RESEARCH ARTICLE

Editorial Process: Submission:04/16/2024 Acceptance:10/08/2024

Indirect Immunofluorescence Test for Detecting HSV-1, 2 in Patients with Esophageal Cancer: A Pilot Cross-Sectional Survey in Kazakhstan

Bakhtiyar Azhken*, Valiy Apsalyamov, Boris Baryshev, Vladimir Grigorevsky, Alina Shelestyuk

Abstract

Objective: To evaluate the detectability of herpes simplex virus type 1 and 2 (HSV-1, 2) antigens in the esophageal mucosa in esophageal cancer. **Methods:** A cross-sectional pilot study with a control group was conducted from December 2022 to May 2023. The patients were divided into two groups: the main group with verified esophageal cancer and the control group without esophageal cancer based on the histological report. Diagnosis using fibroesophagogastroscopy (FEGS), histological analysis, and indirect immunofluorescence reaction (iIFR) was accomplished according to indications at the outpatient center of the endoscopic department of the Multidisciplinary Medical Center, Astana. Descriptive statistics and nonparametric methods were used to analyze the data. **Result:** A total of 30 patients were recruited in the study, with 15 patients in the main group and 15 patients in the control group. HSV-1 and HSV-2 antigens were detected in the 13 cases (86.7%) during the study of biomaterial from patients with confirmed esophageal cancer in the main group and only in one case (6.8%) in the control group (95% CI 7.35 - 1126.9%, p=0.001). The presence of lymphocytic infiltration was detected in 13 cases (86.7%) in the main group and in 5 cases (33.3%) in the control group (95% CI 2.07 - 81.48%, p = 0.008). The process of keratinization was identified in HSV-1,2 antigens positive cases in the main group in 5 cases (35.7%) G1, in 5 cases (35.7%) G2, and in 3 cases (21.4 %) G3 (95% CI 1.66 - 656.23%, p=0.002). **Conclusion:** Overall, the local activity of herpesvirus infection to a certain extent influences the malignant potential of tumor cells and the resistance of surrounding healthy tissues. A large-scale study is still needed to confirm these results. The present pilot study provided an overview of a possible method for detecting HSV-1,2 activity in malignant tissue from esophageal cancer.

Keywords: HSV-1- HSV-2, esophageal cancer, lymphocytic infiltration, keratinization

Asian Pac J Cancer Prev, **25 (10)**, 3559-3565

Introduction

Esophageal cancer is one of the most deadly and difficult to treat forms of cancer [1]. According to the source Globocan [2], in 2020, the global incidence of esophageal cancer in both sexes and all ages was 604 100 reported cases (7.79 cases in every 100 000 people) and the mortality rate was 544 076 (7.78 cases in every 100 000 people). In Kazakhstan, esophageal cancer can also be classified as one of the diseases with the highest mortality rates. According to Kazakh Research Institute of Oncology and Radiology (KRIOR), the prevalence of esophageal cancer in 2020 in the Republic of Kazakhstan in both sexes was 1 082 cases (5.7 cases per 100 000 people), and mortality was 709 cases (3.8 cases per 100 000 people) [3].

Recent scientific research suggests that microorganisms, including viruses, bacteria, fungi and protozoa, play a role in the etiopathogenesis of malignancies [4]. According to the World Health Organization, 3.7 billion people (<50 years of age [67%]) with HSV-1 and 491 million people (15–49 years of age [13%]) with HSV-2 are infected worldwide [5]. One of the reasons for the malignant transformation of a cell is genetic mutations that occur in the cell as a result of exposure to carcinogens such as viral infections, radiation, chemicals and other factors. Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) are two commonly encountered viruses in the herpesvirus family. Therefore, identifying the connection between herpesvirus infection and neoplastic processes is an important issue in carcinogenesis, including esophageal cancer in humans.

Thus, determining the role of viruses, in particular HSV-1,2, is necessary for a modern approach to the prevention of esophageal cancer, guided by common radical methods of treating this disease. However, there

*Department of Histology and Cytology, of NcJSC «Astana Medical University», Astana, Kazakhstan. *For Correspondence: bakhtiyar.azhken@gmail.com*

is limited data worldwide on the role of these viruses in cancer development. This pilot study aimed to determine the detectability of HSV-1 and HSV-2 in the esophageal mucosa in esophageal cancer, which would allow the identification of potential viral markers involved in or influencing malignancy that could be used to improve diagnosis and treatment esophageal cancer.

