The Use of Office-Based Contact Rhinoscopy for *In Vivo* Real-Time Diagnosis of Nasopharyngeal Carcinoma

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In memory of my father

To Lorraine, Eunice and Charlotte

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LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
CAM	Cellular adhesion molecules
CCD	Charge-coupled device
CI	Confidence interval
CR	Contact rhinoscopy
CT	Computed tomography
DNA	Deoxyribonucleic acid
EA	Early antigen
EBER	Epstein-Barr virus-encoded RNAs
EBNA	Epstein-Barr virus associated nuclear antigen
EBV	Epstein-Barr virus
ERT	External radiotherapy
f	Focal length
FDG	Fluoro-deoxy-D-glucose
FNAC	Fine needle aspiration cytology
GIT	Gastrointestinal tract
Gy	Gray, unit of absorbed radiation
H & E	Hematoxyline and Eosin
HLA	Human leukocyte antigen
IARC	International agency for research on cancer
ICAM	Intercellular adhesive molecules
IgA	Immunoglobulin A
IMRT	Intensity modulated radiotherapy

ISH	In-situ hybridization
LMA	Late membrane antigen
LMP	Latent membrane protein
MDM-2 protein	The murine double minute 2 oncoprotein
MRI	Magnetic resonance imaging
NA	Numerical aperture
NBI	Narrow band imaging
NP	Nasopharynx
NPC	Nasopharyngeal carcinoma
NPCIN	Nasopharyngeal intraepithelial neoplasia
NPCIS	Nasopharyngeal carcinoma-in-situ
NPV	Negative predictive value
NTSC	National Television System Committee
PAL	Phase Alternating Line
Pap	Papanicolaou (smear)
PCR	Polymerase chain reaction
PET	Positron emission tomography
PPV	Positive predictive value
PWH	Prince of Wales Hospital
RBG	Red, blue and green
RE	Radiation encephalopathy
RNA	Ribonucleic acid
RT	Radiation therapy
SD	Standard deviation
SP	Superior performance

S-VHS	Super Video Home System
UICC	Unio Internationale Contra Cancrum or International Union Against Cancer
US	Ultrasonography
VCA	Viral capsid antigen
VCAM	Blood vessel cellular adhesion molecules
WHO	World Health Organization
y	Years of age
ZEBRA	BamHIZ Epstein-Barr virus replication activator

LIST OF PUBLICATIONS RELATED TO THIS THESIS

The following clinical studies used in this thesis have been submitted or published as original articles in indexed journals:

1. **Contact Rhinoscopy and the Pain Perceived – Comparison to Nasal Endoscopy**
   Pak MW, Lee DLY, Vlantis AC, Woo JKS, van Hasselt CA
   Submitted to *The Laryngoscope*

2. ***In vivo* Diagnosis of Nasopharyngeal Carcinoma using Contact Rhinoscopy.**
   Pak MW, To KF, Leung SF, van Hasselt CA.
   The Laryngoscope August 2001; 111:1453-1458.

3. ***In vivo* Diagnosis of Persistent and Recurrent Nasopharyngeal Carcinoma by Contact Endoscopy.**
   Pak MW, To KF, Leung SF, van Hasselt CA.
   T*he Laryngoscope* August 2002; 112: 1459- 1466.

4. **The Choice of Chromogen and Reliability of Contact Rhinoscopy in Irradiated Nasopharynx.**
   Pak MW, Chow S, van Hasselt CA.
   J Laryngol Otol 2008; 122: 177-180.

5. **How Reliable is Contact Endoscopy of the Nasopharynx in Patients with Nasopharyngeal Cancer?**
   Pak MW, Vlantis AC, Chow S, van Hasselt CA.
   The Laryngoscope March 2009; 119:523-527.

The following articles have also been quoted in this thesis:

6. **Nasopharyngeal Carcinoma in Situ (NPCIS) - Pathological and Clinical Perspectives.**

   Pak MW, To KF, Lo DYM, Chan LY, Tong JH, Lo KW, van Hasselt CA.
   Head Neck 2002; 24:989-995.

7. ***In vivo* Real-Time Diagnosis of Nasopharyngeal Carcinoma in Situ by Contact Rhinoscopy.**

   Pak MW, To KF, Lee JCK, Liang EY, van Hasselt CA.
   Head Neck 2005; 27:1008-1013.

8. **Retropharyngeal Abscess. A Rare Presentation of Nasopharyngeal Carcinoma.**

   Pak MW, Lai KL, van Hasselt CA.
   J Laryngol Otol January 1999;113:70-72.

DECLARATION OF ORIGINALITY

The work contained in this thesis is completely original, and has not been submitted for any other degree. I was responsible for the study design, conduction of clinical trials, endoscopic examination, data collection, analysis of results and writing of the reports.

The endoscopic examinations were carried out in the out-patient clinics of the Department of Otorhinolaryngology, Head and Neck Surgery of the Prince of Wales Hospital. The clinical studies were conducted in the Department of Otorhinolaryngology, Head and Neck Surgery of the Chinese University of Hong Kong under the supervision of Professor Charles Andrew van Hasselt, Professor and Chairman of Department of Otorhinolaryngology, Head and Neck Surgery, of the Chinese University of Hong Kong and Professor Alexander Chris Vlantis, Associate Professor of Department of Otorhinolaryngology, Head and Neck Surgery, of the Chinese University of Hong Kong.

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PRÉCIS TO THE THESIS

(I) BACKGROUND

Nasopharyngeal carcinoma (NPC) is a commonly occurring malignancy in southern China. It has a unique genetic predisposition and is closely related with Epstein-Barr virus (EBV) infection and environmental carcinogenic factors. As the early symptoms are usually vague and non-specific, the diagnosis of primary NPC can be difficult and delayed. Traditionally, the detection of primary NPC depends on endoscopic examination of the nasopharynx and the use of investigative procedures including EBV serology, computed tomography (CT), or magnetic resonance imaging (MRI). External radiation therapy (RT) is the typical treatment of a non-disseminated disease and has a five-year local control rate of 70% to 80%. Early detection of a recurrence of NPC is important for the manageable treatment of the disease.

Although primary NPC can often be recognized by conventional nasal endoscopy, minute lesions, sub-mucosal disease, or nasopharyngeal carcinoma-in-situ (NPCIN) lesions may be missed during this procedure. Furthermore, inflammation of the nasopharynx and the tendency for malignant cells to grow submucosally render the clinical detection of early residual and recurrent disease in an irradiated nasopharynx very difficult. Clinical studies have shown that the diagnostic yield of a biopsy guided by conventional endoscopy in an irradiated nasopharynx is less than 50%. Similarly, CT and MRI are not reliable enough to detect signs of early recurrence because fibrosis, inflammation and tissue oedema may simulate a recurrence of the disease. Although [^{18}F]-2-fluoro-deoxy-D-glucose positron emission tomography (PET) has been found to be sensitive and specific enough for the detection of NPC, most of the

false positives arise from inflammatory changes and infection during the early post-radiation period. For this reason caution should be exercised when PET is employed earlier than six months after treatment. The development of a more accurate and reliable diagnostic tool to detect subtle foci of primary and early residual or recurrent disease is warranted.

Contact endoscopy was first introduced by Hamou in 1979, as microcolpohysteroscopy, to examine the surfaces of the female genital tract. The endoscope was designed so that its tip could make direct contact with the surface of living tissues, thus allowing real-time *in vivo* examination of vital-stained superficial cells of the tissues at high magnification. This innovative technique was first used to examine the mucosa of the vocal cords under general anaesthesia by Andrea and Dias in 1995. However, the wide calibre of the contact laryngoscope (Karl Storz, 8715A, 5.5mm∅, Tuttlingen, Germany) made it unsuitable for use in the nose and the nasopharynx.

In 1997, a newly designed contact rhinoscope with a smaller shaft was introduced (Karl Storz, 7215AA and 7215BA, 4mm∅, Tuttlingen, Germany). Its slim design allows easy transnasal examination of the nasopharynx under local anaesthesia. This contact rhinoscope can be used not only for examination of the general appearance of the nasopharynx, in the same way as conventional endoscopy, but also for a real-time, *in vivo* examination of the cellular details of the superficial epithelium. It has thus emerged as an appealing office-based aid for the diagnosis of primary and recurrent NPC.

Since 1999, my colleagues and I have undertaken a series of qualitative and quantitative studies to explore the potential role of contact rhinoscopy in the diagnosis of NPC. It is believed that this is the first series of studies ever published on contact rhinoscopy for the diagnosis of NPC.

(II) OBJECTIVES OF THE STUDY

To study the feasibility, tolerability, acceptability, accuracy and reliability of contact rhinoscopy in the diagnosis of NPC, a series of studies have been undertaken. The objectives of these studies were:

1. To evaluate the patients' tolerance and acceptance of contact rhinoscopy.
2. To investigate the accuracy of contact rhinoscopy in the diagnosis of primary NPC.
3. To investigate the accuracy of contact rhinoscopy in the diagnosis of persistent and recurrent NPC after radiation therapy.
4. To identify the optimal concentration of chromogen to be used in contact rhinoscopy.
5. To investigate the reproducibility of the accuracy of contact rhinoscopy in diagnosing primary and recurrent NPC among different assessors.

(III) HYPOTHESES

It was hypothesized that:

1. Contact rhinoscopy is tolerated and accepted by most patients. There is no significant difference in the pain score between contact rhinoscopy and conventional rigid endoscopy.

2. Contact rhinoscopy can provide a real-time differentiation between normal nasopharyngeal mucosa and primary NPC. The sensitivity of contact rhinoscopy for the detection of primary NPC is as high as that of conventional nasal endoscopy.
3. Contact rhinoscopy can provide a real-time identification of different mucosal patterns in the irradiated nasopharynx. The sensitivity for detecting persistent and recurrent diseases of NPC by contact rhinoscopy is higher than that of conventional nasal endoscopy.
4. There is a significant difference in visual clarity of cellular detail between 0.5% methylene blue and 1% methylene blue when used in contact rhinoscopy.
5. The accuracy of contact rhinoscopy for diagnosing primary and recurrent NPC is reproducible among different assessors.

(IV) CLINICAL STUDIES

1. Comparison of the perception of pain in patients undergoing contact rhinoscopy and those undergoing conventional nasal endoscopy

Nasal endoscopy using rigid endoscopes is one of the standard procedures for the examination of the nasopharynx. Previous studies have shown that after administration of local anaesthetic and vasoconstrictive agents, rigid endoscopy can be performed with minimal discomfort.

Contact rhinoscopy is a new and innovative procedure which allows real-time *in vivo* diagnosis of nasopharyngeal pathology under local anaesthesia. A prospective, matched, case-control study was undertaken to assess the feasibility, tolerance and acceptability of contact rhinoscopy and to test the hypothesis that

there is no significant difference in the pain scores between contact rhinoscopy and conventional rigid endoscopy.

Methodology

From January 1999 to December 2000, a prospective series of 162 patients were recruited to undergo examination of the nasopharynx using contact rhinoscopy under local anaesthesia. The patients were aged between 16 and 80 years and had presented to a tertiary-referral clinic of the Prince of Wales Hospital (PWH) either with symptoms of primary NPC, or a positive family history of NPC, or an elevated level of IgA antibodies against antigens of the Epstein-Barr virus, or had been treated with radiotherapy for NPC.

The procedure was performed by a single endoscopist in a standard manner using contact nasal endoscopes after topical nasal preparation with a maximum of 1 ml of 5% cocaine spray (Karl Storz, Tuttlingen, Germany, 7215 AA, 0^0 and 7215BA, 30^0; 23 cm long; 4mm in diameter). After the procedure, the patient was asked to assign a pain score to the procedure using a 10-cm un-scaled visual analogue scale (0 = no pain, 10 = severest pain). The pain scores were recorded by an independent nurse. The patients were asked whether or not they would be willing to repeat the same procedure in the future if required.

The pain scores were analyzed. Independent factors that might predict a patient's willingness to undergo contact rhinoscopy again in the future were identified.

A prospective, matched, case-control study was then conducted to compare the pain scores of patients undergoing contact rhinoscopy and those undergoing conventional rigid endoscopy. During sample size analysis, a two-tailed power analysis estimated that at least 47 study cases and the same number of control cases would achieve 80% power at a 5% significance level to detect a bioequivalence within 49% in the pain score from the study group.

Among the patients who had undergone contact rhinoscopy, the first 50 non-irradiated patients and the first 50 irradiated patients were selected as the study group. From March 2008 to July 2008, another 100 patients, consisting of 50 non-irradiated patients and 50 irradiated patients, presenting themselves at out-patient clinics with sinonasal symptoms or a history of radiotherapy for NPC, were recruited as the control group. All of these were sex-matched and age-matched (within 5 years) with the patients of the study group. The nasopharynges of all the patients in the control group were examined by an experienced endoscopist using a 4mm 0^{o} rigid endoscope under local anaesthesia. The preparation and procedure were the same as for contact endoscopy except that the nasopharynx was not painted with vital stain and the endoscope did not touch the surface of the nasopharynx. After the procedure, the pain experienced was rated by the patients using a 10-cm un-scaled visual analogue scale (0 = no pain, 10 = severest pain) and recorded by an independent nurse. The pain scores of the 100 pairs of patients were analyzed and compared.

Results

A series of 162 patients (123 of whom were men; mean age: 48.3±12.35 y) had been recruited and underwent contact rhinoscopy. Of these 60 were non-irradiated patients (37%) and 102 were irradiated patients (63%). The procedure was completed for 157 patients (96.91%) but failed for 5 patients. The mean duration of the procedure was 6.16 minutes (S.D.: 2.66 minutes). The pain scores of all patients showed a range from 0.8 to 6.2 with a mean of 2.536 (S.D.: 1.12).

Of the 162 patients, 140 (86.4%) were willing to undergo contact rhinoscopy again in the future if needed. The mean pain score for the patients willing to repeat contact rhinoscopy (2.431; S.D.:1.056) was lower than for those who were unwilling (3.174; S.D.:1.306; p=0.003).

A multiple stepwise logistic regression model showed that an older age (p=0.001; odd ratio (OR): 0.931; 95% confidence interval (CI) 0.892-0.972), a longer duration of procedure (p=0.036; OR: 0.818; 95%CI: 0.678-0.987) and a higher pain score (p=0.005; OR: 0.537; 95%CI: 0.349-0.827) were the independent variables that predicted a patient's unwillingness to undergo contact endoscopy.

Among the 100 pairs of sex- and age-matched patients (mean age: 49.81±11.35y in the study group; male: female = 4:1in both groups) who had undergone either contact rhinoscopy or conventional nasal endoscopy, the pain scores were 2.517±1.073 and 2.282±1.324 respectively. There was no significant

difference in the pain scores between the two groups (p=0.17). There was also no significant difference in the pain scores between the non-irradiated and the radiated patients who had undergone either contact rhinoscopy (p=0.286) or conventional nasal endoscopy (p=0.822).

Conclusions

Office-based contact rhinoscopy can be performed with minimal discomfort under local anaesthesia. It is as well tolerated as the conventional nasal endoscopy and accepted by most of the patients.

2. *In vivo* diagnosis of NPC and normal nasopharyngeal mucosa using contact rhinoscopy

Conventional nasal endoscopy is a standard procedure used to examine the nasopharynx and for the identification of nasopharyngeal carcinoma (NPC). Previous large scale studies showed that the sensitivity of conventional nasal endoscopy to detect primary NPC varied from 95.1% to 99%. However, sub-clinical NPC was present in 5% of the high risk patients whose nasopharynges might have appeared normal during examinations in which conventional nasal endoscopy was used.

Contact rhinoscopy can be used not only for the examination of the general appearance of the nasopharynx, but also for the real-time and *in vivo* microscopic examination of the epithelial cells of the nasopharynx. However, its accuracy for the evaluation of normal nasopharyngeal tissues and primary NPC as compared with that of conventional endoscopy was not known. A prospective, single-blind,

controlled study was conducted to test the hypothesis that the sensitivity of contact rhinoscopy for the detection of primary NPC is similar to that of conventional nasal endoscopy.

Methodology

Between January 1999 and September 2000, a prospective study was conducted to compare the efficacy of contact rhinoscopy and conventional endoscopy for the diagnosis of primary NPC.

During the study period, consecutive patients attending a tertiary-referral clinic and presenting either with symptoms of primary NPC, or a positive family history of NPC, or an elevated IgA level of antibodies against the antigens of the Epstein-Barr virus were recruited for the study.

Each recruited patient underwent conventional nasal endoscopy followed by contact rhinoscopy performed by a single endoscopist using a contact rhinoscope in the following manner: The nasal cavities and nasopharynx were anaesthetised using a maximum of 1ml of a 5% cocaine topical anaesthetic spray. After 10 minutes, the endoscope was slowly advanced through the nasal cavity and into the nasopharynx. The nasopharynx was examined for abnormalities using the conventional endoscopy. Afterwards, a small nasal cotton-ball applicator was used to stain the nasopharynx mucosa with 1% methylene blue via the nasal cavity. The tip of the contact endoscope was made gentle contact with the mucosa of the posterior wall of the nasopharynx. The mucosa was examined at magnifications of x60 and x150 by adjusting the zoom switch on the endoscope. Once the contact

endoscopy had been completed, the areas under examination were then biopsied and studied by an independent pathologist who was blind to the findings of the endoscopies. A video recording was made of the entire endoscopic procedure including the conventional endoscopic examination of the nasopharynx and the contact endoscopic examination of the nasopharynx mucosa.

The endoscopist and a consultant pathologist subsequently analyzed the video images but without prior knowledge of the histology of the corresponding specimens. The diagnoses based on the conventional nasal endoscopy and the contact rhinoscopy were made by the endoscopist and the pathologist respectively. Using the histology of each biopsy specimen as the gold standard for the diagnosis, the diagnostic accuracy of both procedures was then compared.

Results

During the study period, 60 consecutive patients (41 of whom were men, mean age: 48.7±13.2 y) were recruited and underwent both conventional endoscopy and contact rhinoscopy. Five of the eligible patients did not take part in the study, 2 of these refusing a tissue biopsy and the other 3 declining to participate in the study.

Conventional endoscopy was carried out for all of the 60 patients (100%). In 24 of the 60 patients, the nasopharynges looked normal. In 23 of the 24 patients, the histology of the tissue biopsies showed a normal respiratory epithelium. The biopsy of one patient revealed undifferentiated carcinoma with syncytial sheets of malignant cells (4.17%).

In the remaining 36 patients, exophytic malignant tumours were observed during the conventional endoscopy and primary undifferentiated carcinomas were confirmed by the histologic sections of all the corresponding biopsy specimens.

Using the histology of the biopsy as the gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of conventional endoscopy in diagnosing primary NPC were 97.3%, 100%, 100% and 95.8% respectively.

Contact rhinoscopy was carried out for 56 of the 60 patients (93.3%). In three patients, the procedure was abandoned because of bleeding of the tumour on contact with the endoscope. In one patient, the tumour was situated on the roof of the nasopharynx, and this could not be accessed by the endoscope.

In 22 of the 56 patients, 2 patterns of normal respiratory epithelium were shown by contact endoscopy: pseudostratified ciliated epithelium (86.4%) and squamous epithelium (13.6%). These mucosal patterns were subsequently confirmed by the histologic sections of the corresponding biopsy specimens.

In 34 of the 56 patients, 2 patterns of malignant cells were visualized using the contact endoscope: syncytial sheets of malignant cells (44.1%) and atypical cells underneath the normal ciliated cells (55.9%). The malignant mucosal patterns were confirmed by the histologic sections of the corresponding biopsies in 33 of the patients while one of the biopsies showed a normal ciliated epithelium.

Of note is that in one of these 33 histologically proven NPC patients, the nasopharynx looked normal when examined using conventional endoscopy.

Using the histology of the biopsy as the gold standard, the sensitivity of contact rhinoscopy for the diagnosis of primary NPC was 100%, with a specificity of 95.7%, a positive predictive value of 97.1%, and a negative predictive value of 100%.

Conclusion

Contact endoscopy can provide a real-time differentiation between the malignant cells of nasopharyngeal carcinoma and the normal mucosa of the nasopharynx. Its sensitivity and specificity for the detection of primary NPC are as high as that of conventional endoscopy. Moreover, it is also able to detect sub-clinical disease, which was not visualized by conventional endoscopy.

