 100% | ++ | 0 | Sign. (P<0.05) |
| | | | | | +++ | 0 | |
| | Moderately | N | 3 | 0 | + | 0 | |
| | differentiated | % | 13.04% | 0.00% | ++ | 2 | |
| | | | | | +++ | 1 | |
| | Poorly | N | 4 | 0 | + | 0 | |
| | differentiated | % | 17.39% | 0.00% | ++ | 2 | |
| | | | | | +++ | 2 | |
| | Undifferentiated | N | 15 | 0 | + | 1 | |
| | | % | 65.22% | 0.00% | ++ | 3 | |
| | | | | | +++ | 11 | |
| | Total | N | 23 | 2 | | | |
| | | % | 92% | 8% | | | |
| Control | | N | 2 | 8 | | | |
| | | % | 20% | 80% | P=0.05 | | |
| Total | | N | 10 | | Sign. (P=0.05) | | |
| | | % | 100% | | | | |

A significant difference (P≤0.05) by using Pearson's Chi-Square test.

Table 2. Signal Scores Expression of p63-IHC in Relation to Pancreatic Cancer Grading

| Studied Groups | Diagnosis | | P63-IHC signal scores | | Score | | Pearson Chi-Square |
|---|---|---|---|---|---|---|---|
| | | | Positive | Negative | | | (P-value) |
| Pancreatic Carcinomas | Well | N | 1 | 2 | + | 0 | P=0.047 |
| | differentiated | % | 5.26% | 33.33% | ++ | 1 | Sign. (P<0.05) |
| | | | | | +++ | 0 | |
| | Moderately | N | 2 | 0 | + | 0 | |
| | differentiated | % | 10.53% | 0.00% | ++ | 1 | |
| | | | | | +++ | 1 | |
| | Poorly | N | 5 | 3 | + | 1 | |
| | differentiated | % | 26.32% | 50% | ++ | 2 | |
| | | | | | +++ | 2 | |
| | Undifferentiated | N | 11 | 1 | + | 0 | |
| | | % | 57.89% | 16.67% | ++ | 2 | |
| | | | | | +++ | 9 | |
| | Total | N | 19 | 6 | | | |
| | | % | 76% | 24% | | | |
| Control | | N | 4 | 6 | | | |
| | | % | 40% | 60% | P=0.13 | | |
| Total | | N | 10 | | Non-Sign. (P>0.05) | | |
| | | % | 100% | | | | |

Table 3. Spearman's rho Statistical Testing to Evaluate HCMV and P63 Scoring Relationships in Pancreatic Carcinoma

| Spearman's rho Correlation (PC) scores | | HCMV scores | P63 scores |
|---|---|---|---|
| HCMV scores | r. | | 0.009 * |
| P63 scores | P-value | | 0.049 |

*, Correlation is significant at the P < 0.01 level (Highly Significant).

In addition to triggering cell cycle arrest, HCMV infection changed the steady-state concentrations of a number of proteins involved in cell cycle control. HCMV generated higher levels of both cyclins E and B, as well as their respective kinase activity (Branch et al., 2021). In contrast, the production of cyclin A appeared to be suppressed, and the levels of the protein and its associated kinase activity increased only at the late stages

of infection. Additionally, it was shown that the disease causes the accumulation of high quantities of the proteins p53 and retinoblastoma (Groves et al., 2021).

This study's findings are consistent with those of Ali et al. (2019), who found a high prevalence of cytomegalovirus infections in the sinonasal and nasopharyngeal malignant tumors of a cohort of Iraqi patients, and suggest that cytomegalovirus may play an oncomodulatory role in the development of these tumors (Jassim et al., 2020). CMV has been implicated in modifying cellular immune responses in cancer in both human and preclinical investigations (Erkes et al., 2017; Meng et al., 2018).

The majority and most strong immunohistochemical expressions of CMV-pp71 proteins were clearly observed in the undifferentiated grades of the pancreatic tumors investigated (Table1). It is possible that CMV infections play starting and/or cofactor roles in pancreatic carcinomas, combining with other carcinogenic agents.

Many different types of cancer showed positive p63 expression, including squamous cell carcinomas (96–100%), thymic tumors (100%), urothelial carcinomas (81–100%), basal type tumors (e.g., basal cell carcinomas, 100%), and various salivary gland neoplasias (81–100%). Furthermore, malignancies derived from p63-negative tissues showed positive p63 immunostaining in 422 pancreatic tumors, although this was unrelated to an aggressive character (Steurer et al., 2021).

Ito et al., (2001) demonstrated that p63 overexpression has been linked to the development of pancreatic adenocarcinoma. Another research on undifferentiated pancreatic cancer (UC) discovered moderate-to-strong pan-p63 expression in anaplastic (pleomorphic giant cell) UC (n = 4), sarcomatoid UC (n = 2), UC with osteoclast-like giant cells (n = 3), and ductal carcinomas with partial squamous differentiation (n = 3). Pan-p63 expression was observed to be weak and localized in monomorphic UC (n = 3) and in the majority of neuroendocrine carcinomas (6/7 cases) (Liszka, 2020).

Cancer metastasis can be restrained by TAp63, which triggers cell death and senescence. Previous research has established a correlation between p63 deficiency and increased tumor growth and invasiveness (Flores et al., 2005). Recent research has elaborated on this finding, demonstrating that p53 mutation with TAp63 deletion or inactivation increases tumorigenicity through TGF-induced pathways and DNA repair gene alterations (Adorno et al., 2009).

In this context, a study by Jassim et al., (2022) on malignant lung tumors found abnormal expressions of some other tumor suppressor proteins such as BRCA1, BCL2, and the RB. Likewise, (Jassim et al., 2021) on tumor suppressor protein "p53" in breast cancerous tumors.

The findings about how TAp63 works and what happens when it is lost or turned off have also been proven in pancreatic cancer (Jahedi et al., 2019). Some mutant forms of p53 that are produced from tumors are able to downregulate p63 and p73 by making direct contact with the p53 core domain. This interaction between "p63 and p53" is critical in the development of malignancies like pancreatic carcinoma (Gaiddon et al., 2001).

In conclusion, The elevated expression of HCMV infection in cancerous pancreatic tissue could reflect the molecular role of this virus in the disease. The infection may have a primary and/or cooperative function in the development of this kind of cancer, meaning that HCMV inactivation may have a role in cancer treatment. The high expression rates of p63 in malignant pancreatic tumors, on the other hand, reflect an oppressive role or an unlucky mutation that renders it nonfunctional.

## Author Contribution Statement

All authors contributed equally in this study.

## Acknowledgements

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