Pakistan Journal of Otolaryngology 2004, 20: 38-39 **Editorial**

Conventional Biopsy to EBV Amplification Kit in Quick Diagnosis of Nasopharyngeal Carcinoma

Shahid Hassan

Nasopharyngeal carcinoma (NPC) is the second most commonly occurring cancer amongst men after lung cancer in Malaysia [1] The relative frequency in South East Asia and China is reported somewhere around 8% of all malignancies in specialized clinics It is a leading cancer in Southern China and Hong Kong, the highest concentration being 18% in certain racial areas of this part of the world On the average the annual incidence in Hong Kong is 23.3 per 100,000 population.[2]

Epstein Barr Virus (EBV) is associated with all types of NPC and the DNA of the virus is found in every cancer cell. EBV genomes consistently found in undifferentiated and well differentiated lesions have also been reported in pre-invasive state of NPC [3] Current method of diagnosis is by biopsy of nasopharynx and histopathological examination Delay in diagnosis may be attributed to some misleading symptoms of, neck mass (43-55%), rhinologic (20-30%) and otologic (10-13%).[4] Other factors include, late presentation occult primaries (13.3%), false negative biopsies (submucosal disease) and low accuracy for FNAC. Radiotherapy is the primary modality of treatment and now 100% cure has been claimed in T1 No lesions

The conventional diagnostic method by nasopharyngeal biopsies and fine needle aspiration of neck lumps have high rate of false negatives Serological test for EBV antibody titre is also less helpful due to low sensitivity and specificity. EBV DNA in tumor cells detected by polymerase chain reaction (PCR) has been shown to be more reliable and able to detect the cancer before it develops clinically. The author and his team in University Sciences Malaysia has successfully conducted a study to evaluate the validity and reliability of detecting EBV genes in fine needle aspirates and biopsy tissue in NPC by PCR EBV products $EBNA_1$ and $EBNA_2$ and LMP_1 (generally implicated in oncogenesis) together with Beta-actin gene were analyzed with adequate sample in this study for the first time in Southeast Asia This was a step forward towards the ultimate diagnosis of NPC which otherwise may lead to an advance disease with regional metastasis adversely affecting the prognosis due to delay in diagnosis This and a few similar studies have given new hope towards the accurate diagnosis of NPC which has inherent pitfalls in detection by conventional methods based on histopathology.

This study has investigated the presence of EBV DNA in the tissue obtained from primary nasopharynx and the metastasis in the neck. A randomized case control study recently conducted in University Sciences Malaysia by Author et al with highest ever sample size, utilizing polymerase chain reaction method has shown the test statistically reliable with Kappa showing excellent inter-test agreement of 9.71 (HPE the gold standard) and likelihood ratio of >15 with single $EBNA_1$ gene detection In two control subjects of the same study it was established that PCR analysis was helpful in predicting the lesion as NPC with an obvious lesion appearing later in 6 months. Similarly unknown primaries in few cases with positive tissues on the needle aspirates from occult neck metastasis, by this method were also proven to be NPC

The outcome of this study suggests that EBV DNA detection is a highly sensitive and specific technique in diagnosing metastatic NPC and therefore may be used to guide the diagnostic workup of occult primaries. This will necessitate a careful repeat biopsy technique under direct vision from an anatomically occult area of nasopharynx Further studies may be required to detect the rate of EBV DNA detection in other tumors like Hodgkin's, Burkit's and B-cell lymphoma and lympho-epithelial carcinoma of salivary gland from the Head and Neck region However rarity makes it difficult to obtain tissue and investigate the conditions associated with EBV.

Department of Otorhinolaryngology - Head & Neck Surgery, School of Medical Sciences, University Sciences Malaysia

Histopathology remains the gold standard for diagnosing NPC from the primary site, though it is not without limitations as discussed above. EBV DNA detection can help when HPE fails to detect the malignancy. In clinically doubtful cases, the presence of EBV DNA detection should raise the index of suspicions leading to a prompt repeat biopsy under general anaesthesia if necessary.

In non-suspicious cases EBV DNA may indicate pre-invasive stage to be followed up closely with repeat biopsy every 03 months In occult primaries in Malaysian community, the presence of EBV DNA will hugely suggest NPC if lymphoma has been ruled out by cytology as NPC is very common here. Especially in Chinese and Malay races one is almost certain that occult primary with EBV DNA has originated from the nasopharynx.

Current Application - Developing a Diagnostic Kit: After the results obtained from this statistically significant study with a large sample size which has proven the presence of EBV DNA in NPC in Malaysia we can proceed to optimize this test. The very idea has helped us in creating a diagnostic kit that is cheap and simple to use as compared to a PCR based test which requires cold storage, reagent transportation and repeated freezing and thawing giving rise to erroneous results.

To achieve EBV amplification (EBV Amp) diagnostic kit, this test needed to be converted to multiplex form, i.e all genes be amplified at once in a single tube and this is feasible as all 4 genes have discretely different product size and can easily be discerned on ethidium bromide stained electrophoresis gel. We have finally developed the EBV Amp Kit that contains thermostabilized PCR reagents in dry powder form in 0 5ml tube and can be easily transported without an ice box. The test has been proven to be 97% sensitive and 100% specific for EBV. The amplified product sizes are $EBNA_1$, $EBNA_2$, LMP_1 and Beta-human actin gene and an internal control DNA extraction from tissue performed in standard way can be added to thermostabilized PCR mix tube and operated in PCR. machine to be finally analyzed on UV trans-illuminator. The result obtained is interpreted in a specified manner. The test in rapid and a result can be obtained in 3 hours after adding 20 microlitre of extracted DNA into the test tube containing PCR mastermix, followed by polymerase chainreaction (PCR) and gel electrophoresis.

Acknowledgements:

To University Sciences Malaysia for funding this project through short term grant. ITEX (International Invention and Technology Exhibition 2004, for awarding Gold Medal awards for the best innovation for the year 2004.

This kit was also awarded the World Intellectual Property Organization as an overall best invention for year 2004 and the cost award, which covers the patenting cost

References:

1. Parathp K Prasad V Ablash D V. The pathology of nasopharyngeal carcinoma in Malaysia. In nasopharyngeal carcinoma current concept University of Malysia press, Kuala lumpur 1983
2. Huang P. Dolly, Lo Kowk-Kai, Aetiological factors and pathogenesis In Nasopharyngeal carcinoma, 2nd edition The Chinese University Press Hong Kong 1999
3. De The G Epidemiology of Epstein-Barr virus in man and associated disease in man In Herpes virus, vol 1, Ed Roimfzman, B USA Plenum press, 1982, 25-87
4. Van Hassett C Andrew, Leung Fai Sing Clinical picture. In Nasopharyngeal carcinoma, 2nd edition The Chinese University Press Hong Kong 1999