3. *In vivo* diagnosis of persistent and recurrent NPC by contact endoscopy

Traditionally, nasal endoscopy has been the standard procedure used to monitor early recurrence of NPC after radiotherapy treatment. However, previous studies have shown that the sensitivity of conventional nasal endoscopy for the detection of recurrent disease was less than 50%. Since its arrival on the scene, the accuracy of contact rhinoscopy in the diagnosis of persistent and recurrent NPC has not been demonstrated.

A prospective, single-blind, controlled study was conducted to test the hypothesis that contact rhinoscopy can provide real-time identification of different

mucosal patterns of an irradiated nasopharynx and that its sensitivity to detect persistent and recurrent NPC is higher than that of conventional nasal endoscopy.

Methodology

From March 1999 to September 2000, consecutive patients who were attending a tertiary-referral clinic and had been treated with external radiotherapy for NPC were recruited for the study.

On each recruited patient, conventional nasal endoscopy, and subsequently contact endoscopy, was performed by a single endoscopist under local anaesthesia in the usual manner. The procedures were video recorded. After the endoscopic examination, the areas under examination were then biopsied and the histology of the biopsy specimens was studied by an independent pathologist who was blind to the results of the endoscopy. The images obtained by both the conventional endoscopy and the contact endoscopy were analyzed by the endoscopist and a consultant pathologist respectively but without prior knowledge of the histology of the corresponding specimens. Using the histology of the biopsy specimens as the gold standard, the accuracy of contact endoscopy for diagnosing persistent and recurrent NPC was compared with that of conventional nasal endoscopy. The kappa reliability test was employed to evaluate the degree of agreement between the diagnosis of the contact endoscopy and the histological examination.

Results

During the study period, 64 consecutive patients (54 of whom were men; mean age, 42 y) were recruited for the study. Five of the eligible patients did not consent to participation in the study and so were excluded. The procedure was performed at a median interval of 7 weeks after the radiotherapy treatment.

All of the patients recruited (100%) underwent both the conventional endoscopy and the contact endoscopy.

During the conventional endoscopy, the appearance of the nasopharynx was assessed as normal or suspicious in 51 and 13 cases respectively. The histology of the biopsy specimens showed four types of pathology: squamous metaplasia (42 cases), radiation change with cellular atypia (8), granulation tissues (9) and malignancy (5). In those patients with persistent or recurrent disease, the appearance of the nasopharynx was normal in two patients under conventional nasal endoscopy (40%). Using the histology of the biopsy specimens as the gold standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of conventional endoscopy to detect persistent and recurrent disease was 60%, 83%, 23.1% and 96.1% respectively.

During the examination of the mucosal patterns of the nasopharynx using contact endoscopy four patterns were identified: squamous metaplasia (43 cases), post-irradiation atypia (10), radiation change/granulation tissues (6), and malignancy (5). The overall findings of the contact endoscopy correlated well with the histological findings (kappa reliability coefficient = 0.847; $p<0.001$; diagnostic

accuracy, 92.1%). The sensitivity, specificity, PPV and NPV of the contact endoscopic findings for predicting persistent and recurrent NPC were all 100%.

Conclusion

Contact rhinoscopy can provide an accurate real-time identification of different mucosal patterns in the irradiated nasopharynx. It has a higher sensitivity and specificity for the detection of persistent and recurrent disease than conventional endoscopy.

4. The choice of chromogen for contact rhinoscopy in the irradiated nasopharynx

Since the introduction of contact endoscopy, 1% methylene blue (methylthionine chloride) has traditionally been used as a chromogen (vital stain) to outline the cellular patterns of the examined tissues. However, the effect of different concentrations of methylene blue on the diagnostic reliability of contact rhinoscopy has not been clearly demonstrated. Furthermore, to minimize the potential phototoxicity and effects of over-staining, the use of 0.5% methylene blue may be a better alternative. Therefore, a prospective, single-blind study was conducted to test the hypothesis that there is a significant difference in visual clarity of cellular details between 0.5% methylene blue and 1% methylene blue when used in contact rhinoscopy.

Methodology

Between November 2000 and March 2001, consecutive patients who had been treated with external radiotherapy for NPC and were attending a

tertiary-referral clinic of the Prince of Wales Hospital were recruited for the study. The nasopharynges of all the patients were assessed by contact rhinoscopy using 0.5% and 1% methylene blue stain on opposite sides of the nasopharynx. The procedure was performed and recorded by a single endoscopist. Three independent observers (a consultant otorhinolaryngologist, a junior trainee in radiology, and a consultant pathologist) assessed the visual clarity of all images from the contact endoscopy using a 10-cm un-scaled visual analogue scale (0= poor clarity, 10= excellent clarity). The assessment was repeated 2 weeks later and after the order of the images had been reshuffled. The paired visual scores of each assessor were compared and the intra-observer and inter-observer variations of all of the assessors were then analyzed.

Results

During the period of the study, 28 patients (18 of whom were men; mean age, 46.1±11.4 y) who had undergone radiotherapy for NPC were assessed using contact rhinoscopy with 0.5% and 1% methylene blue stain on opposite sides of the nasopharynx. Two patients could not complete the procedure because of bleeding from the contact and the inability to have their nasopharynx examined because of choanal stenosis secondary to radiotherapy. They were excluded from the study. The median time interval after completion of the radiotherapy was 5 years. The general appearance of the nasopharynx in all of the patients was normal showing no signs of a recurrence of malignancy.

Twenty-four and twenty-one images (x150) stained with 1% and 0.5% methylene blue respectively were produced and analyzed by the assessors. All of

the images showed a cellular pattern of squamous metaplasia without recurrence and these were recognized by all of the assessors.

The intraclass correlation coefficients for inter-observer reliability of the assessors were 0.884 and 0.885 in the groups of 0.5% and 1% stains, respectively. The intraclass correlation coefficients were 0.916 to 0.957 and 0.839 to 0.964 for the intra-observer reliability of the assessors in the groups of 0.5% and 1% stains, respectively. The mean scores for clarity of the cellular details were statistically rated higher in the 1% stained group (6.422±1.588) than in the 0.5% stained group (4.167± 2.111) by all of the assessors (p<0.001).

Conclusion

It is concluded that 1% methylene blue provides a better clarity of the cellular detail under contact rhinoscopy and thus enhances the histological diagnosis of an irradiated nasopharynx compared with its 0.5% counterpart.

5. Reliability of contact rhinoscopy in the diagnosis of primary and recurrent NPC

Although it has been shown that contact rhinoscopy can identify malignant cells of NPC, it is still not clear how the clinical experience and knowledge of histopathology of the assessors can affect the diagnostic reliability of this technique. A cross-sectional, randomized, single-blind study was performed to test the hypothesis that the accuracy of contact rhinoscopy in the diagnosis of primary and recurrent NPC is reproducible among different assessors.

Methodology

The study retrospectively reviewed two groups of patients who had undergone contact rhinoscopy in the Prince of Wales Hospital between January 1999 and December 2000: 1) a non-irradiated (non-RT) group (56 patients) consisting of patients with untreated primary NPC and patients presenting with sinonasal symptomatology without NPC; 2) a post-irradiated (post-RT) group (101 patients) consisting of patients who had undergone radiation therapy for primary NPC.

On both groups of patients, contact endoscopy had been performed by a single endoscopist and the images from the procedure had been recorded. After the procedure, the nasopharyngeal tissues under examination were biopsied and studied by an independent pathologist who was blind to the findings of the endoscopy.

Random samples of the contact endoscopic images were retrieved and reviewed by 5 independent observers (a trainee in otorhinolaryngology, a consultant otorhinolaryngologist, a surgical house-officer, a consultant radiologist, and a consultant pathologist). The diagnosis made by each assessor was recorded. The assessment was repeated 2 weeks later. Using the histology of the biopsy as the gold standard of diagnosis, the accuracy of the diagnoses made by the different assessors was assessed and compared.

Results

Between January 1999 and December 2000, 162 patients had been examined using contact endoscopy under topical anaesthesia. Contact endoscopy was feasible in 157 (96.91%) of the 162 patients but failed in 5 patients.

Of the 157 patients studied, 37 were women and 120 were men. Their mean age was 48.7 years (S.D.: 12.093 y; range: 21-80 y). They consisted of 56 non-irradiated patients (non-RT group) and 101 irradiated patients (post-RT group).

In the non-RT group, the overall Kappa value for inter-observer reliability for the differentiation of a normal epithelium from primary NPC by 5 assessors was 0.856 (95%CI: 0.753-0.958). There was no significant difference in the inter-observer reliability between them (Chi square test, p=0.851). The overall Kappa value for intra-observer reliability of 5 assessors was 0.736 (95%CI: 0.590-0.882). There was no significant difference between them (Chi square test, p=0.966).

The proportion of correct diagnoses of primary carcinoma made by the trainee in otorhinolaryngology, the consultant otorhinolaryngologist, the house officer, the radiologist and the pathologist was 95%, 95%, 95%, 85% and 90% respectively, and there were no significant differences in the proportions between them (Chi square test, p=0.704).

In the post-RT group, the overall Kappa value for inter-observer reliability to diagnose recurrent carcinoma, atypia, squamous metaplasia and radiation change by 5 assessors was 0.681 (95%CI: 0.594-0.768). There was no significant difference in the inter-observer reliability among them (Chi square test, p=0.851). The overall Kappa value for intra-observer reliability of the 5 assessors in the diagnosis of 4 mucosal patterns was 0.687 (95%CI: 0.601-0.773). There was no significant difference in the intra-observer reliability among them (Chi square test, p=0.365). The proportion of correct diagnoses of carcinoma and atypia made by the trainee in otorhinolaryngology, the consultant otorhinolaryngologist, the house officer, the radiologist and the pathologist was 88%, 88%, 88%, 82% and 85% respectively, and there were no significant differences in the proportions between them (Chi square test, p=0.938).

Conclusion

The results demonstrate that contact rhinoscopy can reliably identify primary and recurrent NPC by various assessors irrespective of their clinical experience and knowledge of histopathology.

(V) CONCLUSIONS OF CLINICAL STUDIES

Contact rhinoscopy can provide real-time and *in vivo* examination of cellular details of the superficial epithelium of the nasopharynx under local anaesthesia. The procedure can be administered safely to the patients. The pain perceived by the patients is minimal and comparable to that of the conventional endoscopy. The procedure is also accepted by most of the patients.

Contact rhinoscopy can accurately identify different mucosal patterns of primary NPC and contribute to the diagnostic process of primary NPC.

Contrary to conventional endoscopy, contact rhinoscopy can accurately identify different mucosal patterns of the irradiated nasopharynx and diagnose early recurrence of the disease even in a normal-looking nasopharynx.

Compared to its 0.5% counterpart, the use of 1% methylene blue enhances the clarity of the cellular details revealed by the contact endoscopy and thus improves the accuracy of the diagnosis of irradiated nasopharyngeal mucosa.

Moreover, the accuracy in the diagnosis of malignancy and different mucosal patterns of the nasopharynx using contact rhinoscopy is highly reproducible by different assessors irrespective of their clinical experience and knowledge of histopathology.

It is concluded that contact rhinoscopy is a safe, well-tolerated, accurate, and reliable office-based procedure for real-time diagnosis of NPC before and after radiotherapy. It can be used as an adjunct to conventional nasal endoscopy in the management of this disease.

PART I

HISTORICAL AND LITERATURE REVIEW

CHAPTER 1

NASOPHARYNX (NP)

1.1. Anatomy of the nasopharynx

Knowledge of the gross and microscopic anatomy of the nasopharynx helps us to understand the presentation and symptomatology of malignant tumours in the nasopharynx. Similarly, a thorough understanding of the extensive lymphatic system of this region is of great importance in tumour staging and the detection and management of tumour spread.

1.1.1. Gross anatomy of the nasopharynx

The nasopharynx is the part of the pharynx lying behind the nasal cavities. It has anterior, posterior, and lateral walls composed of muscular, fibrous, and mucosal layers. Each wall of the nasopharynx contains different important structures which may be affected by invasive tumours of the nasopharynx (Rosse 1997).

Anteriorly, the nasopharynx communicates with the nasal cavities through the posterior choanae. The lower part of the anterior wall is formed by the soft palate in its resting state. Its posterior wall starts as the roof of the nasopharynx, composed of mucosa overlying the basal portions of the sphenoid and occipital bones of the skull base, extends inferiorly at the level of the soft palate, and continues inferiorly as the posterior wall of the oropharynx. The adenoid is a prominent, convoluted lymphoid tissue invariably present in the roof of the nasopharynx in children. The posterior wall of the nasopharynx is composed of the superior constrictor muscles and the pharyngobasilar fascia. The lateral walls of the nasopharynx, connecting the anterior and posterior walls, are formed by the

superior constrictor muscle and the ostia of the auditory (Eustachian) tubes. This opening is surrounded on its superior and posterior aspects by mucosa-covered cartilage, the torus tubarius, or tubal torus, produced by the auditory tube. Posterior to the ostium of the tube, a recess called the fossa of Rosenmuller is located in the posterolateral portion of the nasopharynx. The fossa of Rosenmuller is of great clinical importance since it represents the most common site of origin of nasopharyngeal carcinoma.

1.1.2. Microscopic anatomy of the nasopharynx

The nasopharyngeal mucosa in the adult has a surface area of about 50cm^2. The size of the nasopharynx is subject to considerable variation caused by muscular contraction. The average anterior-posterior dimension of the nasopharynx in adults is 2-3 cm and the transverse and vertical diameters of a relaxed nasopharynx are around 3-4 cm (Adam 1958).

The distribution of the nasopharyngeal mucosa is characteristic (Figure 1-1). Most of the mucosa of the nasopharynx is carpeted by stratified squamous epithelium (Ali 1965). It predominantly lines the lower portion of the anterior and posterior nasopharyngeal walls, as well as the anterior half of the lateral walls. Microscopically, stratified squamous epithelium consists of a variable number of cell layers which exhibit morphological transition from the cuboid basal layer to the flattened surface layers (Figure 1-2A). The basal cells give rise to a new generation of cells which are progressively pushed upward to replace the cells at the surface (Wheater 1987). It is well adapted to withstand abrasion but poorly adapted to withstand desiccation. In the normal state, it is kept moist by local

secretions from the respiratory epithelium. However, after radiotherapy, the epithelium will undergo squamous metaplasia with granulation tissue formation.

Pseudostratified columnar ciliated epithelium predominantly covers the region of the posterior choanae and the roof of the posterior wall of the nasopharynx, which constitutes about 40% of the surface area of the nasopharynx. The microscopic appearance of pseudostratified ciliated epithelium is characteristic (Figure 1-2B): the individual cells exhibit polarity as their nuclei are disposed at different levels, and the apical cytoplasm does not contain nuclei. The distribution of the nuclei creates the illusion of cellular stratification. Functionally, the cilia of the epithelium continuously propel a surface layer of mucus containing entrapped particles towards the oropharynx.

The remainder of the nasopharynx, including the posterolateral walls and the middle third of the posterior wall, is covered by islands of squamous and respiratory epithelium. Areas of transitional epithelium are encountered at the junction between the nasopharynx and oropharynx. Microscopically, the transitional cells appear basaloid with minimal cytoplasm in a cuboid or round configuration.

1.1.3. Lymphatic drainage of the nasopharynx

The lymphatic drainage system of the nasopharynx is extensive. A dense lymphatic network in the mucosa gives origin to the submucosal superior collecting trunk, and to a lesser extent, the middle collecting trunk (Gibb 1999).

The superior collecting trunk drains the bulk of the nasopharynx, the posterior part of the nasal cavity, the Eustachian tubes, the soft palate, and the oropharynx. It drains to the lateral and median retropharyngeal nodes and the upper deep cervical nodes situated close to the internal jugular vein. The middle collecting trunk drains the soft palate and palatine tonsils and terminates in the lateral retropharyngeal nodes and thus the upper deep cervical nodes. The latter consist of the uppermost cervical nodes located in the retrostyloid compartment of the parapharyngeal space and multiple nodes along the anterior triangle of the neck, where the most important and constant node is the jugulodigastric node. The efferent vessels from the upper deep cervical lymphatic chain drain to the lower deep cervical nodes and the jugular trunk. The right jugular trunk ends at the junction of the internal jugular and subclavian veins, while the left jugular trunk joins the thoracic duct.

1.2. Function of the nasopharynx

The role of the nasopharynx is solely respiratory, probably functioning as a collecting space where the inspired air is filtered of impurities by the lymphoid tissues. Its central position and close proximity to the nose, the pharynx, and the middle ears render it a common hub to the ears, nose, and throat regions. Any pathology that occurs in this region can spread to the surrounding structures.

Figure 1-1. Schematic presentation of the distribution of nasopharyngeal epithelium.

(A) Distribution of ciliated epithelia (shaded area in blue).

(B) Distribution of squamous epithelia (shaded area in green).

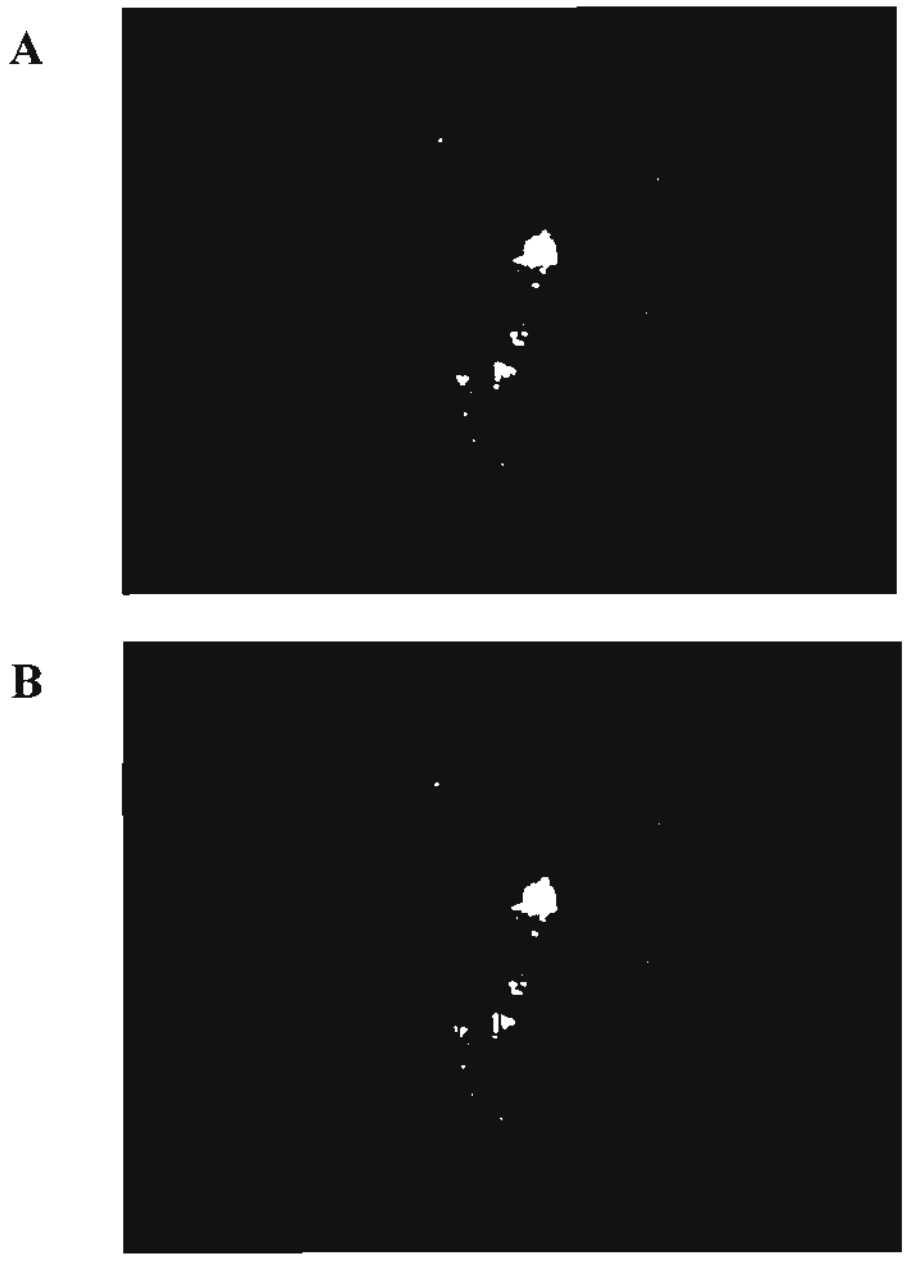
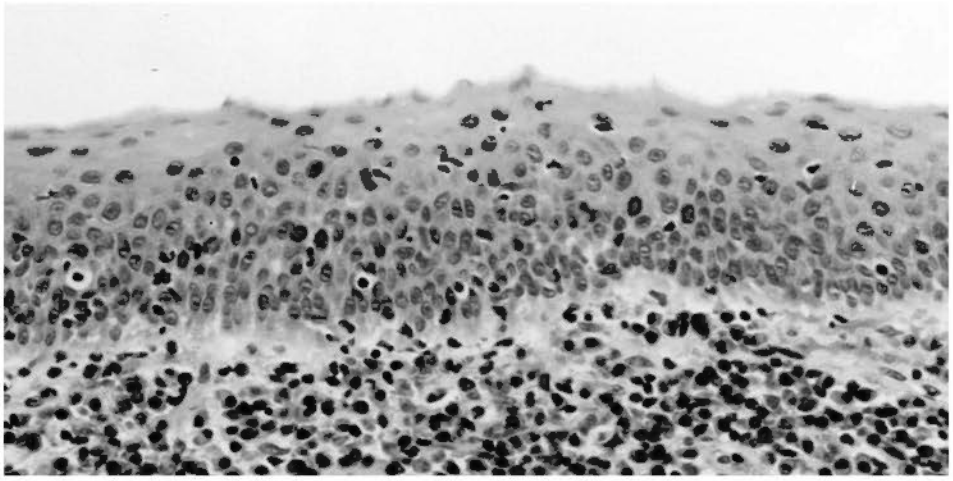


Figure 1-2. Histology of normal epithelium of the nasopharynx.