Materials and Methods

Study design

A population-based cross-sectional pilot study was conducted from December 2022 to May 2023. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. Consent was obtained from all the respondents provided in the study. The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the institution's human research committee. The research was approved and recommended for implementation of the study by the Local Ethics Committee of the NcJSC «Astana Medical University» (Meeting No. 15). All individuals involved have signed the Informed Consent Form.

Participants

We examined 15 patients with esophageal cancer (adenocarcinoma, squamous cell carcinoma) of any stage (0, IA, IB, IIA, IIB, IIIA, IIIB, IIIC, IV) and all ages in the main group and 15 patients with unconfirmed esophageal cancer in the control group, who visited the outpatient center of the endoscopic department of the Multidisciplinary Medical Center (Astana). The conditions for calculating the sample size were the required level of significance at 0.05 and the power of the study at 80%. To calculate the sample size from the general population, Lehr's formula for average values was chosen since it allows us to determine the size of each compared group. The main group of subjects included patients who applied in 2022-2023 of all ages, had verified esophageal cancer of all stages, and had not received previous treatment. In the process of selecting patients from among those examined, attention was paid to the histological conclusion, confirming or excluding esophageal cancer using clinical diagnostic methods, followed by the formation of morphological and virological characteristics.

Collecting materials and conducting research

The study used at stage 1 - the method of fibroesophagogastroscopy (FEGS) to obtain biopsy materials, at stage 2 - methods of histological analysis and indirect immunofluorescence reaction (iIFR) to determine the specific and nonspecific presence of HSV-1,2 and at stage 3 - methods of statistical data processing to evaluate statistical significance.

At the 1st stage - FEGS forceps were used to collect biopsy material from pathological areas of the esophagus in patients suspected of esophageal cancer in the endoscopic department of the «Multidisciplinary Medical Center» (Astana). The collection was accomplished from the mucous membrane of the esophagus in the marginal zone of the pathological focus in an amount of at least 2 pieces.

At stage 2, during histological analysis, preparations of specimens and initial visual assessment of biopsy material were accomplished to determine nonspecific markers of viral infection. All histopathological samples were stained with hematoxylin and eosin. Microphotography of the specimen was accomplished at the Department of Histology and Cytology of the «Astana Medical University» NcJSC using a Leica microscope at a magnification of x100, x400 and x1000.

Next, we prepared smears of tumor tissue on sterile glass slides for subsequent indirect immunofluorescence reaction (iIFR) to detect the presence of HSV-1, HSV-2 antigens. Air-dried fingerprint smears were fixed with 96% chilled ethanol, after which they were subjected to an indirect immunofluorescence reaction to the HSV-1, HSV-2 antigens using standard diagnostic kits «HerpesMonoScan», produced at the Scientific and Production Foundation «Labdiagnostika» (Moscow). Based on the results of this stage of the study, microphotography was accomplished to record tissue samples with foci of luminescence in cells at a magnification of x1000.

Statistical Analysis

Statistical analysis was accomplished using the programs «STATISTICA» and «IBM SPSS Statistics 26». Quantitative indicators were assessed for compliance with normal distribution using the Shapiro-Wilk test (for the number of subjects less than 50). In the absence of a normal distribution, quantitative data were described using the median (Me) and lower and upper quartiles (Q1–Q3). Categorical data were described using absolute values and percentages. A comparison of two groups for quantitative indicators whose distribution differed from normal was performed using the Mann-Whitney U test. Comparison of percentages in the analysis of four-field contingency tables was performed using Fisher's exact test (for expected phenomenon values less than 10). Comparison of percentages in the analysis of multifield contingency tables was performed using the Pearson chi-square test. Statistical significance was determined using the $\chi 2$ test. P values <0.05 were considered statistically significant.

Results

Participant's characteristics

The sample included 15 patients with esophagus cancer (4 females [26.7%] and 11 males [73.3%]) in the main group and 15 patients without esophagus cancer (5 females [33.3%] and 10 males [66.7%]) in the control group. The mean age in the main group was 70.67 + 10.05 years, ranging from 60 to 80 years. The mean age in the control group was 55.8 + 14.69 years, ranging from 41 to 70 years. Histological examination among patients from the main group identified 8 cases (53.3%) of squamous cell carcinoma and 7 cases (46.7%) of adenocarcinoma.

