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# Is Herpes Simplex Virus (HSV) infection a risk factor for nasopharyngeal carcinoma?

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**ABSTRACT**

. **Objective**: The aim of this study was to screen for the presence of HSV in patients with nasopharyngeal Carcinoma (NPC). **Methodology**: Formalin fixed paraffin wax processed NPC tissue were obtained from 150 tissue blocks and retrospectively investigated for the presence of HSV-1 and HSV-2 using polymerase Chain Reaction (PCR). **Results:** Of the 150 NPC tissue specimens, Herpes Simplex Virus type 2 (HSV-2) was identified in 18/150 (12%) samples. Out of the 18 samples infected with HSV-2, 15/97 (15.5%) among males and 3/53(5.7%) were among females. **Conclusion:** The present have shown some weak links between HSV and NPC. The great majority of samples harboring HSV were also found to harbor EBV, which suggests the potentiality of EBV over HSV.

## INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a common Head and Neck Cancer in China, Taiwan and other Asian countries (Waterhouse. *et al*. 1982). While Epstein–Barr virus (EBV) has been closely linked to NPC (Lun, *et al*. 2014; Tsang, *et al*. 2014), hereditary and environmental factors, such as salted fish, Chinese herbs and long-term exposure to sulfuric acid vapor, have also been supposed to be associated with NPC initiation (Armstrong, *et al*. 1983; Ho, *et al*. 1999). However, the etiological factors have yet to be clearly identified. In some studies attempted to investigate the molecular mechanism of NPC pathogenesis, they established nine NPC cell lines (Lin, *et al*.1990; Lin, *et al*. 1993). Most of these cell lines indicate that EBV plays a major role in promoting tumor progression in NPC pathogenesis (Wu, *et al*. 2003; Ryan and Ray, 2004).

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), are two members of the herpesvirus family, that infect humans (Ryan and Ray, 2004). HSV-1 and HSV-2 have developed several mechanisms to escape host detection and immune responses and usually establish lifelong latency (Kenneth, *et al*. 2014) HSV-1 has been identified in benign and malignant thyroid tumours, whereas HSV-2 has been found to be associated with papillary thyroid cancer and the presence of lymph node metastases (Jensen, *et al*. 2010) Moreover, HSV-2 is associated with prostate cancer, melanoma incidence in both men and women (Thomas, *et al*. 2011), and cervical cancer (Haverkos, *et al*. 2000).

Although the probability of an association between HSV and cancer has been proposed [12,13], but so far it has not been likely to demonstrate that HSV can make primary transformation of normal cells in to malignant. Inoculation of HSV-1 and HSV2 into hamsters rarely leads to the induction of a tumor, and it has been difficult to demonstrate herpes viral antigens in a tumor cells (Duff and Rapp, 1971). However, there is a complete lack of literature regarding the relationship between NPC and HSV, therefore, the aim of this study was to screen for the presence of HSV amongst Sudanese patients with PC.

## MATERIALS AND METHODS

In this study 150 formalin fixed paraffin wax processed tissue samples of nasopharyngeal carcinoma were obtained from earlier operated patients from different histopathology laboratories in Khartoum State, Sudan. All tissue samples were from those who had not yet given anti-cancer therapy. The study was approved by the Ethical Committee of the Research Board of Faculty of Medical Laboratory Science, Sudan University for Science and Technology, Khartoum, Sudan.

DNA Extraction: DNA was extracted from paraffin-embedded samples, by immersing tissue section in xylene to dissolve the paraffin from the tissue, and then rehydrated using a series of ethanol washes. Proteins and harmful enzymes such as nucleases were digested by proteinase K. Buffer containing denaturing agent (sodium dodecyl sulfate (SDS)), was added to facilitate digestion (Hilz, *et al* .1975). Nucleic acids were purified from the tissue lysate using buffer-saturated phenol and high speed centrifugation. Following phenol extractions, RNase A was added to eliminate contaminating RNA. Additional phenol extractions following incubation with RNase A were used to remove any remaining enzyme. Sodium acetate and isopropanol were added to precipitate DNA, and high speed centrifugation was used to pellet the DNA and facilitate isopropanol removal. Washing with 70% ethanol was performed to remove excess salts, followed by centrifugation to re-pellet the DNA (Joseph, 2001; Pikor, *et al*. 2011). DNA is re-suspended in distilled water, quantified and stored at -20°C Purified DNA was subsequently used in downstream applications of PCR.