(A) Squamous epithelium (Haemotoxylin & eosin stain, original magnification x 250).

(B) The pseudostratified columnar ciliated epithelium (Haemotoxylin & eosin stain, original magnification x 350). The individual cells exhibit polarity and contain cilia.

A



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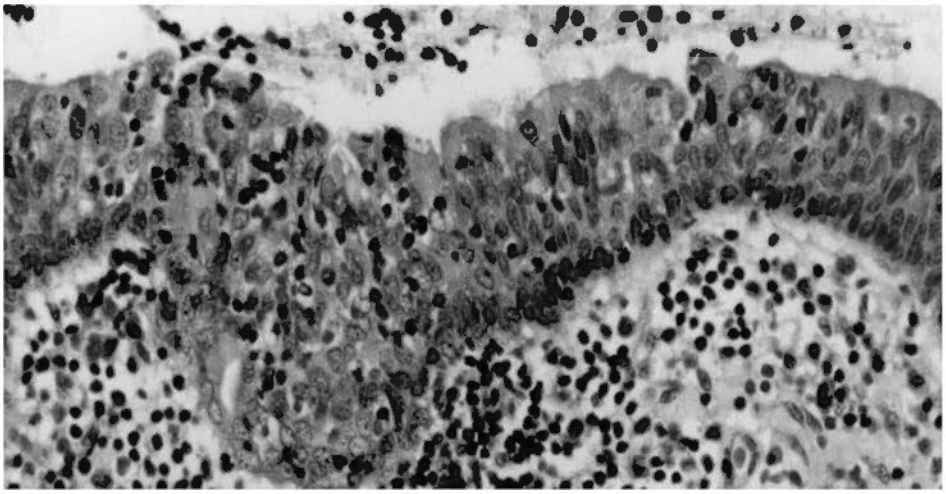


CHAPTER 2

NASOPHARYNGEAL CARCINOMA (NPC)

2.1. Incidence of nasopharyngeal carcinoma (NPC)

Nasopharyngeal carcinoma is a unique malignant tumour with a characteristic racial and geographical distribution. The highest incidence of this disease is observed among southern Chinese who reside in Guangdong province and speak the Cantonese dialect (Ho 1972).

Hong Kong, being part of Guangdong province, is one of the endemic regions of NPC in the world. In Hong Kong, it is estimated that every year about 1000 new cases are diagnosed, and males are affected 2.9 times more frequently than females. Based on the latest annual report of the Hong Kong Cancer Registry in 2005, the crude incidence rates of NPC in Hong Kong are 21.6 per 100,000 for males and 6.8 per 100,000 for females (Hong Kong Cancer Registry 2005). The age-specific incidence rates for both sexes begin to rise at the age of 20, reaching a plateau between 40 and 64 years, and declining slowly thereafter. The peak incidence is at the age of 44, and the median age at diagnosis is 50 for males and 48 for females. For a middle-aged Cantonese man, the incidence is 50 to 100 per 100,000 (Hong Kong Cancer Registry 2007).

Compared to the endemic regions, this tumour is rare in most parts of the world, with an incidence rate for either sex of less than 1 per 100,000 per year. The exceptions are Africa, Canada, Alaska, and Greenland (Mallen 1974, Lanier 1979, Nielsen 1977, Muir 1987).

As with other cancers, the age-standardized incidence rate for nasopharyngeal carcinoma has been falling steadily over the years. The age-standardized rates for NPC were 20.5 per 100,000 in 1985 and 11.8 per 100,000 in 2004 for both sexes. The reason for the falling incidence is unknown and warrants further investigation (Hong Kong Cancer Registry 2005).

2.2. Aetiology of nasopharyngeal carcinoma

As stated, the frequency of NPC is nearly 100-fold higher in southern Chinese than in most Caucasian populations. Since the 1960s, this characteristic racial and geographical distribution has aroused extensive interest in the study of its aetiology (Muir 1967).

To date, epidemiological and experimental studies have linked this tumour with a genetically determined susceptibility associated with specific human leukocyte antigen (HLA) haplotypes, early latent infection by the Epstein-Barr virus (EBV) and its reactivation, and exposure to environmental factors of a chemical nature, especially consumption of salted preserved fish at an early age (Ho 1971a, Simons 1975, Henle 1976, Ho 1976, Huang 1978b, Simons 1982, Armstrong 1983, Chan 1983, Huang 1983, Poirier 1987, Chan SH 1990, Huang 1990, Zhu 1990).

2.2.1. Genetic predisposition

Apart from the fact that the frequency of NPC is nearly 100-fold higher in southern Chinese than in most Caucasian populations (Muir 1987), the

observations of an increased risk of developing NPC in the second generation of southern Chinese who emigrated to low incidence areas in Western countries (Buell 1965, Buell 1974), and a high proportion of familial cases among the first degree relatives of patients with NPC across endemic areas (Brown 1976, Lanier 1979, Huang XL 1980) have highlighted the possibility of an inherited genetic predisposition to NPC.

The alleles for specific HLAs vary in frequency among different populations. In the last two decades, patients with specific haplotypes in the HLA region have been found to have an increased risk of NPC (Simons 1975, Chan 1984, Zhu 1990). Some of these alleles, for example, A2, A33, B46, B58, Cw1, and DR3 are frequently present in patients with NPC (Lu 1990, Ooi 1997). The loci involved are the HLA-A, -B, and -DR loci located on the short arm of chromosome 6. As HLA molecules are responsible for presenting antigenic peptides to T cells, which are the central component of the immune system, it is highly likely that the susceptibility to developing NPC is somehow related to the different abilities of HLA haplotypes in controlling Epstein-Barr virus infection (Murray 1992).

Furthermore, cytogenetic studies also showed that chromosomal aberrations have been associated with NPC. Certain regions of chromosomal loss have been identified in chromosomes 1p,3p,9p,11q,13q,14q,16q, and X. A high frequency of 3p deletion has also been demonstrated in the primary tumour (Huang 1989).

2.2.2. Dietary and non-dietary factors

Volatile nitrosamines, principally N-nitrosodimethylamine and N-nitrosodiethylamine, in salted fish and phorbol esters in plants and oils are mutagenic compounds found in certain traditional Chinese foods (Fong 1973, Fong 1976, Huang 1978b, Huang 1981). These preserved food items have shown a dose-dependent effect and produced mutagenic urine when fed to experimental animals (Fong 1979).

In 1971, Ho first proposed that Chinese salted fish was a possible aetiological factor in the development of NPC if it was fed to young children over a long period of time (Ho 1971b). Case-control studies further suggested that childhood consumption of salted fish is the primary cause of NPC among Cantonese (Anderson 1978, Yu 1986). Ning found that in a non-Cantonese rural population of China, about 50% of NPC cases had consumed salted fish (Ning 1990). Therefore, the close correlation between NPC risk and salted Cantonese food items is more than just a coincidence.

Apart from salted fish, a number of other non-dietary factors including dust, smoke, chemical fumes, and tobacco smoke have sporadically been reported to be associated with the development of NPC. However, no obvious association between the disease and exposure to these inhaled agents has been found (Mabuchi 1985, Yu 1990, Ning 1990). Similarly, studies have described a positive and significant association of chronic ear or nose conditions with NPC, although no causative association has been established between them (Yu 1990).

2.2.3. Epstein-Barr virus (EBV)

An association between NPC and EBV was first discovered by Old et al., who demonstrated high precipitating antibody levels in undifferentiated or poorly differentiated types of NPC (Old 1966). In the next few years, de Schryer et al. and Henle et al. (de Schryer 1969, Henle 1970) noticed that EBV antibody titres, particularly of the IgA class, were higher in patients with NPC than in controls.

With further development of molecular technology, more evidence has been identified to unveil the close association between EBV and NPC. For example, more EBV-specific genes and EBV-associated antigens were found to be consistently expressed in undifferentiated NPC tumours and early dysplastic lesions of southern Chinese patients (Klein 1974, Pathmanathan 1995). The detection of a single form of viral DNA in NPC cells suggested that the tumours are clonal proliferations of a single cell initially infected with EBV. In vitro studies showed that EBV can transform B-lymphocytes and exert a proliferative effect on human epithelial cells (Crawford 1979, Glaser 1980).

Although extensive data in immunological, biological, and molecular studies have shown that there is a strong and consistent association between EBV and NPC, there is still no clear-cut evidence to indicate that EBV causes NPC, and the exact mechanism of tumour genesis by EBV remains to be elucidated.

2.3. Pathology of NPC

2.3.1. Nasopharyngeal intraepithelial neoplasia (NPCIN)

Precancerous change is defined as "a condition of organs and tissues which sometimes, often, or regularly leads to the genesis of cancer" (Henson 1986). This histopathological change has been well documented in the oesophagus, skin, and uterine cervix (Reagan 1953, Richart 1966, Crissman 1989, Rubio 1989). However, the occurrence of non-invasive precancerous changes in the nasopharynx, designated nasopharyngeal intraepithelial neoplasia (NPCIN) or nasopharyngeal carcinoma in situ (NPCIS), is rare.

Although histologically confirmed NPC was recorded as early as 1924, it was not until 1957 that Teo first reported intraepithelial neoplastic changes in the nasopharynx during autopsy of 31 NPC patients (Teo 1957, Shanmugaratnam 1980). Thereafter, more and more cases of dysplasia or NPCIN of the nasopharynx were reported in several retrospective studies that were based on archival biopsies or autopsy examinations (Liang 1962, Shen 1964, Shanmugaratnam 1967, Yeh 1967).

In the two largest series, the prevalence of NPCIN was found to vary from 2% (64/2742) to 3.6% (67/1811) of the nasopharyngeal biopsies taken from symptomatic patients (Zong 1986, Pathmanathan 1995).

However, most of the preinvasive lesions are found adjacent to primary invasive lesions and early recurrence tissues (Nicholls 1993). Pure NPCIN lesions without the associated invasive component of NPC are very rare, reportedly occurring in 0.6% of nasopharyngeal biopsies (Zong 1986, Cheung 1998). To date,

there is little information regarding the prevalence, biological behaviour, and clinical significance of this premalignant entity, which demands further exploration.

Based on the findings of previous studies, the low-power microscopic appearance of NPCIN is similar to that of normal epithelium (Pak 2002a).

Under high magnification, the NPCIN cells resemble those of undifferentiated nasopharyngeal carcinoma. Characteristically, those atypical epithelial cells are present, demonstrating loss of polarity, large vesicular nuclei, clumping of chromatin, prominent eosinophilic nucleoli, and scanty indistinct cytoplasm. The neoplastic cells tend to scatter throughout the full thickness of the mucosa and mix with intra-epithelial lymphoplasmacytic infiltrate (Figure 2-1). The neoplastic changes not only involve the surface epithelium, but also extend to crypts deep within the lymphoid stroma (Shen 1964, Pak 2002a). Zong and Li have further characterized carcinoma in situ lesions after reviewing 2,742 nasopharyngeal biopsies. They identified and subdivided the precancerous lesions into "columnar type" and "squamous type", which showed an increase in nuclear deoxyribonucleic acid (DNA) content over that in normal epithelium and metaplastic squamous epithelium (Zong 1986).

As the lesion may be easily missed under low power examination, it is necessary to have a high index of suspicion when looking for foci of abnormal epithelial cells. Since this preinvasive lesion is rarely reported, it poses a diagnostic challenge to the pathologist. As latent EBV infection can be identified in NPCIN by in-situ hybridization (ISH) for Epstein-Barr virus-encoded RNAs (EBER),

it is recommended that ISH EBER studies should be performed for those suspicious lesions for confirmation of the diagnosis (Zong 1986, Cheung 1998).

With reference to the current concepts on intraepithelial neoplastic changes in other sites of the body, Lee et al. has subdivided NPCIN into different stages (Lee 1986):

NPCIN 1: Isolated atypical cells in the lower 2/3 of the epithelium or numerous undifferentiated cells in the lower 1/3.

NPCIN 2: Undifferentiated cells in the lower 2/3 of the epithelium.

NPCIN 3: Undifferentiated cells in the full thickness of the epithelium.

It is believed that preinvasive lesions may develop into invasive tumours over a period of time. In 2002, from a series study of isolated NPCINs, we found that NPCIN may precede the invasive carcinoma by 3-4 years. Therefore, early detection of these preinvasive lesions is vital to those susceptible individuals before the disease becomes frankly invasive (Pak 2002a).

2.3.2. Primary NPC

NPC has been referred to as "malignant lymphoepithelioma" of the nasopharynx because of the presence of lymphocytic infiltrate at the site of the tumour. With improved immunohistochemical techniques, the tumours were confirmed to be epithelial in origin. The benign reactive lymphocytes that infiltrate the tumour tissue are present in over half the cases of undifferentiated carcinoma as a local tissue reaction to the tumour (Rosai 1996).

In 1978, the World Health Organization (WHO) classified NPC into three categories on the basis of the light microscopic appearances (World Health Organization 1978):

Type I: Well differentiated or keratinizing squamous cell carcinoma.

Type II: Poorly differentiated or non-keratinizing carcinoma.

Type III: Undifferentiated carcinoma.

A modification of this classification system was proposed in 1991, but this has not gained general acceptance. To date, the WHO classification (1978) is the one most commonly used.

Well differentiated squamous cell carcinoma is not common in Asia and accounts for about 25% of cases in non-endemic regions (Dickson 1985). Its histological characteristics resemble carcinoma in other parts of the oropharynx. The malignant cells characteristically reveal unequivocal keratin pearls, definite inter-cellular bridges, or individual cell keratinization. In general, they are less aggressive and radiosensitive than their undifferentiated counterpart.

The non-keratinizing carcinoma has similarities to a transitional cell carcinoma.

Undifferentiated carcinoma is the most common subtype seen in Hong Kong and southern China, and it accounts for over 95% of NPC in these endemic areas.

It is characterized by the presence of the following histological features and immunohistochemistry in Hematoxyline and Eosin (H and E) stained sections:

1. Histology

The tumour cells of primary NPC are always located in the submucosal lymphoid and loose connective tissue, usually separate from the overlying epithelium. As the tumour grows, malignant cells invade and spread extensively in the lamina propria before invading and ulcerating the overlying mucosa. The tumour cells are variable in size and shape without the presence of keratin pearls, intercellular bridges, or individual cell keratinization. The nuclei of the tumour cells are large and round to oval in shape, with well-defined nuclear membranes and one or more prominent, large, eosinophilic nucleoli. The nuclei vary considerably in both shape and size. Mitoses are found and there is coarse and irregular clumping of the chromatin. The cytoplasm of the cells is pale pink in colour.

With reference to the spatial relation between tumour cells and the infiltrating lymphocytes, two histological patterns of nasopharyngeal carcinoma have been recognized (Choa 1991):

The Schmincke pattern consists of syncytial sheets of undifferentiated tumour cells with poorly demarcated cytoplasmic borders. The presence of infiltrating lymphocytes and plasma cells within the sheets of tumour cells prompted the name "lymphoepithelial carcinoma" (Figure 2-2A).

In the less common Regaud pattern, the borders of tumour cells are more clearly defined. The cells are more cohesive and clump together in trabeculae, columns, or nests. The groups of cells are separated by varying amounts of loose connective tissue, lymphocytes, and plasma cells. This tends to form a whirling pattern indicating the basic squamous epithelial nature of lymphoepithelial carcinoma (Figure 2-2B).

In a series of 236 nasopharyngeal carcinomas, McGuire and Lee reported that 91% of the undifferentiated tumours exhibited the Schmincke pattern and 9% the Regaud pattern (McGuire 1990).

2. Immunohistochemistry for epithelial markers

As NPC is an epithelial neoplasm, it virtually always stains positively with anti-keratin AE1/AE3 immunohistochemical stain (Shi 1984) and other epithelial markers, including CAM 5.2 and epithelial membrane antigen. Immunohistochemical stains of NPC biopsies have also demonstrated over-expression of p53 protein (Sheu 1995), bcl-2 protein (Kouvidou 1995a), MDM-2 protein (Kanavaros 1995), EB virus-encoded latent membrane protein (LMP-1) (Vera-Sempere 1996), Ki67 antigen, epidermal growth factor receptor (Zheng 1994), beta-2-microglobulin and Hla-DR proteins (Kouvidou 1995b), the intercellular adhesive molecules (ICAM-1) (Ruco 1994), the blood vessel adhesion molecules (VCAM-1) (de Vincentiis 1996), and c-myc and ras oncogenes (Porter 1994).

The positivity for these immunohistochemical stains in paraffin sections of tissue biopsy helps to differentiate NPC from other malignant and benign diseases (Allen 1999).

3. In-situ hybridization (ISH) studies

In south-east China and Hong Kong, most NPCs contain EBV. In fact, as the EB virus is intimately involved in the pathogenesis, EB viral genomes can be found in tumour cells in 100% of cases by in-situ hybridization for EB virus-encoded RNAs (EBER) (Chao 1996), by Southern blotting for various portions of the viral genome (Pathmanathan 1995), by pulse field gel electrophoresis (Kripalani-Joshi 1994), and by polymerase chain reaction on biopsy tissues (Choi 1993).

Cytological smears from the nasopharyngeal tissues, or biopsies or fine needle aspiration cytology (FNAC) material from cervical lymph nodes can be studied for the presence of EBER by the above mentioned methods. Its presence indicates the likelihood of NPC. However, as benign epithelial cells of the nasopharynx may also harbour EB virus products, positive results of these tests are not diagnostic of malignancy (Chen 1996).

2.3.3. Post-radiation changes or squamous metaplasia

The mucosal cells of the nasopharynx exhibit characteristic histological changes of squamous metaplasia after radiotherapy. Radiation changes include enlargement of cells containing minimally enlarged nuclei with coarse chromatin, multi-nucleation, prominent nucleoli, and cytoplasmic vacuolation (Figure 2-3).

The cilia on the epithelium are absent. These benign squamous metaplastic cells may look atypical and are occasionally misdiagnosed as squamous cell carcinoma. Nevertheless, the nuclear: cytoplasmic ratio is not greatly increased after radiation when compared to cancer cells. In contrast to cancer cells, the radiated benign cells do not show variation in size and the nuclear membrane remains smooth. The cells are cytokeratin negative (Chang 1999).

The presence of granulation tissue is another hallmark of the tissue reaction to radiation. Granulation tissue appears as several layers of fibroblastic cells separated by a collagenous matrix containing capillary buds and inflammatory cells. In some instances, swollen benign proliferating endothelial cells or plasma cells may be misdiagnosed as malignant cells (Figure 2-4).