Table 1. Analysis of the Degree of Differentiation (G) Depending on the Presence of the Virus (HSV-1, HSV-2), Determined Using iIFR

Index	Categories	iIFR (HSV-1, HSV-2)		P.value
		Negative	Positive	
Degree of differentiation (G)	Not evaluated;	14 (87,5%)	1 (7,1%)	< 0,001†
	Well differentiated, low grade (G1);	2 (12,5%)	5 (35,7%)	
	Moderately differentiated, intermediate grade (G2);	0 (0,0%)	5 (35,7%)	
	Poorly differentiated, high grade (G3);	0 (0,0%)	3 (21,4%)	

†, Differences in indicators are statistically significant ($p < 0.05$)

Histology results

Lymphocytic infiltration

In the studied histological specimens stained with hematoxylin-eosin in patients in the main group, areas of severe dysplasia of multilayered epithelium were identified in the walls of the esophagus, sometimes with the presence of polypoid formations and an eroded surface. The formations were represented by merging cords and nests of polymorphic cells with large nuclei, mitoses, and germination into the submucosal and muscular layers. Predominantly lymphocytic infiltration was detected at the border with malignant tissue with the formation of lymphoid follicles in the submucosa of the esophagus (Figure 1 [A-D]). Overall, these histological characteristics indicate the presence of an active immune response to the tumor and indicate a possible association between viruses, including viruses of the Herpesviridae family, and the development of esophageal cancer. Additional research is needed to more accurately determine the role of viruses in the pathogenesis of this disease.

We were able to determine lymphocytic infiltration presence in 13 cases (86.7%) in the main group and 5 cases (33.3%) in the control group. The negative result was 2 cases (13.3%) in the main group and 10 cases (66.7%) in the control group. According to the data obtained, we established statistically significant differences when assessing lymphocytic infiltration depending on the study group (95% CI 2.07 - 81.48%, p=0.008 [method used: Fisher's exact test]). The number of cases in the main group with positive lymphocytic infiltration with a positive iIFR test (HSV-1, HSV-2) was 13 cases (86.67%), while only 2 cases (13.33%) were determined with a negative test (95% CI 2.89 - 283.06%, p=0.001[method used: Fisher's exact test]).

Development of keratinization

In micrographs (Figure 2 [A-D]), it can be seen that the superficial layer of the epithelium is flat and does not