## RESULTS

This study investigated retrospectively tissue samples obtained from 150 patients diagnosed with nasopharyngeal carcinoma, their ages ranging from 17 to 88 years with a mean age of 51 years. Male female ratio was 5:00to 1.00. Of the 150 NPC tissue specimens, Herpes Simplex Virus type 2 (HSV-2) was identified in 18/150 (12%) samples. Out of the 18 samples infected with HSV-2, 15/97 (15.5%) among males and 3/53(5.7%) were among females. The 95% confidence interval and the odd ratio for sex male/female was 3.049 (840-11.059), P <0.06, as indicated in Fig 1.Moreover, of the 18 HSV positive samples 12/18 (66.7%) were found to harbor EBV.

As indicated in Fig 2, the highest frequency of infection rates were seen among age group (51+) representing 10/77 (13%) followed by age range 21-50 and < 20 years, constituting 7/63(11%) and 1/10(10%), in this order.

In regard to the residence and HSV-2 infection, the great majority of infections were identified among Southern populations, representing 7/22 (24%) followed by Khartoum, Eastern, Northern and Western, constituting 4/27 (14.8%), 3/21(14.3%) and 1/18 (5.4%), respectively, though the West represents the greatest participants, as shown in Table1, Fig 3.

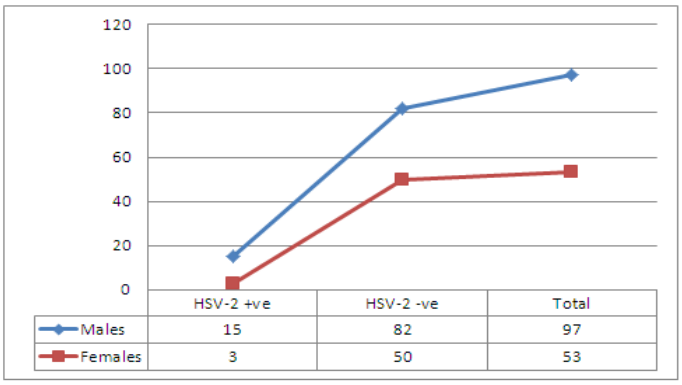


Fig. 1: Description of the study population by sex and HSV-2.

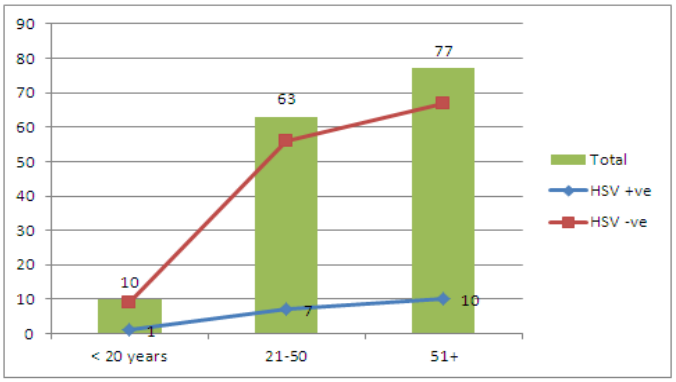


Fig. 2: Description of the study subjects by age

Table 1: Distribution of the study population by residence and HSV-2 infection

| Location | HSV-2 | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Khartoum | 4 | 23 | 27 |
| North | 1 | 17 | 18 |
| South | 7 | 22 | 29 |
| East | 3 | 18 | 21 |
| West | 3 | 52 | 55 |
| Total | 18 | 132 | 150 |

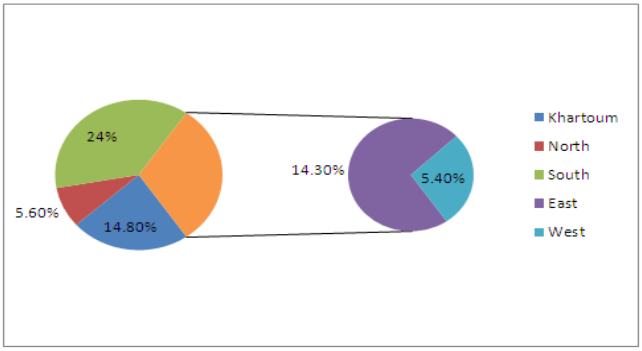


Fig. 3: Description of study population by residence and HSV-2 infection

## DISCUSSION

In the present study we attempted to investigate the relationship between HSV and NPC, though there is an extreme lack of literature in this context. Herpes simplex virus type 1 (HSV-1) is a widespread human pathogen infecting more than 80% of the population worldwide. Its replication involves an essential, poorly understood multistep process, referred to as uncoating (Liashkovich*, et al*. 2011).