However, radiation changes subside after twelve months, and by two years the mucosa of the nasopharynx will return to normal, leaving little residual evidence of the treatment.

2.3.4. Recurrent nasopharyngeal carcinoma

The histology of recurrent nasopharyngeal carcinoma is identical to that of primary untreated nasopharyngeal carcinoma. However, squamous cell differentiation is more commonly seen in recurrent tumours than in primary tumours (Allen 1999).

2.4. Management of primary NPC

2.4.1. Diagnosis of primary NPC

The deep-seated position of the nasopharynx, combined with the problems of examining the nasopharynx, poses a diagnostic challenge to the clinicians. In general, early primary NPC or residual NPC after treatment is often asymptomatic. Hence, clinical examination, EBV serology, imaging, endoscopy, and histological study are indispensable procedures for the early diagnosis of primary and recurrent disease.

2.4.1.1. Clinical presentation

1. Nasopharyngeal intraepithelial neoplasia (NPCIN)

The clinical presentation of NPCIN has not been well documented. Cheung et al. have identified two patients with purely preinvasive NPC lesions without evidence of an invasive component (Cheung 1998). The presenting symptoms were unilateral tinnitus and blood-stained post-nasal drip. The lesions were described as small bulges in the nasopharynx. However, in our previous study, the presenting symptoms of patients with NPCIN were quite different (Pak 2002a). In our series, transient middle ear effusion, cervical lymphadenopathy, and cranial nerve palsy were the clinical features which caught the attention of the clinicians. Moreover, the nasopharynx can look normal under the direct vision of experienced otorhinolaryngologists. The avenue to diagnosis relies on a high index of clinical suspicion and elevated EBV serology.

2. Primary NPC

As the nasopharynx is a deeply-seated structure situated as a part of the air passage within the head, the early presenting symptoms of primary NPC are often vague and non-specific. This can lead to delay in referral to secondary care and a definitive diagnosis. In fact, only around 10% of cases are diagnosed as stage I disease (Skinner 1991).

Painless neck swelling is the most frequent presentation of nasopharyngeal carcinoma. In both endemic and non-endemic areas, 40-50% of patients with nasopharyngeal carcinoma presented with unilateral painless cervical lymphadenopathy (Grammatica 1999, van Hasselt 1990). Skinner et al. found that upper cervical nodes are more bulky than the lower cervical nodes, reflecting a tendency of an orderly downward spread of the disease. Nodal metastases commonly affected level II nodes, from which lymphatic spread extended down in an orderly manner to involve level III, level IV, and the supraclavicular fossa nodes, or extended posteriorly to involve level V nodes (Skinner 1991). In 3-5% of patients, disease may spread to mediastinal or abdominal nodes, usually in association with supraclavicular nodal metastases (Ng 2004a). Similarly, Sham et al. further found that involvement of lymph nodes in the lower neck is associated with a poorer prognosis (Sham 1990b). The frequency of skip metastases was 7.9%.

Blood-stained nasal discharge and unilateral nasal obstruction are the next most common presenting symptoms of NPC, and they account for about 30% of cases. As nasal symptoms are non-specific and may mimic rhinitis and upper

respiratory infection, they are often dismissed and neglected by patients or even medical practitioners. As a consequence, the mean duration of nasal symptoms prior to the first consultation with a physician is longer than for other complaints (Skinner 1991).

Approximately 17% of patients complain of ear symptoms, including hearing loss, tinnitus, otalgia, and otorrhoea (Skinner 1991). Most of them are unilateral. Examination usually reveals conductive hearing loss and middle ear effusion caused by malfunction of the Eustachian tube.

As the disease progresses, symptoms will present as a result of malfunctions of the structures adjacent to the nasopharynx or metastases to regional or distant parts of the body. Headache, diplopia, facial paraesthesia, facial palsy, hoarseness, dysphagia, vertigo, and blindness remain the indications of advanced disease invading the cranial nerves III, V, VI, VII, VIII, IX, and XII (Kao 1993, Chong 1996a, Chong 1996b, Chong 1997).

Distant metastasis at presentation is rare. Nevertheless, the bones and lungs are the most common sites for secondary deposits. Presenting symptoms related to distant metastasis are extremely uncommon, but appear in 5% of patients; bone pain is by far the most frequent symptom related to distant metastasis. Other presentations are very rare. Nevertheless, sporadic case reports have demonstrated that vertigo (Krause 2007), trismus (Ozyar 2005), parapharyngeal abscess (Tan 2007), and retropharyngeal abscess (Pak 1999) may also be the presenting signs or symptoms of primary NPC.

2.4.1.2. Nasal endoscopy and tissue biopsy

The nasopharynx is literally at the centre of the head and detailed examination is not always possible. Traditionally the nasopharynx was examined by a reflecting mirror placed in the oropharynx. This method is limited by variations in oral anatomy and the sensitivity of the gag reflex. Examination was usually brief and depended very much on the relaxation of the patient.

After the advent of modern endoscopy, nasal endoscopy became an important and indispensable diagnostic tool for primary NPC. With the use of rigid transnasal 0^0 or 30^0 nasal endoscopy with a Hopkins rod lens system (2.7 mm or 4.0 mm in diameter) under topical anaesthesia, an undistorted and clear view of the nasopharynx can be easily attained (Hopkins 1966) (Figure 2-5). This allows detailed examination of the nasopharynx and biopsy from selected sites under direct visualization, which makes the biopsy results more reliable (Shanmugham 1985). The sensitivity of this technique in diagnosing primary NPC has been found to be as high as 95% to 99.7% (Waldron 1992, Kwong 2001a).

Following the administration of a topical anaesthetic and vasoconstrictive agent, rigid rhinoscopy can be performed with the minimum of discomfort for most patients as an office-based procedure. Previous studies have shown that the mean pain scores experienced by patients vary from 2.0 to 2.3 when using a variety of different topical anaesthetic agents prior to rigid nasal endoscopy with a 2.7mm endoscope (Midwinter 2001, Douglas 2006). However, in some cases where anatomical variations exist, such as a deviated nasal septum or coexistent

nasal polyps, it may be difficult to advance the instrument through the nose into the nasopharynx. In these circumstances, a retrograde view of the nasopharynx can be obtained by passing a rigid 90^0 endoscope through the mouth (Woo 1997).

To those patients who cannot tolerate rigid instrumentation, the use of a flexible fibre-optic nasal endoscope is another alternative (Midwinter 2001). The panoramic and magnified view provided by a flexible fiberscope allows the detection of small tumours and documentation of the extent of the lesions, as well as mapping and photography of the site and extent of the tumour. Coupled with the latest digital photographic technology, digital pictures and video for teaching and explanation to patients can now be captured with ease (Yanagisawa 1993).

Although nasal endoscopy can accurately identify most of the tumours in the nasopharynx, the gross appearance of the tumour in no way indicates the extent of disease infiltration. A study had found that approximately 5% of primary NPCs are submucosal, and this is often associated with a normal-looking nasopharynx or a very mild degree of asymmetry (Sham 1989). Therefore, there is a tendency for early tumours to present as inconspicuous or innocuous-looking lesions, which are often missed by examination using nasal endoscopy (Low 2000). In cases where submucosal NPC is suspected, multiple deep biopsies from both fossae of Rosenmuller and the posterior wall and vault of the nasopharynx should be undertaken.

2.4.1.3. Cytological diagnosis

Exfoliative cells of NPC can be collected and studied from tissues scraped from the nasopharyngeal mucosa (Huang 1978a). The first paper studying the use of cytology as a diagnostic procedure for NPC was reported in 1949. It reported a success in seven out of eight cases of NPC (Morrison 1949).

The typical cytological features of undifferentiated NPC are cohesive clumps of large tumour cells with oval to round vesicular nuclei, a high nuclear: cytoplasmic ratio, and prominent nucleoli (Figure 2-6) (Dong 1983, Chan 1988, Chan MKM 1990). Cytoplasm is usually scanty or poorly stained. Lymphocytes are usually seen adjacent to the tumour cells.

With the use of different collecting devices, a variation of detection rates between 26% and 86% has been reported (Chang 1999). The single largest series to detect NPC by cytology was performed in 1,138 patients and the detection rate was reported as 89% (Dong 1983). However, the sensitivity of the test depends very much on the experience of the individual cytopathologist (Chang 2001).

EBV genomes are present in most undifferentiated NPC tumours. To enhance the diagnostic accuracy, the nasopharyngeal smear can be examined for EBV-associated nuclear antigen (EBNA) and EBV-encoded RNA (EBER) using immunohistochemical stains (Khan 1992, Ambinder 1994). In a large scale study, using a polymerase chain reaction (PCR) for amplification of deoxyribonucleic acid (DNA) of EBV in nasopharyngeal brush specimens, the sensitivity and

specificity for the diagnosis of NPC were reported as 90% and 99%, respectively (Tune 1999).

Subsequent studies also showed that quantitative analysis of EBV DNA and its associated tumour markers in NP brush samples can accurately diagnose primary NPC and monitor the efficacy of treatment (Tong 2002, Hao 2003, Mäkitie 2004).

Nevertheless, inadequate and non-representative specimens for study are the limiting factors in cytodiagnosis. This is related to the choice of collecting instruments and the technique of collection. Poor cellular preservation and inexperienced examination are the other limiting factors in the accurate cytodiagnosis of NPC (Chang 2001). Therefore, more studies have to be performed to confirm the role of cytological study of NP brush method in the diagnosis of primary NPC.

2.4.1.4. Epstein-Barr virus (EBV) serology

1. Epstein-Barr virus and its associated antigens

Epstein-Barr virus is a double-stranded DNA virus which is classified as a member of the *Herpesviridae* family. It has a DNA-containing icosahedral viral nucleocapsid enclosed in a lipoprotein envelope with virus-specific membrane glycoproteins (Epstein 1964). EBV infection is unique in the human population and the EBV has adapted a unique method of survival throughout the journey of evolution. Primary infection with EBV in human beings usually occurs in the first decade of life in lower socio-economic groups, whereas infection occurs

commonly in older children and young adults in higher socio-economic groups, as infectious mononucleosis. Human B lymphocytes were found to be the only susceptible cells for EBV infection in vitro (Jondal 1973). Infection of B cells by EBV may lead to two different outcomes: transformation of B cells into latent infection, or activation of the latent cells into the lytic cycle, leading to cell death with or without the production of infectious virions (Paganal 1981).

In the latent mode of infection, the EBV DNA remains in cells as a closed circular episome or integrated into the cellular DNA. During the process of transformation, a number of latent antigens are expressed. These include LMP-1, LMP-2A/B, EBNA 1, 2, 3A, and3C and LP. EBNA proteins are the only EBV-specific antigens expressed persistently in carcinoma cells of NPC biopsies (Fahraeus 1988). However, the latent infection can be disrupted and activated spontaneously or induced by super-infection or chemicals. Initiation of lytic infection is mediated by the expression of the BamHIZ EBV replication activator (ZEBRA) encoded in the BZLF1 protein. A number of gene products have been identified in the lytic replication cycle of EBV. These products include early antigens (ZEBRA, DNAase, polymerase, etc.) and late antigens (viral capsid antigen, late membrane antigen, etc.) (Ernberg 1974, Henle 1971, Hummel 1982).

The early antigens consist of a group of non-structural polypeptides, the classification of which is based on the location and appearance of the antigen in the cells as well as their stability after methanol fixation (Henle 1971).

The viral capsid antigen (VCA) and late membrane antigen (LMA) are viral-specific late proteins and are integral components of the full virion. The VCA is the main building block of the virus nucleocapsid, while LMAs are associated with the viral envelope and are responsible for the induction of virus-neutralizing antibodies.

2. EBV serology in the diagnosis of undifferentiated NPC

It has been shown that different EBV-specific antigens are expressed in the immunological response in patients with different EBV-associated diseases (Tam 1999).

Since the early seventies, specific markers of latent EBV infection have been identified in the tumour cells and blood lymphocytes of NPC patients (Klein 1974). The serum of patients with NPC regularly exhibit high levels of immunoglobulin A (IgA) against EBV-specific antigens (Fahraeus 1988, Brooks 1992). Among the different EBV-specific antigens, VCA and early antigen (EA) are of most diagnostic significance. From the studies in endemic regions, it is found that the sensitivity of IgA anti-VCA (97% endpoint at >5) is higher than that of IgA anti-EA (79% endpoint at >5), whereas the specificity of IgA anti-EA (97% endpoint at >5) is higher than that of IgA anti-VCA (67.2% endpoint at >5) in the diagnosis of primary NPC. Therefore, IgA anti-EA appears to be a more specific but less sensitive marker than IgA anti-VCA. However, when the two serological markers are used together, the sensitivity and specificity for the diagnosis of NPC are elevated to 80% and 98%, respectively, using a serological endpoint of > 5 for both markers (Tam 1999). Therefore, IgA EBV titres are important and reliable markers for

undifferentiated NPC. IgA titres to VCA and EA are now used routinely as diagnostic criteria for undifferentiated NPC (Ho 1976).

Apart from its diagnostic value in primary NPC, EBV serology is also useful in detecting patients at risk of NPC. It can be used to estimate the risk of subsequent development of NPC for those patients with a positive test without clinical evidence of NPC. Among those patients who had raised serum IgA anti-VCA titres without evidence of NPC, 2% subsequently developed NPC and 69% showed seroconversion to negative titres within follow-up of a median of 54 months (Lo 2004).

3. EBV serology as a screening tool for undifferentiated NPC

EBV serology has been used as a screening test for NPC in the general population of an endemic region. Several studies have been conducted in China to screen the asymptomatic population using mostly IgA anti-VCA titres. The sero-positive incidences were 2.65 % in 21 cities of southern China (Deng 1995), 5.3% in Guangxi (Zeng 1982), and 9% in Guangdong (Sham 1990d). The incidence of NPC among the sero-positive subjects was between 0.37% and 6.2%.

It has been shown that false negative rates for EBV IgA of either VCA or EBNA 1 are 10-15% (Chan 2003, Tsang 2004). Therefore, 10-15% of asymptomatic NPC patients will be missed by EBV serological screening. In addition, the poor sensitivity (60%) and unacceptable false negative rate (10-15%) of EBV EA titres in stage I NPC makes it unsuitable as a screening marker (Leung

2003a). Considering the cost-effectiveness of EBV serology, it is not an ideal screening tool for NPC in the general population.

Nevertheless, EBV serology seems to be valuable in screening family members of NPC patients. Ng et al. (Ng WT 2005) conducted a study in which asymptomatic first-degree relatives of NPC patients were screened. Among 929 family members, 9% were found to have raised IgA against EBV. After examination of those subjects with positive EBV serology, 1.2% of the first degree relatives of NPC patients were found to have NPC at the first visit or subsequent follow-up. More early-stage NPC cases (42%) were diagnosed from screening than from those with symptoms (2%). The study confirmed that early NPC can be detected by serological screening in subjects with a positive family history of NPC.

4. Plasma EBV DNA

Using a real-time quantitative PCR method, Lo et al. demonstrated that cell-free EBV DNA was detectable in the plasma of 96% of NPC patients in contrast to 7% of controls (Lo 1999a). Further studies have shown that plasma EBV DNA is a more sensitive and specific marker than the serum IgA VCA titre for the diagnosis of NPC. These findings provide convincing evidence for the use of plasma EBV DNA measurements for the diagnosis and staging of NPC as well as for monitoring recurrence and metastasis of this tumour (Leung 2004, Shao 2004).

Compared to EBV IgA serology, plasma EBV DNA measurement has a lower false positive rate of 5%. Furthermore, it identifies almost all false-negative IgA anti-VCA cases and gives 99% diagnostic sensitivity when combined with IgA anti-

VCA serology (Leung 2004). In the screening setting, EBV DNA identifies three-quarters of false-positive IgA anti- VCA cases. Therefore it is postulated that the selective application of plasma EBV DNA measurement in an EBV serology-based screening protocol could improve screening accuracy (Leung 2004). A few studies have also found that the pre-treatment level of circulating EBV DNA is a prognostic indicator in early stage NPC (Leung 2003b, Lin 2004).

However, as the number of EBV DNA copies detected in the blood indirectly reflects the tumour volume, this assessment carries a 10-15% false negative rate for early stage I primary NPC (Leung 2003a).

2.4.1.5. Radiological diagnosis of primary NPC

Compared to clinical examination, serology tests, and nasal endoscopy, imaging plays a lesser role in the diagnosis of primary NPC. Nevertheless, as the nasopharynx is relatively inaccessible to physical examination and surgical procedures, imaging can help to identify minor mucosal disease when endoscopy and biopsy are negative. In addition, it can help in the assessment of the size of the tumour, the staging of the tumour, radiotherapy planning, and detection of recurrent disease. Among all modern imaging techniques, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and ultrasonography (US) are the principal modalities of imaging in the diagnosis of NPC.

1. Computed tomography (CT)

Being useful to define the soft tissue planes of the nasopharynx, CT is an established, accurate, and the most widely available technique for evaluating patients with primary NPC. This multiplanar imaging is especially useful in the assessment of the involvement of the skull base by tumours. Coronal and axial CT with bone settings has the advantage of being able to depict early cortical bone invasion, especially around the neural foramina. Therefore, CT has been selected as the method of choice for the assessment of the tumour extent as well as the degree of skull base erosion (Olmi 1995). Nevertheless, its role is limited in the diagnosis of small volume disease of NPC confined in the fossa of Rosenmuller, as evaluation of the symmetry of the nasopharynx on CT is notoriously difficult. Therefore, since the advent of magnetic resonance imaging (MRI), which has a superior contrast resolution, the diagnostic role of CT has been gradually superseded by MRI.

Nevertheless, as CT is less expensive and more sensitive than MRI in the detection of nodal necrosis and extranodal spread, it still remains the gold standard for staging of disease and radiotherapy planning in many centres (Chong 1996a, Yousem 1992).

2. Magnetic Resonance Imaging (MRI)

MRI has the advantage of having better soft tissue resolution and multiplanar capacity. Compared to CT, it is more accurate in the detection of early primary tumours, the assessment of skull base erosion, staging of the disease, detection

of recurrent disease, and even screening of asymptomatic patients. However, its value is limited by the relative lack of bony detail it provides, and by its high cost.

The strength of MRI is most evident in demonstrating early perineural infiltration, basal foraminal infiltration, meningeal involvement, and intracranial extension (Crawford 1989, Mineura 1991). The ability of T2-weighted sequences of MRI showing good contrast between NPC and the pharyngobasilar fascia and the adjacent structures in the nasopharyngeal mucosal space gives it a further advantage over CT in the evaluation of early disease. MRI has been found to be superior to CT in delineating the infiltration of the marrow spaces in skull base erosion, while the interpretation of CT often underestimates the frequency and extent of skull base involvement (Poon 1996, Wong 1996).

Furthermore, as MRI seems to provide the most detailed imaging of soft tissue invasion outside the nasopharynx and of retropharyngeal node involvement, Chung et al. concluded that MRI is better than CT for the staging of NPC (Chung 2004, Ginsberg 1998, Chong 1996c).

Apart from the evidences that MRI is more accurate than CT in detecting and staging NPC (Ginsberg 1998), a preliminary study has also shown that MRI had a sensitivity of 100%, specificity of 95%, and accuracy of 95% in detecting NPC as a screening device in a group of asymptomatic patients and has the potential to direct the site of biopsy in small cancers that may be missed by endoscopy (King 2006).

However, the value of MRI is limited by the relative lack of bony detail it provides, and by its high cost.

3. [^{18}F]-2-fluoro-deoxy-D-glucose positron emission tomography (PET) (^{18}F-FDG PET)

Positron emission tomography is an advanced form of radionuclide imaging. It measures cellular glycolysis, which reflects cellular proliferation, growth, and cell type de-differentiation.

The assessment of locoregional metastasis is relevant to the management of NPC patients. As there is a tremendous overlap of positive and negative nodes based on mere assessment of their size, CT scan is not an ideal diagnostic tool. In this aspect, ^{18}F-FDG PET has been found to be more accurate than CT in detecting cervical nodal metastases (Kao 2000).