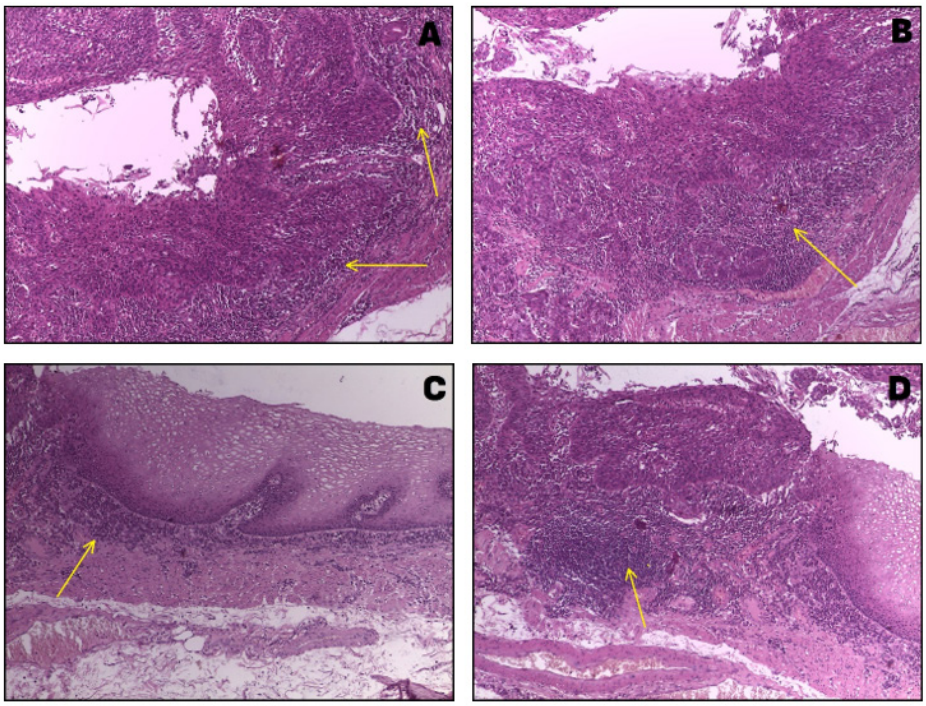

Figure 1. Biopsy Material from Patients with Esophageal Cancer. A, low-grade squamous cell carcinoma of the esophagus without keratinization with invasion into the submucosal layer and pronounced lymphocytic infiltration (arrows); B, moderately differentiated squamous cell carcinoma of the esophagus with leukocyte infiltration (arrow); C, pronounced leukocyte (mainly mononuclear) infiltration of the epithelium, lamina propria and submucosa in the border zone of the tumor and relatively intact tissues of the esophagus (arrow); D, pronounced leukocyte infiltration of tumor tissue with areas of necrosis with the formation of a lymph node (arrow). Staining: Hematoxylin-eosin, Magnification: x100.

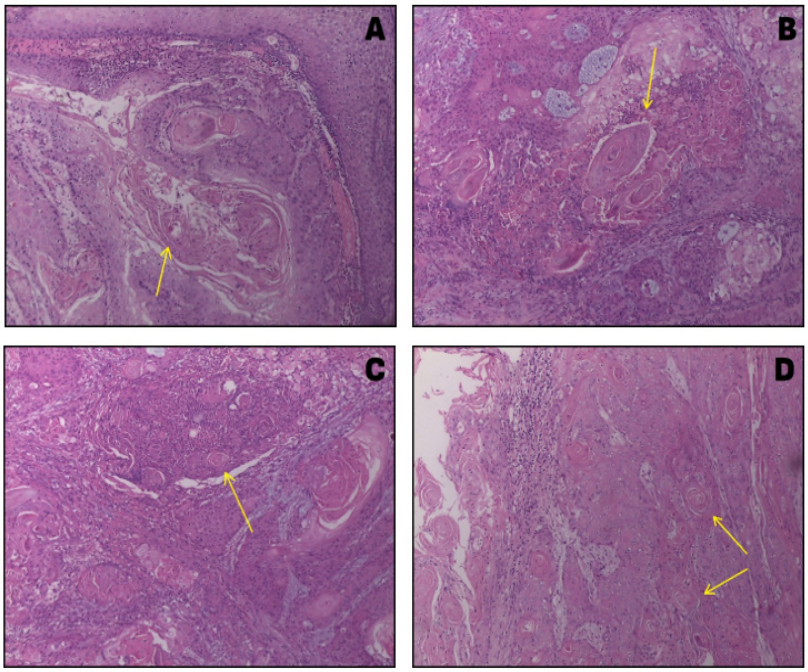

Figure 2. Keratinization Processes in Biopsy Material of Tumor Tissue from Patients with Esophageal Cancer. A, processes of atypical keratinization of tumor cells at the border with normal tissues are visible (arrow); B, C, areas of layering of horny scales on top of each other are visible with the formation of foci of keratosis (cancerous pearls) (arrow); D, multiple areas of keratosis in esophageal cancer (arrows); Staining: Hematoxylin-eosin, Magnification: x100.