However, HSV impair the immune system by disturbing T-cell receptor signalling. These viruses inhibit the T-cell receptor (TCR)-stimulated formation of a linker required for the activation of a T-cell signalling complex. As a consequence, TCR-stimulated NF-κB activation and selective TCR-stimulated interleukin-10 synthesis are inhibited, which in turn favoured viral replication and blocked cellular immunity (Sloan, *et al*. 2007). HSV was found to inhibit the type I interferon response (Murphy, *et al*. 2003), and HSV-1 infected cells can resist T-cell induced apoptosis through expression of the Us5 gene product gJ (Jerome, *et al*. 2001). The relationship between HSV immune disturbance and cancer can be expressed by the hypothesis of tumour immunosurveillance. It was stated that the human body employs its natural defences and exhibits immunological resistance to the development of cancer. This immunological response happens during the early stages of cancer growth. The clinically noticeable signs of tumour disease can only be seen after the cancerous cells have already escaped the immune response (Peto, 2001).

However, although the percentage of infection with HSV is relatively low among NPC, but most of these cases have a co-infection with EBV prime suspect risk factor for NPC. *In vitro* and animal models suggest that the HSV-1 may play a role in the development of oropharyngeal squamous cell carcinoma (OSCC) (Jacqueline*, et al*. 2000). The persistence of the HSV in the oral cavity and its capability to stimulate host DNA synthesis and repair during reactivations proposes that it may contribute to OSCC development. In fact, *in vitro* studies have clarified specific mechanisms over which HSV1 may induce the transformation of human cells: HSV1 infection of human cell cultures has been revealed to be mutagenic (Das, *et al*. 1998), and inhibit apoptosis (Jerome, *et al.* 1998), which may contribute to carcinogenesis (Polverini and Nor, 1999). Some studies found that HSV1 was particularly associated with OSCC risk when other risk factors, such as cigarette smoking or a history of HPV infection, are present (Jacqueline*, et al*. 2000). Furthermore, the significant presence of co-infection between HSV and EBV, may have synergistic effects in the development of NPC.

Regarding age most of HSV infections were observed among elder patients. Also regarding residence, most infections were seen among those patients coming from South.

In conclusion: The present have shown some weak links between HSV and NPC. The great majority of samples harboring HSV were also found to harbor EBV, which suggests the potentiality of EBV over HSV.

## REFERENCES

Armstrong RW, Armstrong MJ, Yu CM, (1983). Salted fish and inhalants as risk factor for nasopharyngeal carcinoma in Malaysian Chinese. *Cancer Res.,* 43: 2967–2970.

Das C M, Zhang S, Shillitoe E J. (1994). Expression of the mutagenic peptide of herpes simplex virus type 1 in virus-infected cells. Virus Res., 34: 97-114.

Duff R and Rapp F. (1971). Oncogenic Transformation of Hamster Cells after Exposure to Herpes Simplex Virus Type 2. Nature new biology., 233:48-50.

Haverkos H, Rohrer M, Pickworth W. (2000). The cause of invasive cervical cancer could be multifactorial. *Biomed Pharmacother*, 54(1):54-59.

Hilz H, Wiegers U and Adamietz P. (1975). Stimulation of proteinase K action by

denaturing agents: application to the isolation of nucleic acids and the degradation of 'masked' proteins. *Eur J Biochem*, 56:103-108.

Ho CK, Lo WCH, Huang PH. (1999). Suspected nasopharyngeal carcinoma in three workers with long-term exposure to sulfuric acid vapor. *Occup Environ Med*, 56:426–428.

Jacqueline R Starr, Janet R Daling, E Dawn Fitzgibbons, (2001). Serologic Evidence of Herpes Simplex Virus Infection and Oropharyngeal Cancer Risk Cancer Res., 61:8459.

Jensen K, Patel A, Larin A, Hoperia V, Saji M, Bauer A, Yim K, Hemming V, Vasko V. (2010). Human herpes simplex viruses in benign and malignant thyroid tumours. *J Pathol*, 221(2):193-200.

Jerome KR, Fox R, Chen Z, Sarkar P. and Corey, L. (2001). Inhibition of apoptosis by primary isolates of herpes simplex virus. *Arch Virol*, 146(11): 2219-2225.

Jerome K. R., Tait J. F., Koelle D. M., Corey L. (1998). Herpes simplex virus type 1 renders infected cells resistant to cytotoxic T-lymphocyte-induced apoptosis. J. Virol., 72:436-441.