In NPC patients, the most common site for distant metastatic disease is bone (20%), followed by the lungs (13%) and liver (9%) (Sham 1990a). Nevertheless, most of these patients are asymptomatic. ^{18}F-FDG PET is able to detect distant metastases that are otherwise clinically occult (Ng 2004a).

Chang et al. found that the sensitivity, specificity, positive predictive value, and negative predictive value of ^{18}F-FDG PET for distant metastases were 100%, 90.1%, 63.6%, and 100%, respectively (Chang 2005). It appears that PET is more accurate than the conventional work-up to stage the nodal and metastatic diseases of NPC.

Recently, fused PET-CT emerged as a valuable imaging tool for the staging of NPC. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of PET-CT studies for staging NPC were 96%, 94.4%, 95.4%, 96%, and 94.4%, respectively. Therefore, PET-CT is more accurate than PET alone or CT alone for the depiction of NPC (Chen 2006). In addition, PET-CT allows the development of biological radiotherapy, in which tumour areas with intense metabolic activity can be identified and given a higher daily radiation dose compared to the remaining target areas using a conformal technique.

4. Ultrasonography (US)

Cervical nodal metastasis is a common presenting feature of NPC. However, reactive lymphadenopathy of inflammatory origin or tuberculous cervical adenitis may mimic the metastatic lymph nodes of NPC patients. Compared to clinical examination, high resolution ultrasound is more sensitive and accurate in the diagnosis of metastatic lymph nodes (Bruneton 1984, Vassallo 1992).

In contrast to benign reactive lymph nodes, the metastatic neck nodes from NPC always appear hypoechoic on US relative to surrounding tissues. Most of them are situated in the upper cervical chain (22%) and in the posterior triangle (64%). They are usually round in shape with a sharp border and without an echogenic hilus or intranodal necrosis. Nodes with these echoic features are considered to be diagnostic of malignancy in a patient with known NPC (Ahuja 1999).

US combined with fine needle aspiration cytology (FNAC) of neck nodes has a high specificity (97.2%) in distinguishing benign from malignant disease and thus provides the first clue to the presence of NPC (de Jong B 1989).

2.4.2. Treatment of primary NPC

The choice of treatment modality depends on the pathology of the tumour, patients' factors, and the stage of the disease.

Based on the advance in imaging technology and incorporation of significant prognosticators, various stage-classifications for NPC have been proposed over the decades (Ho 1982, Green 2002). Nevertheless, there is still no consensus as to which is most appropriate. To date, a modified Ho's classification is considered the most effective classification in separating patients into different prognostic groups (Table 2-1). The UICC/AJCC classification is the other commonly used classification preferable for publications (Table 2-2).

With knowledge of the stage of the disease, the treatment strategy can be tailor-made to individual patients for better control and survival rates.

2.4.2.1. External radiotherapy (ERT)

NPC is a radiosensitive tumour. Over decades, radiotherapy has remained the mainstay of curative treatment for all stages of NPC without distant metastases (Lee 1992a, Lee 1993a, Wang 1989). There are two types of radiotherapy: external radiotherapy (ERT) and brachytherapy.

External radiotherapy is employed as the principal treatment of primary NPC; it delivers a radical tumoricidal dose of large field radiation from both sides of the head to irradiate the primary tumour in the nasopharynx and cervical nodal metastases.

So far, there has been a lack of common consensus on the optimal time-dose-fractionation. Conventionally, in most of the centres, fractionated radiotherapy involving the administration of 1.8 or 2 Gy per fraction, five fractions per week for seven weeks, to a total dose of 60-70 Gy is employed to treat NPC (Wang 1989). Although there has been evidence to favour the use of higher doses beyond the conventional tumoricidal level for advanced stages of NPC, the rate and severity of complications increase with the dosage, and so the total tumour radiation dose cannot be increased indefinitely without prohibitive radiation morbidity (Teo 1996a, Shanmugaratnam 1978, Yan 1989).

In general, ERT is effective in controlling the disease. Retrospective studies by major centres using ERT alone showed that the cumulative 5-year local control rate was between 67% and 79% for tumours confined within the nasopharynx (Lee 1993b). However, the cumulative local control rate falls to 44% when the skull base is eroded and 31% when there is infiltration of the cranial nerves.

Nodal control is relatively less problematic. With prophylactic neck irradiation, more than 90% of node-negative patients remained free of regional disease. To patients with more overt lymphatic involvement, a control rate of 85% to 90% can be achieved with an adequate total dose.

The effective locoregional control by conventional ERT yields an overall 5-year survival rate of 75%, varying from 85% for stages I–II disease to 66% for stages III–IVb disease (Lee 2005a).

However, radiotherapy has limitations. The most sinister problem is the exceptionally high incidence of haematological dissemination, which occurs in 40% of patients presenting with advanced T-stage disease and 60% of those with nodes extending down to the supraclavicular fossa. This may eventually result in locoregional failure.

Furthermore, one of the adverse side-effects of ERT is collateral damage of the surrounding normal tissues. The nasopharynx is surrounded by an array of radiosensitive normal tissues such as the brainstem, spinal cord, temporal lobes, optic and auditory pathways, and parotid glands (Lee 1992b). Conventional ERT employs large field radiation coming from both sides of the head to irradiate the nasopharynx. The dose differential between tumour and normal tissues is low. The high dose radiation which is required to eradicate the disease can result in irreversible radiation damage to normal tissues (Lee 1992b). Although some of the complications are transient, severe acute reactions can sometimes lead to late irreversible damage (Maciejewski 1992). A series of studies have shown that the median incidence of late complications of radiotherapy is 37%. The serious morbidity rate has been found to be 7% (Lee 1992b) and the treatment mortality rate 3% (Haghbin 1985).

Intensity modulated radiotherapy (IMRT) is an advanced form of conformal radiotherapy, conforming a high dose to the tumour while conforming a low dose to normal tissues. Besides employing multiple beams conforming to the shape of the target in three dimensions, IMRT also allows for fine modulation of radiation intensity within each radiation beam. As a result, there are thousands of beamlets, each with a calculated intensity to deposit a defined dose of radiation at each specific target point. A good therapeutic ratio can be achieved by giving a high dose to the tumour while keeping down normal tissue complications by limiting the radiation dose to normal tissues.

For early disease, IMRT has been shown to achieve improvement in local control and to reduce xerostomia. (Kwong 2006, Pow 2006, Kam 2007). For locally advanced disease, dose escalation with IMRT has been used in combination with chemotherapy. Early results showed improvement in local control with reduced toxicity. To date, IMRT is accepted as the new standard of care for the treatment of NPC (Kwong 2004a, McMillan 2006).

2.4.2.2. Brachytherapy

Brachytherapy is another form of radiation therapy which allows local delivery of a high dose to a limited volume of the nasopharynx, while at the same time restricting the irradiation of normal tissue to a minimum.

All along, different types of intracavitary applicators have been designed and various isotopes have been implanted in the nasopharynx. With the advent of remote afterloading machines, nasopharyngeal brachytherapy has been adopted

as adjuvant treatment with ERT for primary NPC. Several clinical trials have incorporated elective brachytherapy into the primary treatment of patients with early stage NPC, and a 90-95% local control rate has been demonstrated (Teo 2000, Wang 1991, Chang 1996, Levendag 2002, Lu 2004, Ng T 2005, Ozyar 2002). However, these results do not show additional improvement in local tumour control by brachytherapy. Therefore the extra benefit of brachytherapy to primary ERT has remained unproven.

2.4.2.3. Chemotherapy

NPC is both radiosensitive and chemoresponsive. Recent data suggest that chemotherapy administered concurrently with radiotherapy improves the effect of radiotherapy in patients with locoregionally advanced NPC through volume reduction, increase in radiosensitization, and reduction in the number of micrometastases, thus decreasing the possibility of distant metastases and improving overall survival (O'Sullivan 2007).

So far, 15 randomized clinical trials and four meta-analyses of chemotherapy combined with radiotherapy have been reported in the English literature for patients with locally advanced NPC (Huncharek 2002, Langendijk 2004, Thephamongkhol 2004, Baujat 2006, Afqir 2009). Among all clinical trials, the timing of administration of chemotherapy is highly variable. With respect to the time of application relative to radiotherapy, chemotherapy can be described as neoadjuvant, concurrent, or adjuvant to radiotherapy.

Although the literature has suggested that induction chemotherapy can cause

a significant reduction in the incidence of locoregional failures and distant metastases, no improvement in overall survival was observed (Chan 1995, Chua 1995, International Nasopharyngeal Cancer Study Group 1996, Roussy 1996, Chua 1998, Ma 2001, Hareyama 2002). Of all the trials on induction chemotherapy, only one trial using cisplatin, epirubin, and bleomycin achieved significant improvement in disease-free survival at the expense of high treatment mortality (International Nasopharyngeal Cancer Study Group 1996). Nevertheless, as reduction in the size of the primary tumour should help to achieve better locoregional control with radiotherapy (Lee 2005b), further investigations are necessary to recommend this method for use in practice.

Similarly, the results of adjuvant chemotherapy have been disappointing; none of the three trials achieved significant benefit in any end points (Chi 2002, Kwong 2004b, Al-Sarraf 1998).

In contrast, data of eight recent randomized trials and four meta-analyses have demonstrated that patients with advanced locoregional NPC can be better treated by concurrent chemoradiation than by radiotherapy alone. All randomized trials demonstrated an improvement in survival or locoregional control with concurrent cisplatin-radiotherapy in patients with stage III/ IV NPC (Al-Sarraf 2001, Huncharek 2002, Lin 2003, Kwong 2004, Langendijk 2004, Thephamongkhol 2004, Chan 2005, Lee 2005c, Zhang 2005, Lee 2006, Wee 2006). Although there is a higher incidence of acute mucosal and haematological toxicities, concurrent cisplatin-radiotherapy has now been adopted as the standard treatment for patients with advanced NPC. Nevertheless, the optimal regimens of concurrent

administration of chemotherapy and radiotherapy are yet to be determined (O'Sullivan 2007).

2.4.2.4. Surgery

As NPC is both radiosensitive and chemosensitive, the role of surgery in the management of primary NPC is limited. Radical surgical resection of a primary tumour is difficult because of the deep-seated anatomical location of the nasopharynx and its proximity to adjacent critical structures. Moreover, as the disease tends to be submucosal, it may not be possible to define margins of resection adequately.

2.5. Management of persistent and recurrent NPC

To improve the overall survival rate of NPC patients, early diagnosis of local relapses after radiotherapy is important. Therefore, close monitoring of progress after the primary treatment of NPC patients is essential. The majority of recurrences occur in the first three years (Wang 1987, Lee 1999) and salvage treatment is feasible in most of the patients with clinically isolated local failure. A multi-centre study suggested that salvage treatment only prolongs survival in patients with T1 to T2 recurrent disease (Yu 2005). Therefore, patients who have an early and small recurrence have a more favourable prognosis.

2.5.1. Diagnosis of persistent and recurrent NPC

Early detection of relapses depends on clinical surveillance and regular monitoring with investigations including nasal endoscopy, imaging, and EBV serology.

2.5.1.1. Clinical presentation

In general, the presenting symptoms of persistent disease or local relapse after radiotherapy are vague and more poorly differentiated than those of primary carcinoma. Nasal bleeding, pain, and diplopia caused by the infiltration of the recurrent tumours usually occur late. Rare presentations, such as facial palsy (Low 2002), facial lymphadenopathy (Chong 2000), diffuse neck swellings, and dermatitis (Leong 2001) have also been reported.

Moreover, the clinical detection of cervical nodal relapse is notoriously difficult, with a false positive rate of about 25% and a false negative rate between 10 and 15% (de Jong 1989).

As most of the recurrent diseases can only be revealed during routine clinical examination and routine investigations, regular follow-up and a high index of clinical suspicion are prerequisites to the early diagnosis of persistent and recurrent NPC (Wei 2007).

2.5.1.2. Nasal endoscopy and tissue biopsy

Following radiotherapy, repeated surveillance of the status of the nasopharynx with nasal endoscopy is important to detect residual disease and early recurrence. However, after radiotherapy, excessive crust formation, general

mucosal oedema, and fibrosis of the nasal mucosa make evaluation of the nasopharynx with rigid endoscopy difficult to interpret (Croft 1988) (Figure 2-7). Therefore, identification of residual and recurrent disease in the irradiated nasopharyngeal tissues is much more difficult than in the primary disease (Figure 2-8). Although the use of fibre-optic endoscopy in the biopsy of suspicious lesions in the irradiated nasopharynx is found to be more accurate in identifying residual tumour than the conventional indirect nasopharyngeal mirror (Sham 1990c, Sham 1990d), its efficacy in detecting the small lesions is unsatisfactory.

Unlike primary disease, early residual or recurrent disease may appear as submucosal lesions underneath the normal-looking nasopharynx, which may escape the surveillance of conventional nasal endoscopy (Figure 2-7B). In Hong Kong, Sham et al. have found that the positive pick-up rate of persistent and recurrent lesions by conventional endoscopy during follow-up of patients was less than 50% (Sham 1992). Another small scale retrospective study on 57 NPC patients showed that the sensitivity, specificity, and positive and negative predictive values of conventional endoscopic examination for recurrent disease were 75%, 94.3%, 50%, and 98%, respectively (Chao 2003). In another larger series, it was found that after radiotherapy, the sensitivity of conventional endoscopy in predicting recurrent disease was as low as 29%, compared to 99.7% before the radiotherapy (Kwong 2001a). Similarly, in non-endemic regions, Ragab et al. also showed that, at 12 weeks after radiotherapy, the sensitivity, specificity, positive predictive value, and negative predictive value of conventional endoscopy in detecting recurrent NPC were 66.6%, 95%, 66.6%, and 95%,

respectively (Ragab 2008). These results have clearly shown that the accuracy of conventional nasal endoscopy in detecting early local recurrence is limited.

Due to the limitations of conventional endoscopy, it is recommended that endoscopy should be performed meticulously to identify suspicious lesions, where repeated and multiple biopsies should be performed under local or general anaesthesia.

2.5.1.3. Cytological diagnosis

Cytological examination of irradiated nasopharyngeal tissues collected by nasopharyngeal brush is possible. However, the detection of tumour recurrence in irradiated tissues using optical microscopy may be difficult. Only an experienced cytologist can differentiate between radiation changes in benign cells and malignant cells. Under microscopy, the nasopharyngeal cells after radiation appear as enlarged cells with minimally enlarged nuclei containing coarse chromatin, multi-nucleation, prominent nucleoli, and cytoplasmic vacuolation. The nuclear: cytoplasmic ratio is not greatly increased after radiation in comparison to cancer cells. The cells do not show variation in size and the nuclear membranes remain smooth (Chang 1999). These features of irradiated cells may mimic the appearance of malignant cells and the presence of inflammation and tissue necrosis creates further diagnostic difficulties for the cytopathologist. Therefore, the cytopathological diagnosis of recurrent NPC is largely operator dependent.

To solve this problem, an objective test to detect recurrent cancer cells has been introduced. As the tumour cells are known to harbour EBV in a latent state

and EBV is expressed by most of the NPC tissues, quantitative analysis of EBV-specific genomes in nasopharyngeal brush samples using real-time PCR is an attractive diagnostic method for recurrent NPC (Stewart 1993).

Latent membrane protein 1 (LMP-1) is one of the specific antigens expressed only by NPC rather than other head and neck tumours. Regression of LMP-1 is demonstrated in patients soon after treatment with chemotherapy, and the quantity of LMP-1 is inversely proportional to the time after treatment (Lin 2005). Therefore, re-expression of LMP-1 in the nasopharyngeal brushings of irradiated patients may be an early sign of recurrence. Tsang et al. have shown that, by detecting re-expression of LMP-1 in nasopharyngeal swabs, NPC recurrence was diagnosed with a sensitivity of 100% and a specificity of 98.4% (Tsang 2003).

These findings suggest that early detection of the recurrence of NPC can be achieved by this rapid and non-invasive method. However, the reliability of cytodiagnosis relies heavily on the availability of adequate and representative specimens for study. This method also suffers from the major drawback of being costly. Therefore, the method is not widely used.

2.5.1.4. Epstein-Barr virus (EBV) serology

1. IgA antibodies against EBV VCA and EA

Although EBV serology is useful in the detection of primary NPC, its role in the diagnosis of recurrent tumour has not been established.

Although sporadic cases have shown that a four-fold rise in EBV titres, particularly of antibodies to the early antigens of the virus, was a significant predictor of relapse before clinical presentation of local and regional recurrence, neither persistently high titres nor falling titres after treatment were found to be reliable indicators of relapse or survival (Wyatt 1993). Furthermore, as IgA titres of EBV remain high after external radiotherapy, they are useless for screening for residual or recurrent NPC.

2. EBV DNA

Circulating EBV DNA analysis has been shown to be valuable in the detection, prognostication, and monitoring of patients with primary NPC. It has been shown that after treatment, plasma EBV DNA levels of NPC patients would decline exponentially with a median half-life of 3.8 days (To 2003) and remain undetectable in remission (Lo 1999b). Previous reports had also indicated that patients with recurrent NPC had elevation of plasma EBV DNA levels (Lo 1999b, Hsiao 2002). Therefore, in principle, the analysis of circulating EBV DNA is useful in monitoring the development of tumour recurrence following treatment (Chan 2002). Using a 50-cycle PCR-based approach, high sensitivity and a high negative predictive value for detecting recurrent disease can be obtained from the detection of the cell-free EBV genome in sera from patients with NPC (Hsiao 2002).

Nevertheless, as the number of EBV DNA copies detected in the blood indirectly reflects the tumour volume, for small recurrences that could be surgically removed for cure, the incidence of elevated EBV DNA copies was only about 61%

(Wei 2004). Another large scale study also showed that EBV DNA analysis carried a missing rate of 50-62% in patients with surgically salvageable stage I and II locally recurrent NPC (Leung 2003a). Therefore, the role of quantitative analysis of EBV DNA for the diagnosis of early recurrence has yet to be established. There is still room to develop more sensitive and specific molecular markers for screening for early primary and recurrent NPC.

2.5.1.5. Radiological diagnosis for recurrent NPC

1. Computed tomography (CT)

Before the advent of MRI, CT was the modality of choice in the follow-up of patients after radiotherapy. Most cases of recurrence take the form of a recurrent mass, bony destruction or the appearance of a growing tumour in the region of the nasopharynx. However, the diagnosis of recurrence is more difficult if there is no soft tissue extension showing in the CT scan.

Likewise, asymmetry of the nasopharynx on CT is not a reliable criterion for recurrence as this can be confused by post-radiotherapy changes. Abnormal density in the irradiated nasopharynx can be contributed to by post-radiotherapy oedema, inflammation, or fibrosis, as well as by recurrent tumour. Therefore, routine CT scanning has no added clinical benefit in the detection of recurrence in irradiated patients (Chao 2003).

2. Magnetic resonance imaging (MRI)

Compared with CT, based on the differences in signal intensity on T2-weighted images, MRI is superior in differentiating post-radiation changes from

recurrent tumours, except in those cases showing subtle bone erosions on initial CT scan (Olmi 1995). Coronal sections of MRI are also useful in diagnosing the extent of recurrent tumour invading the skull base or hypopharynx (Fujii 1994). Therefore, MRI is now becoming the modality of choice for the follow-up of patients who have undergone radiotherapy.

However, the signal intensity pattern of the tumour in MRI is not specific and may still be seen in radiation oedema and infection. The overlap in signal intensity can preclude differentiation of benign lesions from malignant counterparts (Gong 1991, Olmi 1995). Although MRI is more accurate than CT, with less false positive diagnoses (Olmi 1995), it has a 17% false positive rate in the detection of recurrent disease (Fujii 1994).

In essence, although MRI is more useful in identifying mature scarring, tumour recurrence, and post-radiation complications, it still cannot reliably demonstrate mucosal recurrence or differentiate tumour recurrence from post-radiation tissue changes. Comparison with previous images is essential to facilitate a definitive diagnosis.

3. Ultrasonography (US)

Following radiotherapy, the incidence of persistent or recurrent disease in the neck nodes is around 18% (Bedwinek 1980). It is not easy to detect residual or recurrent lymphadenopathy clinical examination after radiotherapy. Furthermore, due to post-radiation induration, nodes are frequently not palpable and persistent disease may proceed to distant metastases before they are detected clinically.