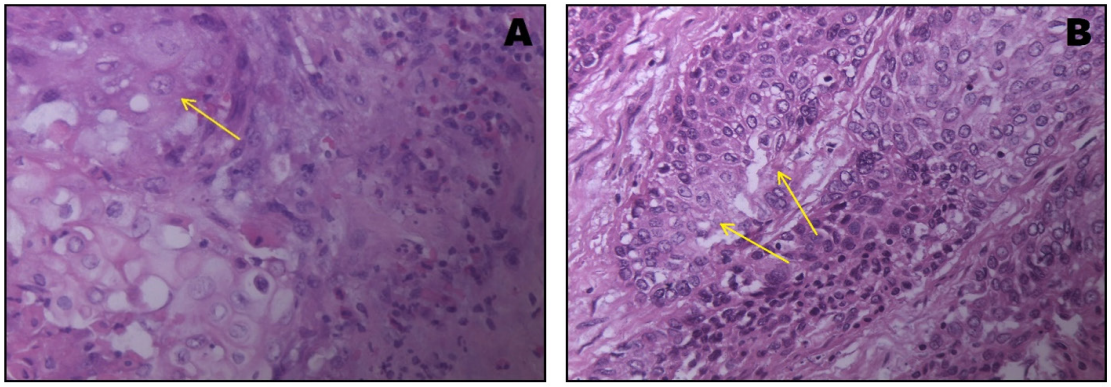

Figure 3. Biopsy Material of Tumor Tissue from Patients with Esophageal Cancer. A, B, giant nuclei and multinuclear elements are visible in cells with esophageal cancer (arrows); Staining: Hematoxylin-eosin, Magnification: x400.

contain nuclei, indicating that it has already undergone a process of pathological keratinization. Keratinization is a process in which superficial epithelial cells lose their nuclei and become a rigid structure with the presence of keratinized remains of dead cells (horny scales). Cells stop dividing and begin to produce keratin, a tough protein matrix that fills the cell's cytoplasm. Keratin forms a dense network inside cells, making them hard and durable. During keratinization, the nuclei and other cellular organelles disappear, so that ultimately only dead cells filled with keratin remain in the tissue. However, the micrographs also show areas of the epithelium where varying degrees of atypia of cell nuclei are still visible, where horny scales, layering on top of each other, form zones (foci of keratosis) called «cancerous pearls», which may indicate the presence of pathological changes associated with the development of cancer. It is also possible to form a structure resembling a bird's eye (early cancer pearls). The number of cases of determination of keratinization in biopsy materials for highly differentiated esophageal cancer was 6 cases, where areas of keratosis were noticeable, which were presented in the form of small grayish-white grains and cancerous pearls. Consequently, based on the data obtained (Table 1), it was found that only in 7 out of 8 cases with verified squamous cell carcinoma of the esophagus, foci of keratinization were detected, while in adenocarcinoma this process was not determined. When studying the materials at the cytological level, nonspecific signs of infected cells were identified, which were multinucleation, the formation of giant nuclei with ground glass chromatin, and eosinophilic intranuclear inclusions (Figure 3 [A-D]). The described cell changes may be accompanied by diffuse lymphocytic infiltration of surrounding tissues, or even accompanied by local

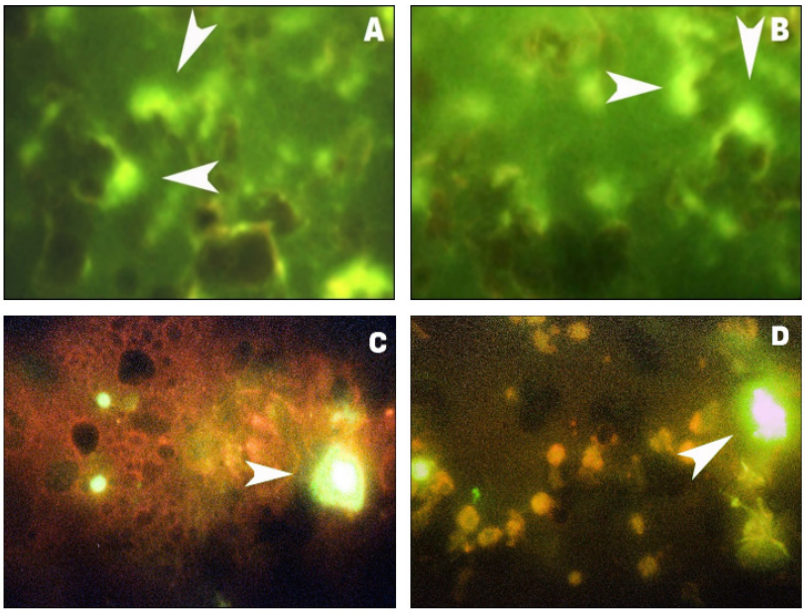

Figure 4. Indirect Immunofluorescence Reaction to Detect the Presence of HSV-1, HSV-2 Antigens (Positive Result). A, B, specific glow in the cytoplasm and nuclei of individual cells of a smear-imprint of biopsy material from a patient with esophageal cancer (arrow) [low contrast], Magnification: x1000. C, D, specific luminescence in the cytoplasm and nuclei of individual cells of a smear-imprint of biopsy material from a patient with esophageal cancer (arrow) [high contrast], Magnification: x1000.