Joseph Sambrook, DR. (2001). *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Kenneth Alibek, Yeldar Baiken, Ainur Kakpenova, (2014). Implication of human herpesviruses in oncogenesis through immune evasion and supression. *Infectious Agents and Cancer*, 9:3.

Liashkovich I, Hafezi W, Kühn JM, Oberleithner H, and Shahin V. (2011). Nuclear delivery mechanism of herpes simplex virus type 1 genome. J Mol Recognit., May-Jun; 24(3):414-21.

Lin CT, Wong CI, and Chan WY. (1990). Establishment and characterization of two nasopharyngeal carcinoma cell lines. *Lab Invest*, 62:713–724.

Lin CT, Chan WY and Chen W. (1993). Characterization of seven newly established nasopharyngeal carcinoma cell lines. *Lab Invest* 68:716–727.

Lin CT, Kao HJ and Lin JL. (2000). Response of nasopharyngeal carcinoma cells to Epstein–Barr virus infection *in vitro*. *Lab Invest*, 80:1149–1160.

Lun SW, Cheung ST. and Lo KW. (2014). Cancer stem-like cells in Epstein-Barr virus-associated nasopharyngeal carcinoma. Chin J Cancer., 33(11):529-38.

Murphy JA, Duerst RJ, Smith TJ. and Morrison LA. (2003). Herpes simplex virus type 2 virion host shutoff protein regulates alpha/beta interferon but not adaptive immune responses during primary infection in vivo. *J Virol*, 77(17):9337-9345.

Peto J. (2001). Cancer epidemiology in the last century and the next decade. Nature 411(6835):390-395.

Pikor LA, Enfield KSS, Cameron H, Lam WL. (2011). DNA Extraction from Paraffin Embedded Material for Genetic and Epigenetic Analyses. J. Vis. Exp., 49: e2763.

Polverini PJ, Nor JE. (1999). Apoptosis and predisposition to oral cancer. Crit. Rev. Oral Biol. Med., 10: 139-152.

Ryan KJ, Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 555–62.

Sloan DD, Jerome KR. (2007). Herpes simplex virus remodels T-cell receptor signaling, resulting in p38-dependent selective synthesis of interleukin-10. J Virol, 81(22): 12504-12514.

Thomas F, Elguero E, Brodeur J, Le Goff J, Missé D. (2011). Herpes simplex virus type 2 and cancer: a medical geography approach. Infect Genet Evol, 11(6):1239-1242.

Tsang CM, Deng W, Yip YL, Zeng MS, Lo KW, Tsao SW. (2014). Epstein-Barr virus infection and persistence in nasopharyngeal epithelial cells. Chin J Cancer., 33(11): 549-555.

Waterhouse L, Muir C, Shanmugaratnam K. (1982). Cancer Incidence in Five Continents, International Agency for

Research on Cancer Scientific Publication 42,Vol. IV. International Agency for Research on Cancer: Lyon,

Wu HC, Lin YJ, Lee JJ. (2003). Functional analysis of EBV in NPC cell. *Lab Invest* 83:797–812.

## ARABIC SUMMARY

**هل الإصابة بفيروس الهربس البسيط (HSV) عامل خطر لسرطان البلعوم؟**

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**الهدف**: كان الهدف من هذه الدراسة للكشف عن وجود فيروس الهربس البسيط في المرضى الذين يعانون من سرطان البلعوم (NPC) .

**الطريقة**: تم الحصول على ١٥٠ كتله نسيجية باثر رجعي من شمع البرافين المثبت بالفورمالين، تم التحقق من فيروس هربس البسيط HSV-1 و HSV-2 باستخدام تفاعل البلمره التسلسلي (بى سى ار).

**النتائج**: من ١٥٠ عينه نسيجية من سرطان البلعوم ، تم الكشف عن فيروس هربس البسيط من النوع ٢ (HSV-2) في ١٥٠/١٨ (١٢٪) عينة. من ١٨ عينة مصابة ب فيروس هربس البسيط ٢ ( HSV-2)، ٩٧/١٥ (١٥.٥%) كانوا ذكورا و٥٣/٣ (٥.٧٪) كانوا إناثا.

**الخلاصة**: التقرير الحالي اظهر بعض الروابط الضعيفة بين HSV وسرطان البلعوم. تم العثور فيروس الهربس البسيط (HSV) وايضا فيروس ابشتاين بار(EBV) على الغالبية العظمى من العينات مما يشير إلى إمكان حدوث EBV خلال HSV.