Therefore, ultrasound appears to be an ideal initial investigation to evaluate the status of cervical nodes after radiotherapy. However, owing to the fact that the features of previously abnormal nodes remain unaltered for at least three months following radiotherapy, it is hypothesized that a surveillance scan for the neck nodes should be performed one year after radiotherapy. Any change in the appearance and distribution of the lymph nodes in the scan should alert the clinician to the possibility of recurrence (Ahuja 1996).

In contrast to the case with primary NPC, ultrasound-guided fine needle aspiration cytology (FNAC) is not useful after radiotherapy. It is often difficult to obtain positive samples from the irradiated nodes. Moreover, the interpretation of the cytology of the scanty cells is very difficult.

Although US is useful in evaluating the neck for recurrent disease and may help to identify an enlarging node prior to its becoming palpable, regular comparisons with previous findings are still important.

4. ^{18}F-FDG PET

^{18}F-FDG PET is valuable in detecting cervical nodal and distant metastasis, and is also useful and accurate in detecting a locally recurrent tumour. A study has shown that the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of PET images for the diagnosis of recurrent NPC and distant metastasis were 92%, 90%, 92%, 90%, and 91%, respectively (Yen 2005a). Better differentiation between recurrent NPC and benign lesions by PET makes it valuable in avoiding unnecessary locoregional radiotherapy in some NPC patients

by the revelation of occult distant metastases, especially in patients with primary disease at nodal stages of N2 and 3 (Kao 2002, Yen 2005b).

However, false negative findings with PET may arise from nodal micro-metastases or small mucosal recurrences, and false positive results may arise from inflammatory changes, infection, or lymphoid hyperplasia. Since a majority of false positives occur during the early post-therapy period, caution should be exercised when using PET earlier than six months after treatment.

Apart from its valuable role in the diagnosis of recurrent and metastatic diseases, PET is also useful in identifying post-radiation complications. With the significant improvement in the survival of patients with NPC undergoing radiotherapy and the growing availability of sophisticated imaging modalities, the number of radiation encephalopathy (RE) cases relating to NPC radiotherapy is increasing. Delayed RE patients exhibit significant hypometabolic changes in the inferior temporal lobe, which is more accurately picked up by PET (Wang 2007).

2.5.2. Treatment for recurrent NPC

2.5.2.1. External radiotherapy and brachytherapy

In NPC, local persistent disease diagnosed at 4-5 weeks after completion of ERT has been associated with a significantly higher rate of ultimate local failure in advanced tumours (Teo 1996b). Yan et al. showed that 36% of the patients with histologically proven residual disease within two weeks of completion of ERT would subsequently develop local recurrence (Yan 1990).

Re-irradiation at a tumoricidal dose of no less than 60 Gy has been the most commonly employed method to treat local recurrences, although other methods such as nasopharyngectomy and photodynamic therapy are possible alternatives in selected cases. However, in view of the high radiation dose given in the primary therapy to the nasopharynx and surrounding tissues, re-irradiation carries a much higher morbidity. Furthermore, taking all recurrent T-stages together, the overall outcome of re-irradiation is poor, with 5-year and 10-year actuarial cancer-specific survival rates of only 14% and 9%, respectively (Lee 1993b).

To avoid the complications of ERT, brachytherapy is a better alternative for early recurrence. In theory, brachytherapy can deliver a very high and localized dose to the recurrence with relative sparing of the normal organs. The limitation of brachytherapy is that it cannot adequately irradiate a bulky tumour that erodes the skull base.

To date, brachytherapy is commonly employed as salvage treatment for local persistent disease. Studies have showed that patients with early T1 locally persistent disease could be effectively salvaged by brachytherapy alone or in combination with ERT (Leung 2000, Teo 2000, Kwong 2001b, Law 2002, Zheng 2004, Leung 2005).

Recently, there was more preliminary evidence suggesting that T2 patients with local persistent disease could also be effectively treated by brachytherapy (Leung 2005). In general, an overall 50-60% 5-year local tumour control can be

achieved from re-irradiation with brachytherapy alone. In well-selected patients, a 5-year local control of 80% can also be achieved with various interstitial implants.

To those patients with regional nodal recurrence, brachytherapy is also indicated as an adjunctive treatment to radical neck dissection, the combination giving reasonably good results (Ngan 1996).

2.5.2.2. Chemotherapy

Historically, the role of cytotoxic chemotherapy has been confined to the palliative treatment of metastatic or locally recurrent disease. Combination chemotherapy regimens have shown higher response rates than single agent chemotherapy.

One report has given an overall response rate of 59% with acceptable toxicity using a combination of paclitaxel and carboplatin in patients with local recurrent or metastatic NPC (Yeo 1998). To ascertain the role of chemotherapy in this group of patients, a multi-centre, prospective randomized study is needed.

2.5.2.3. Surgical treatment

Radical surgical resection of primary NPC is difficult because of the deep-seated anatomical location and the proximity to adjacent critical structures. However, surgery is an acceptable alternative in the management of locoregional relapse. Compared to radiotherapy, surgery is a better treatment modality to eradicate residual or recurrent disease in the nasopharynx and neck lymph nodes.

1. Neck dissection

Despite the sensitivity of the tumour to radiotherapy, there is a recurrence rate of 9% to 10% in the neck after therapeutic or prophylactic neck dissection (Huang SC 1980, Yu 1983). In the presence of persistent and recurrent lymph node metastasis, radical neck dissection is the treatment of choice. It gives a better survival rate of 40% to 80% compared to 19% to 28% with a second course of radiotherapy. Together with surgical salvage for persistence or recurrence, few died of uncontrolled nodal disease alone.

2. Nasopharyngectomy

Although the majority of local recurrences were treated with either a second course of ERT or with brachytherapy, more and more studies have shown encouraging results with nasopharyngectomy. There have been several approaches to this region (Fisch 1983, Belmont 1988, Uttley 1989, Wei 1991). So far, the commonly accepted technique for nasopharyngectomy has been via the maxillary swing approach, which allows wide exposure for adequate excision of the tumour in the nasopharynx (Wei 1991).

The results of nasopharyngectomy are variable. Reports have shown that patients' 3-year actuarial survival rate after nasopharyngectomy was 42.5% (Wei 1995) and its local control rate was 31% (Fee 1991). As most of the series involved carefully selected patients, the definitive benefit of the surgery to the patients has yet to be determined.

Table 2-1. Modified Ho's classification (1982)

Primary tumour (T)

T1 Nasopharynx only
T2n Nasal fossa
T2o Oropharynx
T2p Parapharyngeal region
T3a Bony involvement of below the skull base including floor of sphenoid
T3b Bony involvement of the skull base
T3c Cranial nerve(s) palsy
T3d Orbit, laryngopharynx (hypopharynx) or infratemporal fossa
T3p Parapharyngeal region

Regional lymph node (N)

N0 No nodes
N1 Node(s) above the skin crease at laryngeal cartilage
N2 Node(s) below the above skin crease but above the supraclavicular fossa
N3 Supraclavicular fossa node(s)

Metastasis (M)

M0 No distant metastasis
M1 Distant metastasis

Staging grouping

Stage I	(T1,T2n,T2o) N0M0
Stage IIa	(T1,T2n,T2o) (N1,N2)M0
Stage IIb	(T2p,T3,T3p) N0M0
Stage IIIa	(T2p,T3,T3p) (N1,N2)M0
Stage IIIb	(T1,T2n,T2o) N3M0
Stage IVa	(T2p,T3,T3p) N3M0
Stage IVb	M1 (any T, any N)

Table 2-2. UICC/AJCC classification (2002)

Primary tumour (T)

TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Tis Carcinoma in situ
T1 Confined to nasopharynx
T2 T2a Oropharynx and/or nasal fossa
T2b Parapharynx extension
T3 Bone invasion and/or paranasal sinuses
T4 Intracranial extension and /or cranial nerves, infratemporal fossa, hypopharynx, orbit involvement

Regional lymph node (N)

NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Unilateral ≤6 cm above supraclavicular
N2 Bilateral ≤6 cm above supraclavicular
N3 N3a Above supraclavicular >6 cm
N3b Supraclavicular fossa nodes

Metastasis (M)

MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

Stage grouping

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIa	T2a	N0	M0
Stage IIb	T1-2a	N1	M0
	T2b	N0-1	M0
Stage III	T1-2b	N2	M0
	T3	N0-2	M0
Stage IVa	T4	N0-2	M0
Stage IVb	Any T	N3	M0
Stage IVc	Any T	Any N	M1

Figure 2-1. Histology of NPCIN.

(A) The overlying epithelium is replaced by atypical cells that exhibit enlarged oval-shaped pleomorphic nuclei with prominent nucleoli and indistinct cytoplasmic borders (indicated by arrows). No invasion is present. (H & E stain, original magnification x200).
(B) Atypical cells demonstrate positive nuclear signals for Epstein-Barr virus RNA by in situ hybridization for EBV-encoded mRNA (EBER).

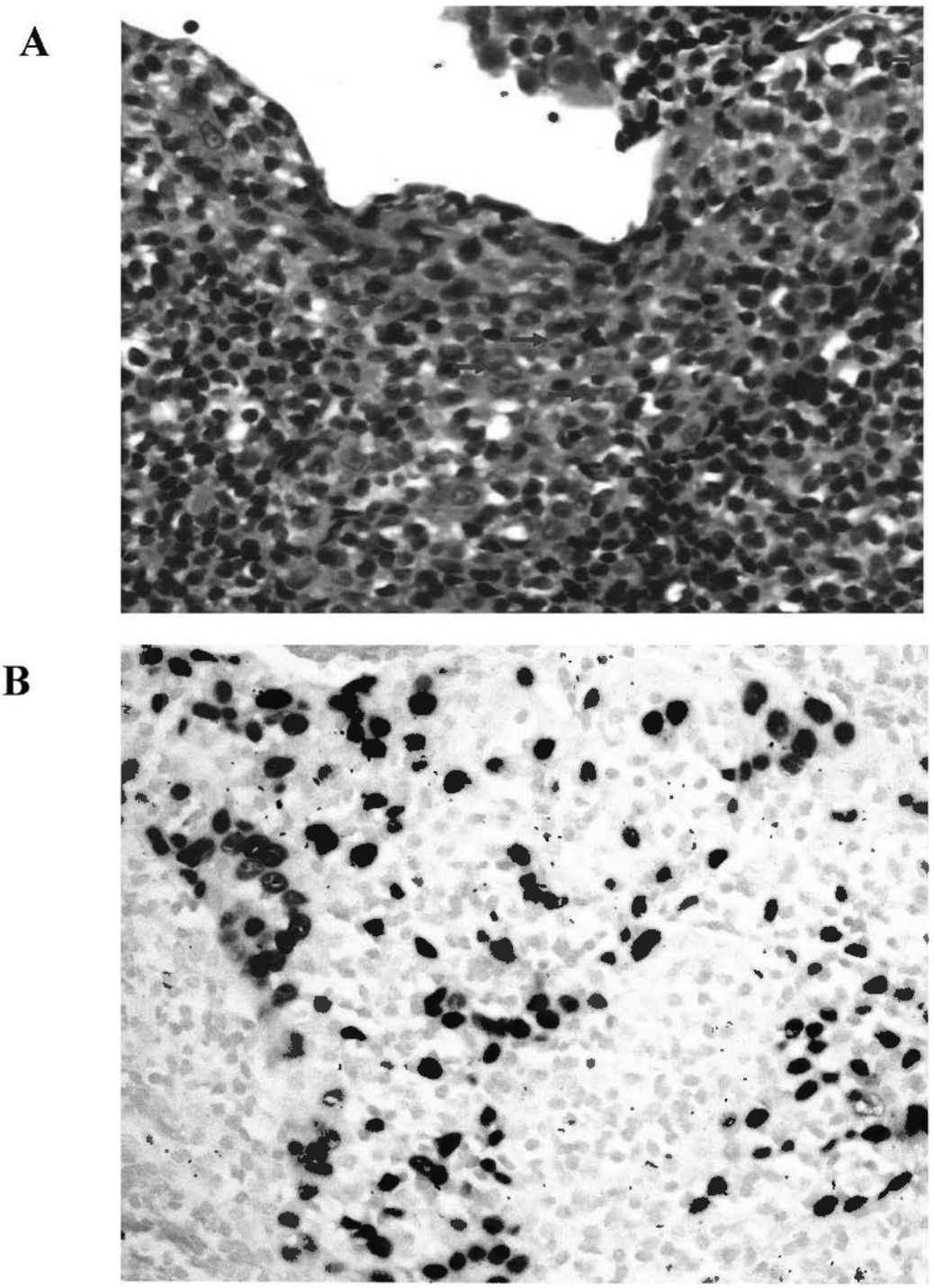
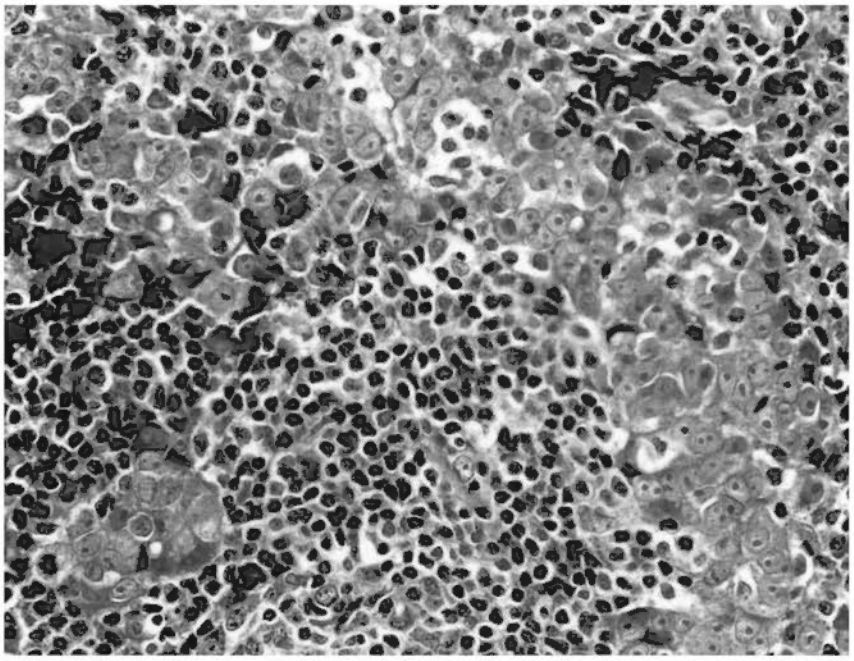


Figure 2-2. Histology of undifferentiated carcinoma of the nasopharynx.

(A) Schmincke pattern consisted of syncytial sheets of carcinoma cells demonstrating oval-shaped vesicular nuclei, prominent nucleoli, and scanty indistinct cytoplasm, admixed and surrounded with dense lymphoplasmocytic infiltrate (H & E stain, original magnification x400).
(B) in Regaud pattern, the borders of the tumour cells are better defined (H & E stain, original magnification x400).

A



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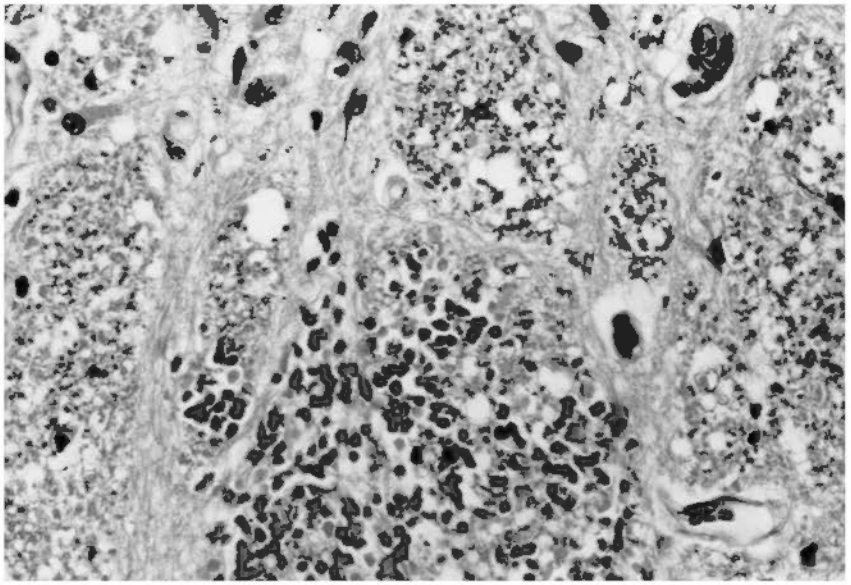


Figure 2-3. Normal epithelial cells with radiation induced cytomegaly.

The nuclear to cytoplasmic ratio is slightly increased but the cells exhibit homogeneity in size and shapes (Papanicolaou stain, original magnification x400).

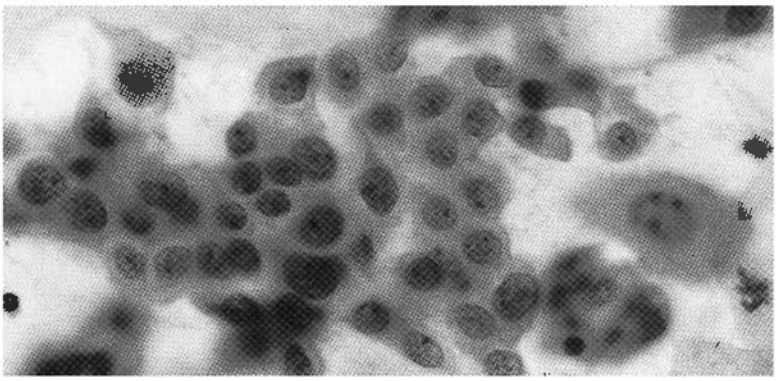


Figure 2-4. Histology of recurrent nasopharyngeal carcinoma.

The tumor is composed of syncytial sheets of malignant cells. The carcinoma cells exhibit oval pleomorphic nuclei with prominent nucleoli and scanty indistinct cytoplasm. (Haemotoxylin & eosin stain, original magnification X400).