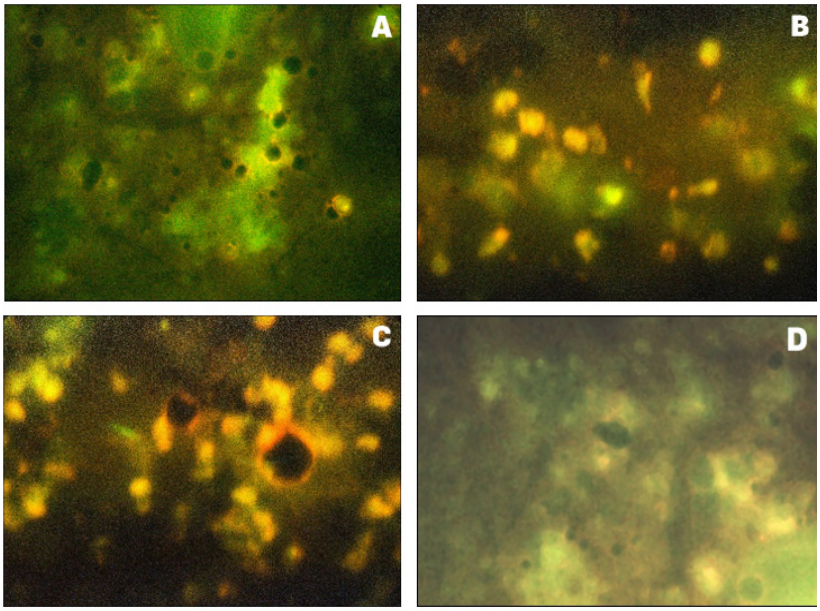

Figure 5. Indirect Immunofluorescence Reaction to Detect the Presence of HSV-1, HSV-2 Antigens (Negative Result). A, D, absence of specific luminescence in the cells of the smear-imprint of the biopsy material of a patient with esophageal cancer (low contrast), Magnification: x1000. B, C, absence of specific luminescence in the cells of the smear-imprint of the biopsy material of a patient with esophageal cancer (high contrast), Magnification: x1000.

formation of lymphoid follicles, which was also noted in precancerous conditions.

Indirect immunofluorescence reaction (iIFR)

The specific fluorescence obtained using an indirect immunofluorescence reaction suggests the presence of antigens of the HSV-1 and HSV-2 viruses in the cells of the esophageal mucosa (Figure 4 [A-D]). This glow occurred in 13 cases in the main group and 1 case in the control group (95% CI 7.35 - 1126.89%, p<0.001 [method used: Fisher's exact test]). The absence of luminescence during microscopy determined the absence of viral activity in the cells of the studied materials (Figure 5 [A-D]). In squamous cell carcinoma, a positive result of iIFR (HSV-1, HSV-2) was determined in 7 cases (87.5%) and 1 case (12.5%) with a negative result. In adenocarcinoma, the positive result of iIFR (HSV-1, HSV-2) was similar to squamous cell and amounted 6 positive cases (85.7%), and the negative result was in 1 case (14.3%). For unconfirmed cancer in control group, a positive result was determined

in 1 case (6.7%) and negative in 14 cases (93.3%), respectively ($p<0.001$) (method used: $\chi 2$ test).

Discussion

Herpes simplex virus types 1 and 2 interact with epithelial cells of the digestive tract through specific receptors on their surface [6]. These receptors are usually found on the cell membrane and may differ depending on the type of cell the virus is attacking. The viral glycoproteins gB, gD, and gH/gL operate sequentially, where each can interact with multiple receptors on the membrane of target cells. We can indicate at least four target cell receptor molecules that are known to bind only to gB and gD [7]. When a virus enters a cell, it begins to replicate using the cellular machinery and resources found within the cell. The viral genes then cause the cell to produce new viral particles, which can then spread to other cells or exit the cell, causing it to die. It is not possible to accurately identify such cells using FEGS. To increase the chances of identifying viruses, a method was used to collect biomaterial from the lesion at the border with healthy tissue.

