Expression of MSH2 in Head and Neck Lymphomas
(A Study Utilizing Immunohistochemistry and Real-time Polymerase Chain Reaction)

Safa Fathi Abdel-Ghani1, Rehab Fawzi Kasem 2, Dina Soliman Khater1

1&3 Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University. 2Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University and the Head of the Oral Pathology Department, Faculty of Dentistry, October University of Modern Science and Arts (MSA).

Introduction: **Lymphomas are divided into Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). In Egypt, lymphomas have represented an important oncologic problem, especially NHL which was considered as one of the five most common cancers, accounting for 4.64% of all cancers. The DNA mismatch repair (MMR) genes are responsible for maintaining the fidelity of DNA replication by recognizing mis-incorporation errors and facilitating their excision. Of this family, Mut-S homolog gene located on chromosome 2p22, MSH2 is principally involved in a phenomenon known as microsatellite instability (MSI). Studies have proposed that mutations in MSH2, could be one of the critical genes that underlie a novel pathway of tumourogenesisi for some cancers. MSI and MMR proteins might be involved in the pathogenesis of lymphomas.**

Aim: **This study was conducted to invstigate the expression of MSH2 at the gene and protein levels in HL and NHL of the head and neck which could contribute to better understanding of its role in development, tumorigenesis, prognosis and treatment of these malignancies.**

Materials and Methods:

1-Case selection: **A total of 26 specimens were used (6 cases of normal lymph nodes (NLs) obtained from elective neck dissection of oral squamous cell carcinoma with a clinical staging N=0 (used as control group), 6 cases of lingual tonsils with lymphoid hyperplasia (LH), and 14 cases of lymphomas of the submandibular and sublingual lymph nodes (HL, 6 cases of mixed cellularity type and NHL 8 cases of diffuse B cell lymphoma classified according to the REAL classification (Revised European American Lymphoma Classification)) . All cases were retrieved from the archival paraffin blocks from files of Pathology Department, National cancer institute, Cairo University.**

2. Immunohistochemical staining protocol: **Paraffin embedded sections of 4ml of all specimens were mounted on positively charged glass slides Optiplus;Biogenex, Milmont Drive, CA, USA) to be stained with primary antibody mouse monoclonal anti-MSH2 antibody (Cat #MS-1498-R7, Thermo Scientific, Labvision, Kalamazoo, MI, USA).**

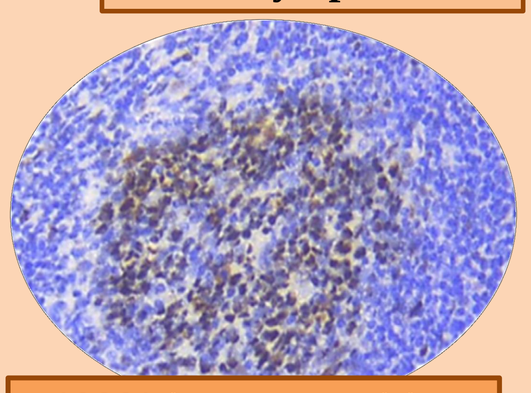
3. Immunohistochemical evaluation: **Light microscope was used to detect and localize the immunostaining of anti-MSH2 antibody. Cells with nuclear staining were considered positive. An image analyzer computer system using the software Leica Qwin 500 (Leica Microsystems Imaging Solutions Ltd, Cambridge, UK). Were used to count MSH2 positive cells in the five fields at magnification of X400.**

***4. Quantitative real-time PCR (qRT-PCR) analysis for MSH2 expression*:MSH2 mRNA were extracted from paraffin sections. The cDNA was amplified for the expression of MSH2 and β-actin with SYBR Green Universal Master Mix (2X) (Applied Biosystems, Warrington, WA1 4SR, UK) according to the manufacturer's protocol. The relative quantification of MSH2 gene was determined using the comparative CT method. Relative expression of the target gene was calculated by the equation $2^{-\Delta\Delta Ct}$, which was the amount of MSH2 product, normalized to the endogenous control (β-actin) and relative to the control sample.**

Results:

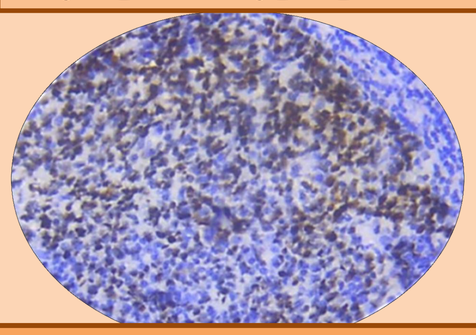
1-Immunohistochemical detection of MSH2

Normal lymph nodes



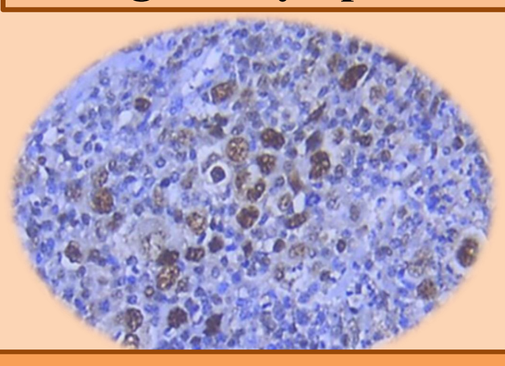
100% immunopositive

Lingual tonsils with lymphoid hyperplasia



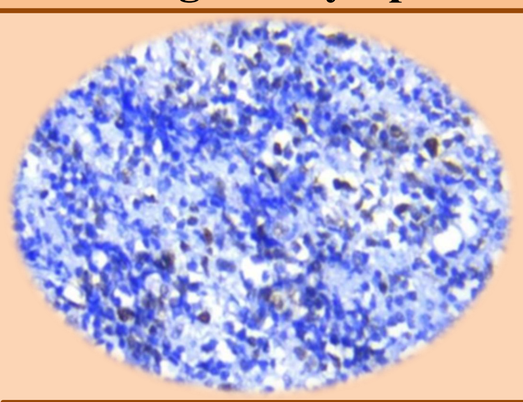
-100% immunopositive
-Positivity was detected within germinal centers as well as within diffuse lymphocytes

Hodgkin's lymphoma



83% immunopositive. The immunopositivity was mainly localized to malignant histocytes, however, some malignant lymphocytes were positive

Non-Hodgkin's lymphoma

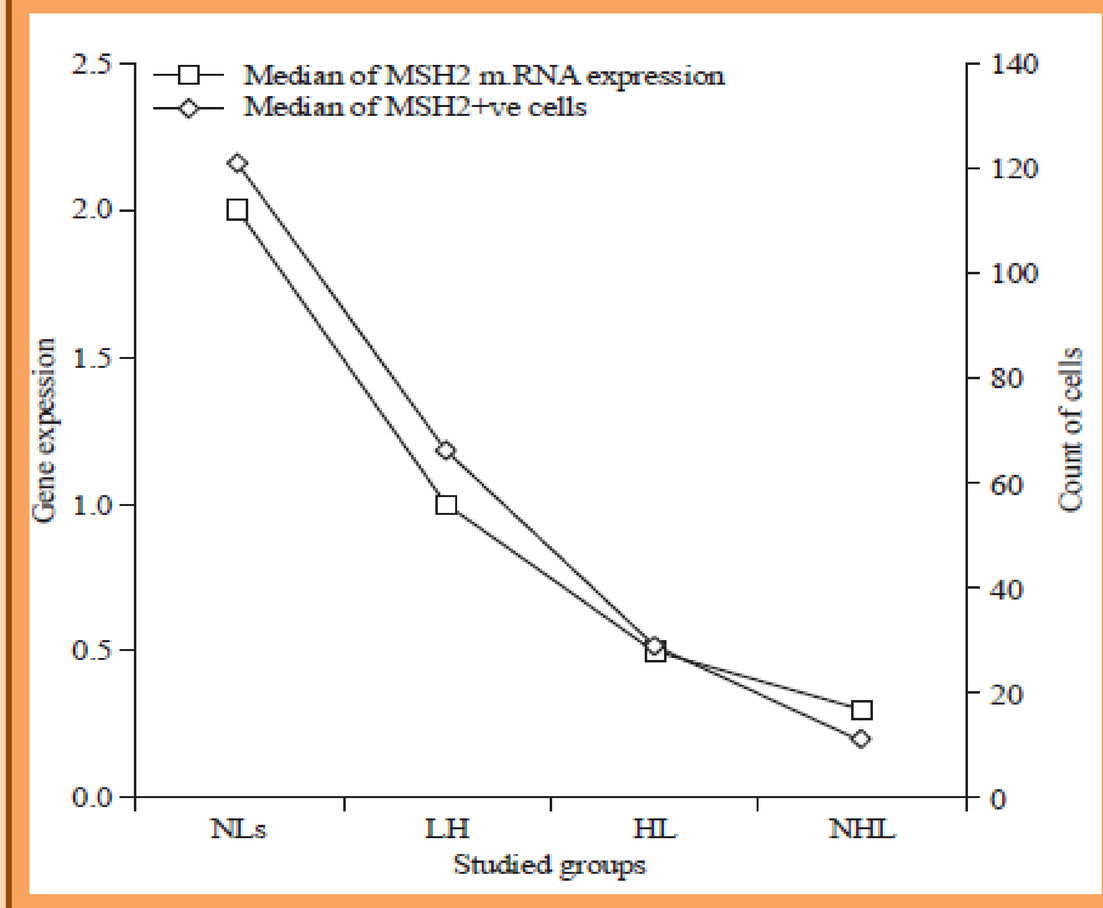


62.5% immunopositive. Few malignant lymphocytes were anti-MSH2 positive.

A significant decrease in the count of MSH2 positive cells was detected from normal lymph nodes (control group) to lymphoid hyperplasia, HL and finally NHL ($p \leq 0.0046$).

2-Expresssion of MSH2 mRNA by qRT-PCR

MSH2 mRNA was detected in all studied cases. statistically significant decrease in mRNA concentration was detected among the studied cases ($p \leq 0.007$) from normal lymph nodes (control group) followed by lymphoid hyperplasia, HL, and the lowest levels were observed in NHL cases.



A strong positive linear correlation was detected between the expression of MSH2 protein and its mRNA among the studied cases (R=1), (Spearman's correlation test)

Conclusion: **The reduced expression of MSH2 in malignant lymphocytes could be considered as a biomarker for loss of MMR activities in malignant cells of lymphoma, and might aid in prediction of prognosis of such lesions. Moreover, it might be used as a potential diagnostic marker to assess the risk of malignant transformation in lymphoid tissues.**