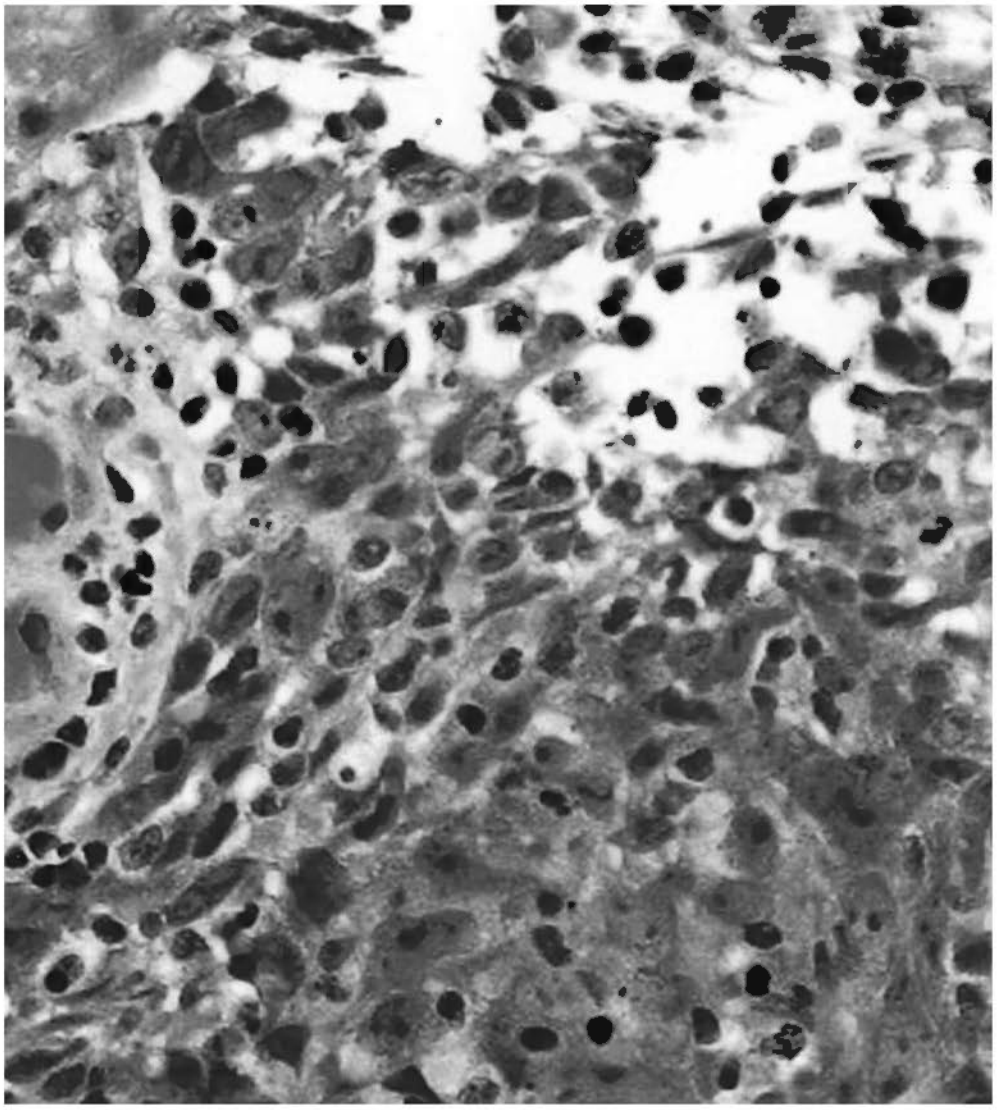
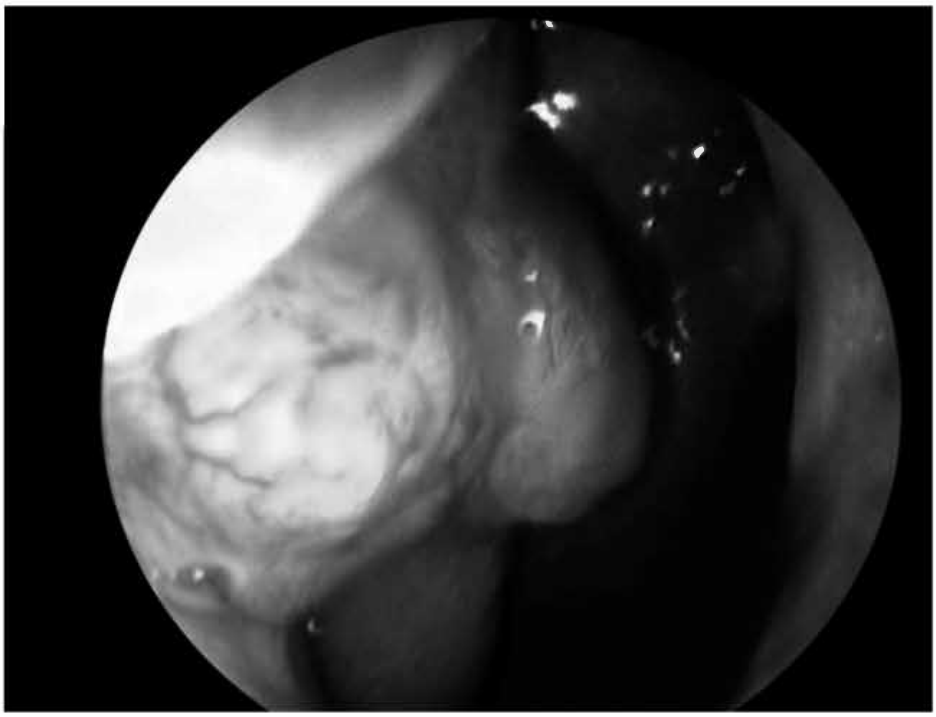


Figure 2-5. Endoscopic view of nasopharyngeal carcinoma.

(A) A prominent tumour situated in the right fossa of Rosenmuller.
(B) An early tumour situated in the centre of the nasopharynx as indicated by an arrow.

A



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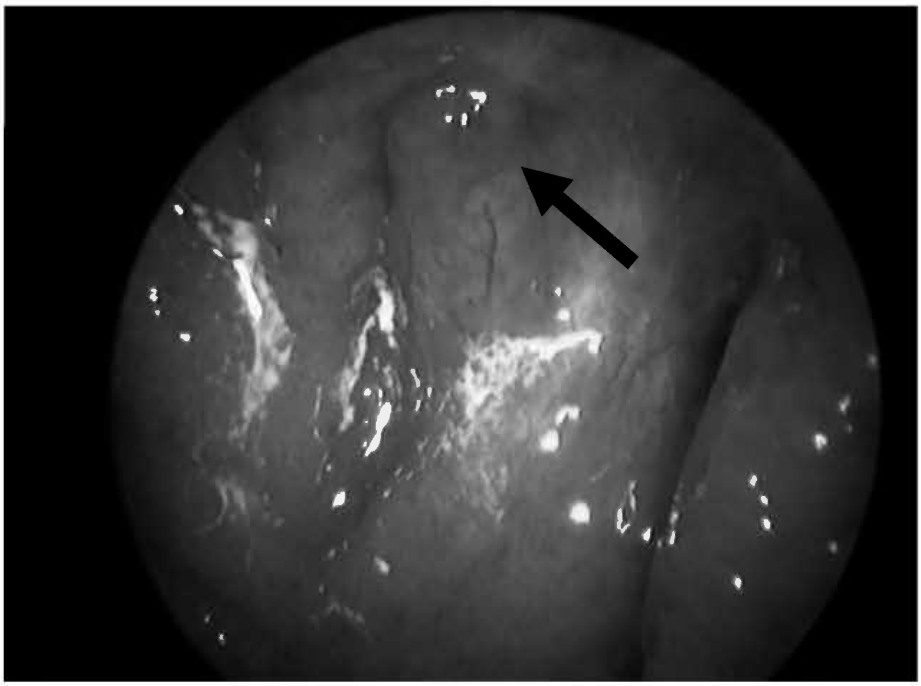


Figure 2-6. Exfoliative cytology of undifferentiated carcinoma of the nasopharynx.

The cells exhibit variation in nuclear size, vesicular nuclei and prominent nucleoli (Papanicolaou stain, original magnification x400).

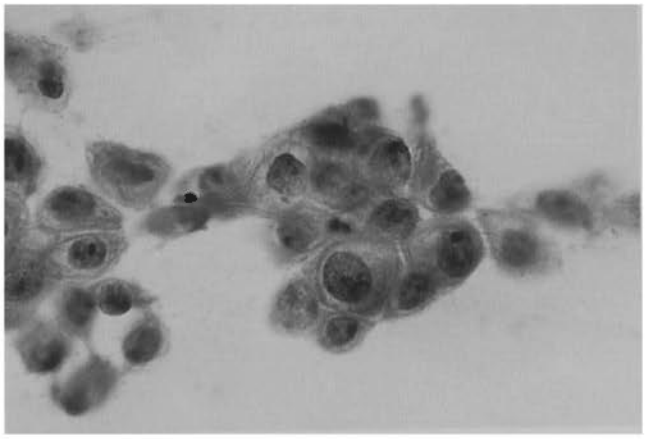
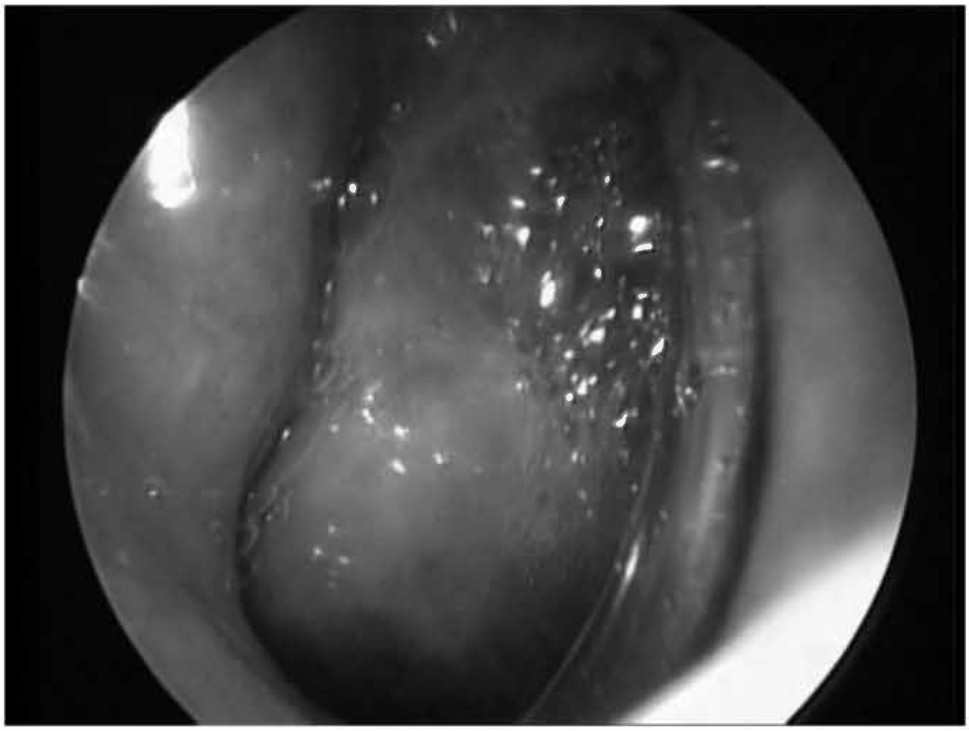


Figure 2-7. Endoscopic view of the irradiated nasopharynx.
(A) The mucosa is edematous and covered with mucus.
(B) The nasopharynx is carpeted with extensive exudate.

A



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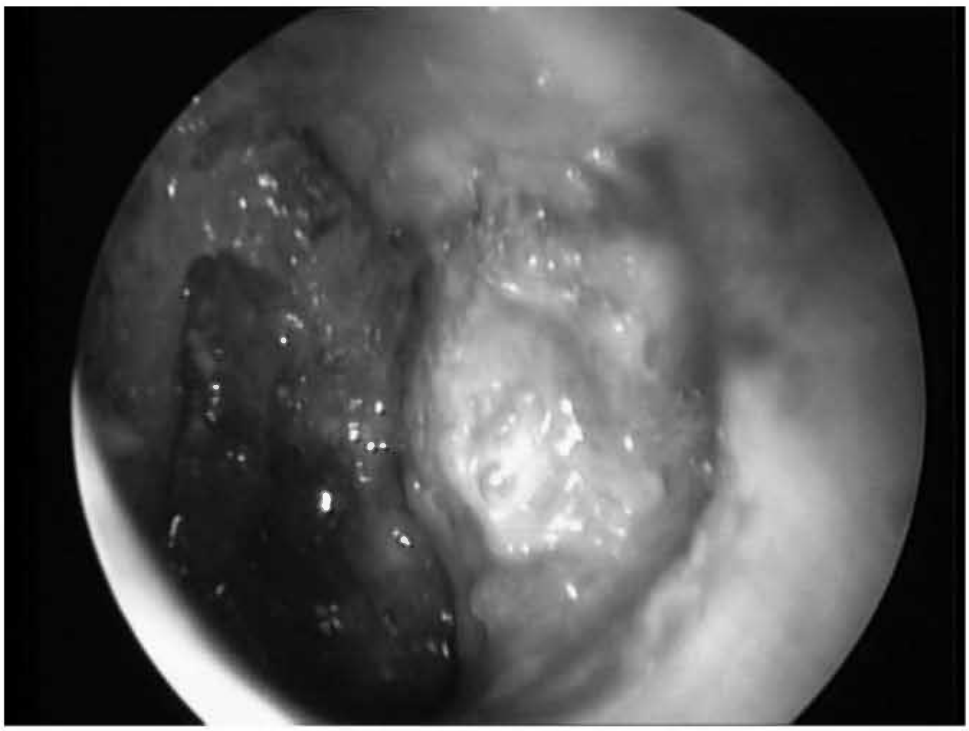
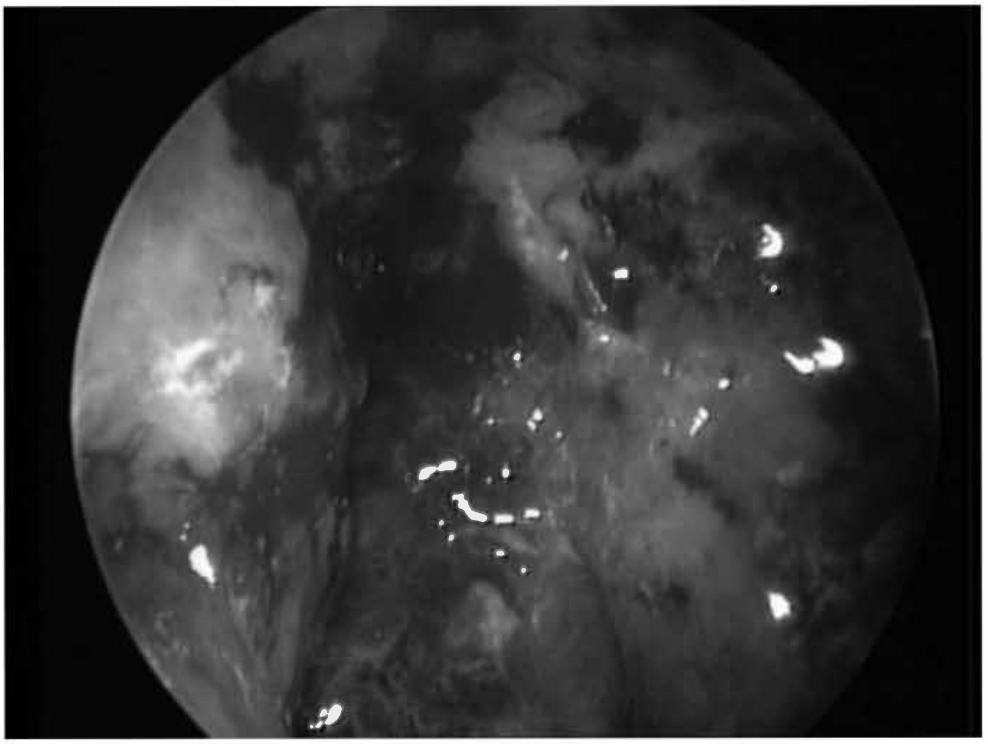


Figure 2-8. Endoscopic view of local recurrence.

(A) The mucosa is rough with contact bleeding. Biopsy showed recurrent tumours.
(B) Presence of persistent NPC in a normal looking irradiated nasopharynx.

A



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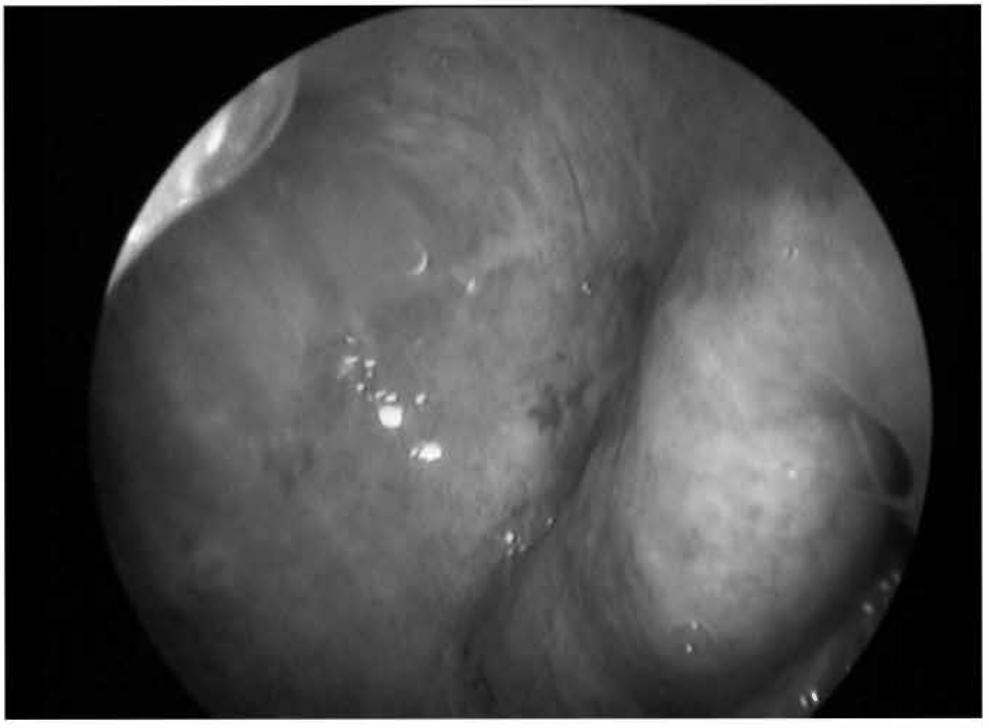


CHAPTER 3

CONTACT ENDOSCOPY (CE)

3.1. History of conventional nasal endoscopy

Endoscopy was used as early as the ancient Greek and Roman periods. An instrument considered to be a prototype of an endoscope was discovered in the ruins of Pompeii (Walk 1966). In 1805, Philip Bozzini made the first attempt to observe the living human body directly through a tube he created known as a "Lichtleiter" (light guiding instrument) to examine the urinary tract, rectum, and pharynx, including the nasopharynx (Bush 1974). In 1838, Baumes described his technique using a mirror to examine the larynx and choanae.

In 1853, Antoine Jean Desormeaux of France developed an instrument to examine the urinary tract and the bladder. He named it an "endoscope". This was the first time this term was used (Draf 1978).

In 1879, Max Nitze, employing the principle of a telescope with a conventional optical system, invented his cystoscope to examine the bladder and urinary system (Nitze 1879). A year later, Leiter and Zaufal used the Nitze cystoscope for nasal and nasopharyngeal examinations (Zaufal 1909). However, the device did not receive much attention because the hot platinum wire light source required a bulky built-in water cooling system.

In 1901, Hirschmann viewed the maxillary sinus with a modified Nitze cystoscope through an enlarged dental alveolus (Hirschmann 1903). After Hirschmann, various otorhinolaryngologists began to develop maxillary antrostomy, approaching the maxillary sinus primarily through the inferior meatus.

In 1925, Maltz coined the term "sinuscopy" and described techniques to examine the maxillary sinuses via inferior meatal and canine fossa routes (Maltz 1925). However, because of technical limitations encountered at the time, this technique was not widely accepted.

John Logie Baird, who invented the television, patented the idea of transmitting images through a flexible glass cable in 1926. These ideas influenced Harold H Hopkins, a mathematician and physicist who invented the zoom lens in 1948. By 1956, Hopkins introduced his new rigid rod lens endoscope with glass rods separated by "air lenses" (Hopkins 1966). This new optic system improved the previous Nitze system of a train of glass lenses by interspersing neutral gas between them instead of air. The mechanism of the light transmission involved the principle of total reflection, which minimizes the loss of visual clarity of the observed images. The optic efficiency was improved by lowering the refractive index and increasing the functional diameter of the lenses. Hopkins' rod lenses had clear advantages over the Nitze system in that they provided greater light transmission, a wider view, better image quality, and better contrast. Since then, rigid nasal endoscopy has become the standard procedure for the diagnosis of nasal and nasopharyngeal pathologies (Kaluskar 1997).

With the development of fibre-optic and glass-wool light conductors, fibre-optic endoscopy was introduced in the late 1970s. Since then, clinicians have had a choice between a flexible and a rigid endoscope for examining the nose. Some clinicians find rigid instruments harder to use and less acceptable to

patients, and they therefore prefer flexible instruments for examination of the nose and nasopharynx (Mohr 1991).

Modern nasal endoscopy allows a thorough evaluation of intranasal anatomy and identification of pathology that cannot be seen using standard techniques of anterior rhinoscopy. Levin has found that nasal pathology was identified by nasal endoscopy in almost 40% of patients deemed normal by traditional rhinological examination (Levin 1990). Using either type of endoscope, endoscopic examination was found to have a high sensitivity (84%) and specificity (92%) in the diagnosis of sinonasal diseases (Hughes 1998).

The extension of the use of nasal endoscopy for diagnostic purposes to minimally invasive sinonasal surgery was initiated and popularized by Messerklinger in the late 1960s (Messerklinger 1969, Messerklinger 1978). Following the modification of Messerklinger's techniques, modern endoscopic surgery has extended its application to the management of lacrimal, orbital and skull base diseases through the work of other rhinologists, including Draf, Stammberger, Kennedy and Wormald (Kennedy 1985, Stammberger 1986, Wormald 2006).