Recent studies [8, 9] have demonstrated that HSV infections may have a higher association with the pattern of keratinization of cells compared to other viral infections, which confirms our study, where the probability of involvement of HSV-1, HSV-2 was higher in moderately differentiated (keratinized type) squamous cell esophageal cancer. Non-keratinizing squamous epithelium is the epithelial tissue that typically lines the lining of the esophagus, and changes can be associated with a variety of factors, including smoking, alcohol consumption, and poor diet, as well as hereditary factors. Viruses from the Herpesviridae family, such as herpes simplex viruses type 1 and 2, Varicella-Zoster, Epstein-Barr, human herpes virus type 8 associated with Kaposi's Sarcoma, etc., after primary infection can often remain latent in different cells and tissues of the human body, and can be reactivated in an immunodeficiency state, stress, and also in cancer. Depending on the state of the immune system, especially cellular immunity, subject to prolonged and intense functional suppression of T cells, the risk of viral reactivation increases [10, 11].The speed of this process may vary depending on the underlying verified disease [12, 13], its activity and severity [14], the presence of antitumor therapy [12, 13], and age [15].

Reactivation of herpes simplex viruses type 1 and 2 can occur not only with the appearance of local pathognomonic symptoms, but also lead to dissemination of the virus, which, provided the virus is able to infect cells of any tissue, can cause cerebral or visceral diseases [10, 16]. The latent period of HSV-1 and HSV-2 possibly occurs in the intermuscular nerve plexuses (Meissner's plexus, Auerbach's plexus) and with antegrade transport of viruses through dendrites can lead to reinfection of the primary lesion or another site, including esophageal tissue [17]. Although infiltration of inflammatory cells into the myenteric plexus and degeneration of enteric nerves are commonly observed in patients with functional bowel disorders, an association with HSV-1 has not yet been proven, largely due to insufficient understanding of the underlying mechanisms [18]. It is noted that infection is asymptomatic in more than 80% of cases when retrograde transport is involved to damage nervous tissue and subsequent activation of the latent period, where, due to the long lifespan of neurons, the virus can persist for quite a long time [19, 20]. It is worth noting that polymerase chain reaction (PCR) detection of viral DNA by isolating viruses in cell culture and identifying viral antigens in collected tissue samples demonstrates higher rates in post-chemotherapy patients compared to pre-chemotherapy patients, although statistically significant no difference was determined [20, 21]. We believe that the affected neurons may also be cells of the autonomic nervous system (nodes, plexuses), which are also involved in the innervation of the esophagus.

Author Contribution Statement

Study design and conceptualization: BA, VA; Data curation and supervision: BA, VA; Project administration: BA, VA; Formal analysis and validation: BA, VA, BB, VG, AS; Writing- original draft review & editing: BA, VA, BB, VG, AS; Approval of final manuscript: BA, VA, BB, VG, AS. All authors read and approved the final manuscript.

Acknowledgements

General

The authors gratefully thank all study participants who took part in this study. We also thank all health unit coordinators for their help and support.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Ethical Declaration

This study was approved by the ethics committee of NcJSC «Astana Medical University» (Meeting No. 15). Informed consent was obtained from all participants prior to participation.

Approval

It is part of an approved master's thesis at NcJSC «Astana Medical University».

Conflict of Interest

All the authors declare that no conflict of interest.

References

1. Cancer today. Accessed March 6, 2023. http://gco.iarc.fr/today/home.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.
3. Indicators of the Oncology Service of the Republic of Kazakhstan, JSC "Kazakh Research Institute of Oncology

and Radiology", p.168.
4. Xu W, Liu Z, Bao Q, Qian Z. Viruses, other pathogenic microorganisms and esophageal cancer. Gastrointest Tumors. 2015;2(1):2-13. https://doi.org/10.1159/000380897.
5. Herpes simplex virus. Accessed April 7, 2023. https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus.
6. Arii J, Kawaguchi Y. The role of hsv glycoproteins in mediating cell entry. Adv Exp Med Biol. 2018;1045:3-21. https://doi.org/10.1007/978-981-10-7230-7_1.
7. Pellett PE, Roizman B. Herpesviridae. In: Knipe DM, Howley PM, Cohen JI et al (eds) Fields virology, 6th edn. Lippincott-Williams & Wilkins, Philadelphia. Fields virology. 2013;2(6):1802-22.
8. He S, Han J. Manipulation of host cell death pathways by herpes simplex virus. Curr Top Microbiol Immunol. 2023;442:85-103. https://doi.org/10.1007/82_2020_196.
9. van der Beek MT, Laheij AM, Raber-Durlacher JE, von dem Borne PA, Wolterbeek R, van der Blij-de Brouwer CS, et al. Viral loads and antiviral resistance of herpesviruses and oral ulcerations in hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2012;47(9):1222-8. https://doi.org/10.1038/bmt.2012.2.
10. Lee HS, Park JY, Shin SH, Kim SB, Lee JS, Lee A, et al. Herpesviridae viral infections after chemotherapy without antiviral prophylaxis in patients with malignant lymphoma: Incidence and risk factors. Am J Clin Oncol. 2012;35(2):146-50. https://doi.org/10.1097/COC.0b013e318209aa41.
11. Levin MJ, Weinberg A, Schmid DS. Herpes simplex virus and varicella-zoster virus. Microbiol Spectr. 2016;4(3). https://doi.org/10.1128/microbiolspec.DMIH2-0017-2015.
12. Chen SY, Suaya JA, Li Q, Galindo CM, Misurski D, Burstin S, et al. Incidence of herpes zoster in patients with altered immune function. Infection. 2014;42(2):325-34. https://doi.org/10.1007/s15010-013-0550-8.
13. Ruiz-Camps I, Aguilar-Company J. Risk of infection associated with targeted therapies for solid organ and hematological malignancies. Ther Adv Infect Dis. 2021;8:2049936121989548. https://doi.org/10.1177/2049936121989548.
14. Teh BW, Harrison SJ, Worth LJ, Thursky KA, Slavin MA. Infection risk with immunomodulatory and proteasome inhibitor-based therapies across treatment phases for multiple myeloma: A systematic review and meta-analysis. Eur J Cancer. 2016;67:21-37. https://doi.org/10.1016/j.ejca.2016.07.025.
15. Luxton GW, Haverlock S, Coller KE, Antinone SE, Pincetic A, Smith GA. Targeting of herpesvirus capsid transport in axons is coupled to association with specific sets of tegument proteins. Proc Natl Acad Sci U S A. 2005;102(16):5832-7. https://doi.org/10.1073/pnas.0500803102.
16. Tan IL, McArthur JC, Venkatesan A, Nath A. Atypical manifestations and poor outcome of herpes simplex encephalitis in the immunocompromised. Neurology. 2012;79(21):2125-32. https://doi.org/10.1212/WNL.0b013e3182752ceb.
17. Brun P, Qesari M, Marconi PC, Kotsafti A, Porzionato A, Macchi V, et al. Herpes simplex virus type 1 infects enteric neurons and triggers gut dysfunction via macrophage recruitment. Front Cell Infect Microbiol. 2018;8:74. https://doi.org/10.3389/fcimb.2018.00074.
18. Gilden DH, Mahalingam R, Cohrs RJ, Tyler KL. Herpesvirus infections of the nervous system. Nat Clin Pract Neurol. 2007;3(2):82-94. https://doi.org/10.1038/ncpneuro0401.
19. Herget GW, Riede UN, Schmitt-Gräff A, Lübbert M, Neumann-Haefelin D, Köhler G. Generalized herpes simplex virus infection in an immunocompromised patient--report of a case and review of the literature. Pathol Res Pract. 2005;201(2):123-9. https://doi.org/10.1016/j.prp.2004.12.003.
20. Djuric M, Jankovic L, Jovanovic T, Pavlica D, Brkic S, Knezevic A, et al. Prevalence of oral herpes simplex virus reactivation in cancer patients: A comparison of different techniques of viral detection. J Oral Pathol Med. 2009;38(2):167-73. https://doi.org/10.1111/j.1600-0714.2008.00684.x.
21. Tayyar R, Ho D. Herpes simplex virus and varicella zoster virus infections in cancer patients. Viruses. 2023;15(2). https://doi.org/10.3390/v15020439.