3.2. History of contact endoscopy

3.2.1. Application of contact endoscopy in gynaecology

The principle of contact endoscopy was first introduced in the field of gynaecology by JE Hamou in 1979 (Hamou 1980a). The first contact endoscope was coined as "the microhysteroscope" by Hamou (Hamou 1980b).

The microhysteroscope is a 4 mm diameter endoscope (Karl Storz 26156B, Tuttlingen, Germany) which allows *in vivo* examination of the female genital tract at four different magnifications (x1, x20, x60, and x150) with or without contacting the surface of examined tissues. The instrument is designed so that its 30^0 angulated tip can make contact with vitally stained tissue in the frontal and lateral walls of the genital tract and allow visualization of the cellular morphology of the superficial linings at high magnifications. It can also be incorporated with an outer sheath (5 mm in diameter, Karl Storz 26156E, Tuttlingen, Germany) containing a working channel to facilitate endoscopic surgery within the cavity. Hamou has used this instrument as a diagnostic and therapeutic device to manage diseases in the cervix, the uterine endometrium, the fallopian tubes, and even in embryos (Hamou 1981).

Hamou has incorporated microhysteroscopy with colposcopy and found that it allowed detailed and accurate *in vivo* evaluation and grading of the dysplastic and neoplastic changes in the transformation zone of the cervix (Hamou 1984).

Hamou has also extended its use to the diagnosis and treatment of intrauterine adhesions as an office-based procedure. In the study, the size and nature of the adhesion was assessed by contact endoscopy before adhesiolysis was performed. Guided by the contact magnified view provided by the microhysteroscope, adhesiolysis was successfully performed in 59 out of 69 patients under local anaesthesia (Hamou 1983).

3.2.2. Application of contact endoscopy in laryngology

The credit for introducing this innovative technique to the world of otolaryngology should be given to Professor M Andrea and Dr. O Dias. In 1994, inspired by the work of Hamou, Andrea and Dias started to examine the superficial layers of the vocal cords using Hamou's microhysteroscope (Andrea 1994a, Andrea 1994b, Andrea 1995a). During the procedure, contact endoscopy was performed using the conventional Kleinsasser technique under general anaesthesia.

In 1995, a specifically designed contact microlaryngoscope was manufactured to examine the laryngeal and hypopharyngeal tissues. It offers significant advantages for the clinical use of contact endoscopy in the larynx, with better lighting and imaging and easier manipulation. The contact microlaryngoscopes consist of telescopes with different angles of vision: straight forward telescope (0^0), forward oblique telescope (30^0), lateral telescope (70^0), and retrospective telescope (120^0) endoscopes (8715AA, 8712BA, 8712CA, 8712E, Tuttlingen, Germany, 23 cm long, 5.5 mm in diameter, magnification of x1,

x60, and x150). When in contact with the tissue, they allow x60 and x150 magnifications.

During the procedure, the larynx is first evaluated with the surgical microscope and angled rigid telescopes. Placing the contact endoscope close to the mucosal surface, a panoramic examination of the mucosal surface is attained. The vocal cords are then stained with 1% methylene blue and the cellular details of the superficial cells are examined when the endoscope is gently put in touch with the mucosa.

Since 1995, Andrea and Dias have extensively investigated the application of contact microlaryngoscopy in the diagnosis of normal and pathological conditions of the vocal cords (Andrea 1995b, Andrea 1995c, Andrea 1996a, Andrea 1996b).

During the examination of the normal vocal cords, the appearance of the normal squamous cells is recognized by their characteristic polyhedral shape and contiguous pattern of cellular layout. The nuclei are round and darkly stained, and the cytoplasm has a light blue tone. The nuclear: cytoplasmic ratio is normal, and the overall morphological pattern is homogenous. Similarly, ciliated epithelium present in the larynx can also be identified as filamentous structures.

Contact endoscopy is also valuable to identify different stages of malignant transformation in vocal cords. In carcinomas, contact endoscopy shows the extreme heterogeneity of the cells in distribution, shape, and colour, with darkly

stained nuclei and prominent nucleoli. A high nuclear: cytoplasmic ratio is demonstrated.

In papillomatosis, contact endoscopy allows the visualization of multiple papillae covered by squamous epithelium. Microscopically, ballooned koilocytes are observed. Characteristically, they have a vacuolated cytoplasm with the nucleus displaced to the periphery.

Under contact laryngoscopy, distinct microvascular patterns with exuberant anastomoses, vascular ectasia, thrombosed vessels, and spiral vessels are also seen associated with various pathologies in the vocal cords.

Andrea and Dias' qualitative studies in contact laryngoscopy have opened a door to the detailed mapping and *in vivo* study of normal and abnormal laryngeal tissues.

To further investigate the usefulness of contact laryngoscopy in the diagnosis of laryngeal pathology, Wardrop et al. have performed a partially blind controlled study of eight cases with a variety of laryngeal pathologies to compare the diagnoses made by contact laryngoscopy with the histology of the biopsy specimens (Wardrop 2000). He found that the diagnoses were all correct but one. In the false negative case, the endoscopy showed normal epithelium and histology confirmed haemangioma. The encouraging results of this small scale study have suggested that this technique is useful for the diagnosis of laryngeal diseases and further validation of its reliability is warranted.

3.2.3. Application of contact endoscopy in rhinology

The first attempts to perform contact endoscopy of the nasal mucosa were performed by Andrea and Dias in 1996 using the contact microlaryngoscope (Karl Storz 8715 A) to visualize the superficial layers of the nasal epithelium (Andrea 1998). Using the contact microlaryngoscope, they examined 50 patients with normal and diseased nasal mucosa. Squamous epithelium, ciliated epithelium, glandular ostia, mucus secretions, microvascular networks, inflammatory cell infiltrates, tissue inclusions, nuclear abnormalities, and fungal hyphae were visualized during the contact endoscopy (Andrea 1997).

In 1997, a narrower contact endoscope which was specifically designed for examination of the nose and nasopharynx was manufactured (Karl Storz, 7215AA and 7215BA, 4 mm in diameter, Tuttlingen, Germany) (Andrea 1998) (Figure 3-1). The slim size of the endoscope allows its use in the assessment of the sinonasal regions under local anaesthesia. This contact rhinoscope (CR) can be used not only for examination of the general appearance of the nasopharynx, in the same way as conventional endoscopy, but also for a real-time, *in vivo* examination of the cellular details of the superficial epithelium of the nasal structures in clinic settings.

Using 70^0 Hamou's contact microhysteroscope (Karl Storz, Hamou 26156B), Xiaoming et al. were able to visualize and characterize the superficial cells of different benign and malignant nasopharyngeal diseases in the nasopharynx under local anaesthesia. The diseases included chronic nasopharyngitis, nasopharyngeal cyst, dysplasia and carcinoma (Xiaoming 2001).

These studies have widened the horizon of the practical use of contact rhinoscopy in the diagnosis of various nasopharyngeal disorders. Since 1999, our colleagues and I have started a series of qualitative and quantitative studies on the application of diagnosis of nasopharyngeal carcinoma using the specific contact rhinoscope (Pak 2001, Pak 2002b, Pak 2005, Pak 2008, Pak 2009). Nevertheless, the efficacy of this technique in the management of nasopharyngeal carcinoma still needs to be substantiated.

Recently, nasal endoscopy has also been found useful to identify lesions in Rendu-Osler-Weber syndrome (Folz 2007) and reliable to differentiate inverted papilloma from inflammatory polyps (Romano 2007).

3.2.4. Application of contact endoscopy in general surgery

Since the introduction of contact endoscopy, a few general surgeons have used this instrument for microscopic tissue examination in head and neck surgery. Richtsmeier et al. explored its use in the intra-operative identification of normal visceral tissues in the neck of pigs, dogs, and patients before undergoing necessary therapeutic operations. The contact endoscopic images of the fatty tissues, thyroid gland, and parathyroid tissues were documented in this preliminary study. This was the first article to document that this technique is safe and rapid, and that the results are reproducible in differentiating parathyroid from thyroid tissues, and nerves from blood vessels in canine and human tissues. It appears to be useful for practical differentiation between different tissues during surgery. Nevertheless, its practical use still requires further standardization for

normatives in staining and the appearance of normal and neoplastic tissues confirmed by histological sections (Richtsmeier 1999).

Given that the parathyroid glands are sometimes difficult to differentiate from other structures, non-invasive identification of the parathyroid tissues may help to minimize its unnecessary damage. In 2003, Dedivitis et al. explored the possibility of using contact endoscopy to identify and preserve parathyroid glands during thyroid surgery. It was concluded that contact endoscopy is an efficient auxiliary method for identifying the parathyroid tissues intra-operatively (Dedivitis 2003).

3.3. Principle of contact endoscopy

Contact endoscopy is accomplished by a contact endoscope designed to examine the cellular details of superficial cells of living tissues. To increase the visibility of the cells and distinguish them from the tissues in the background, vital stain is applied to the surface prior to the examination. Strictly speaking, contact endoscopy is a combination of endoscopy and microscopy. To understand how the contact endoscope works, one has to study the principle of the microscope.

3.3.1. Fundamentals of light microscopy

A compound light microscope is an optical instrument that uses visible light to produce a magnified image of an object that is projected onto the retina of the eye. Three microscope components are of critical importance in forming the images (Murphy 2001).

1. Objective lens

This collects light diffracted by the specimen and forms a magnified real image at the real intermediate image plane near the eyepieces in the following manner:

In a microscope, the objective lens is focused on a specimen. The focus of the image is adjusted by changing the distance between the specimen and the objective lens. The image is both real and magnified, meaning that the object is located at a distance between 1*f* and 2*f* (*f* = focal length). Since the focused objective is very near to the specimen, it is deduced that the focal length of the objective must be very short, only a few millimetres (Figure 3-2). The function of the eyepiece or ocular is to magnify the primary image another 10-fold, and together with the lens component of the eye, to produce a real magnified image of the intermediate image on the retina. Thus the object of the eyepiece is the intermediate image made by the objective lens.

Simple lenses have spherical surfaces, but a spherical lens is associated with many intrinsic optical faults called aberrations that disturb the image in various ways. To minimize the distortion, modern microscopes incorporate corrective measures in the lens system including the use of compound lens designs, glass elements with different refractive indexes and colour dispersion, and aspherical lens curvatures.

2. Condenser lens

Imaging performance by a microscope depends on the light delivery system, which includes the illuminator and its collector lens as well as the condenser. The condenser lens focuses light from the illuminator onto a small area of the specimen. The resolution and contrast of the image are changed by adjusting the field and condenser diaphragms.

3. Ocular lens

Oculars are needed to magnify and view the image produced by the objective.

3.3.2. Principle of magnification

Owing to the wave nature of light, the image of a point produced by a lens is actually an extended spot surrounded by a series of rings, and a focal plane is contained in a three-dimensional slab of finite thickness. These properties are due to the diffraction of light.

In the microscope, when light is projected onto the specimen on the stage, the specimen acts as a diffraction grating and diffracts or scatters the light. When magnified, the pattern is observed to consist of a central spot or diffraction disk surrounded by a series of diffraction rings (Airy disk). The size of the central disk is related to the wavelength λ and the aperture angle of the lens. The radius *d* of the diffraction spot is described by a related expression:

$$d = 1.22\ \lambda\ (f / D),$$

where D is the lens diameter, *f* is the focal length, and λ is the wavelength of the light.

The diffracted rays are then collected by the objective, where the diffracted and non-diffracted rays are recombined by the mechanism of interference and focused in an image plane where waves constructively and destructively interfere to form a contrast image. According to Abbe's theory, interference between zero-order and higher-order diffracted rays in the image plane generates image contrast and determines the limit of spatial resolution that can be provided by an objective (Inoue 1986). It is found that the larger the number of diffracted rays collected by the objective and the relay lenses, the sharper and better resolved are the details in the image (Figure 3-3).

As mentioned, the image of an illuminated object is a diffraction pattern created by the action of interference in the image plane. The key elements in this optical system of the microscope are the objective lens and the relay lens, which determine the precision with which these actions are affected. The degree of magnification of an optical instrument is related to its resolution and human eye resolution. The critical factor in obtaining a crisp and detailed image with the microscope is its resolution power, which is defined as the smallest distance between two particles at which they can be seen as separate objects. The spatial resolution power of the objective lens depends on its diameter and angular aperture. The angular aperture is the angle over which the objective can collect diffracted rays from the specimen.

In the light microscope, the angular aperture is described in terms of the numerical aperture (NA) as

$$NA = n \sin \theta,$$

where θ is the half angle of the cone of specimen light accepted by the objective lens and n is the refractive index of the medium between the lens and the specimen. For dry lenses used in air, n = 1; for oil immersion objectives, n = 1.5.

As the resolution power of the microscope is related to the NA of the objective lens, the resolving power of the microscope is defined as

$$d = 0.61\lambda / NA$$

or

$$d = 0.61\lambda / n \sin \theta,$$

where *d* is the minimum resolved distance in μm, λ is the wavelength in μm, and n is the refractive index between the specimen and the objective lens.

If the angular aperture of a microscope is increased, as occurs when opening the condenser diaphragm or when changing the objective of a higher NA, the image is better resolved and magnified.

3.3.3. Mechanism of contact endoscopy

3.3.3.1. Optics of traditional rigid endoscope

The optical design of the rigid endoscope is similar to that of a telescope. The traditional endoscope employs a series of achromatic doublet lenses for the transmission of images (Figure 3-4). When the endoscope is connected to a light source, the objective lens system forms an inverted image of the object observed. A lens placed near the image redirects the optic ray towards the centre of a series

of relay lenses. Another field lens directs the optic ray within the tube of the endoscope to the ocular lens, which further magnifies the image.

3.3.3.2. Optics of modern rod lens rigid endoscope

Contrary to the traditional endoscopes, modern endoscopes utilize much longer lenses (Figure 3-5), with length-to-diameter ratios as high as 10. The lenses are polished on the circumference and ground on both ends. These are the so-called rod lenses of the Hopkins design (Hopkins 1966). The advantage of the rod lens design is that the chief ray bundle can be confined more tightly to the optical axis by reducing the ray divergence in the air gaps between the lenses. The other advantage of the rod lens system is that the mounting of rod lenses is simpler than that of thin achromatic lens. The rod lenses tend to tilt less when they are assembled into the inner lens tube. Also, since the spacers are shorter in a rod lens design, they can be made thinner, which increases the numerical aperture of the lenses.

Moreover, to increase the field of view of the endoscope, a small prism is placed at the tip of the endoscope to redirect the images from the side to the central lens relay system. As the brightness of the image in the endoscope is proportional to the numerical aperture and diameter of the relay lens, the design of the rod lens system allows greater light transmission, better image resolution, a wider field of view, and a greater image magnification.

3.3.3.3. Optics of contact endoscope

As the contact endoscope is a device combining the action mechanisms of the endoscope and the microscope, its functions are accomplished by incorporating the optic lens system of light microscopy with the Hopkins rod lens system in the instrument. Therefore, the mechanism of light microscopy mentioned above also applies to this device. One exception is that the specimen is no longer illuminated from the lamp filament underneath and there is no field stop diaphragm to control the intensity of illumination. Instead, the examined tissue is illuminated by the endoscope in the front and the contrast of the image is enhanced with the use of vital stain.

The contact rhinoscope was developed from the prototype of the microhysteroscope invented by JE Hamou. The specification of the microhysteroscope has been clearly listed and compared with other similar devices in one of his earliest papers (Hamou 1981). It was stated that the diameter of the lens was 0.15cm and the refractive index (n) of mucus and plasma as the medium filling the object space was 1.5. Incorporating these parameters in the formula $d = 0.61\lambda / n \sin \theta$, it was calculated that contact endoscopy can achieve a resolution power as low as 2μm (Hamou 1981). This can give amplifications of x60 at a depth of field of 80μm and x150 at a depth of field of 30μm. Given that cilia measure 7-10μm in length and the size of a single epithelial cell is 20-30μm, contact endoscopy is able to visualize the details of the cells in the epithelium (Figure 3-6) (Wheater 1987).

According to the description of Hamou's patent claim, the device is specifically designed to incorporate a sliding lens between the ocular lens and the

eyepiece within the distance of the focal length of the former (Hamou 1980a). Changing the position of this sliding lens allows maximization of the optic efficiency of the objective lens and thus enhancement of the resolution and magnification of the observed images.

Apart from the specific optic lens arrangement, the use of the xenon light source is another factor that may increase the resolution power of the device. A xenon lamp produces a monochromatic and continuous spectrum of light across the entire visible range from 400-750 μm, with peaks of output in the ultraviolet and infrared range. Monochromatic light eliminates the chromatic aberration of the objective lens and keeps all the diffraction spots uniform in size. This helps to sharpen the image and increase contrast for objects with inherently low contrast.

3.4. Instrumentation for contact rhinoscopy (CR)

The equipment used for contact rhinoscopy consists of contact rhinoscopes, video cameras, light sources, video recorders, video monitors, local anaesthetic agents, vital stain, and equipment for basic nasal examination and biopsy (Table 3-1) (Figure 3-7).

The following equipment was used for the clinical studies in Part II of this thesis.

3.4.1. Contact rhinoscopes

Two models of contact rhinoscopes were used. They are 18cm long with a diameter of 4 mm and different angulations of views (Figure 3-1).

a) 7215AA Contact Micro-Rhinoscope with HOPKINS ® autoclavable Straight-forward telescope 0^0 with magnifications of x1, x60, and x150.
b) 7215BA Contact Micro-Rhinoscope with HOPKINS ® autoclavable Forward-oblique telescope 30^0 with magnifications of x1, x60, and x150.

The endoscopes were designed to be advanced into the nasal cavity through the inferior nasal meatus and allow direct access to the nasopharynx. The two angulations, 0^0 and 30^0, permit the endoscopes to explore different walls of the nasopharynx. The 0^0 rhinoscope allows the examination of the posterior wall and lower roof the nasopharynx, whereas the 30^0 rhinoscope reveals the upper roof and lateral walls of the nasopharynx.

By turning the zoom switch, the magnification of the view can be changed from x60 to x150 and *vice versa*. Slight adjustments of the zoom switch permit focusing and defocusing on specific levels of the examined tissues.

3.4.2. Video camera

Colour video cameras have evolved in the last decade. The development of solid-state colour image capture devices and the use of digital video cameras were all important improvements. The image capture device is a critical component of the video camera. The commonly used charge-coupled devices or CCDs use integrated circuits or computer chips to produce video images by

simultaneously accumulating light intensity from all the picture elements (pixels) that cover the pickup surface. The advantage of this system is that the chips are small, light, and durable. In order to improve the colour sensitivity, brightness, and resolution of the images, a 3-CCD system was developed, which is able to divide the incoming light into three primary colours, each focused on a separate pickup device. However, it has the disadvantages of being expensive and fragile.

In the studies of contact rhinoscopy, we used a Karl Storz Three-Chip video camera with an integrated image processing module to capture the video images (Figure 3-8A). During the procedure, the endoscope was mounted with the video camera, and the real-time images were displaced and examined on the video monitor.

3.4.3. Light source

Illumination is often the limiting factor in producing an acceptable video image. Each video camera has a minimum required illumination to produce qualified images with a better colour saturation.

As mentioned above, the intensity of the light illumination also affects the resolution of the endoscope; the use of a monochromatic light source is thus highly recommended.

To produce high quality video images, a Karl Storz xenon light source (150W) was used (Figure 3-8B). It was connected to a fibre-optic cable and the

endoscope. It gives out a colour temperature of 6000K and allows manual or automatic adjustment of light intensity.

3.4.4. Video recorder

There are multiple formats for video recording, namely, 3/4-inch (U-matic), 1/2-inch (VHS, S-VHS, and Betacam SP), and 8 mm (Hi8). The S-VHS format produces excellent images, but videotapes have a limited life span (van Wezel, 1987).

A Sony S-VHS video recorder was used in our study of contact endoscopy (Figure 3-8C). All the images of the procedure were stored in pneumatic videotapes and were studied and revised after the procedure. Another copy of the S-VHS video images was transferred into digital images by video capture software for long-term storage.

3.4.5. Video monitor

The Sony Trinitron was the model used as the video monitor. It supports the colour systems of both the Phase Alternating Line (PAL) and the National Television System Committee (NTSC).

3.4.6. Vital stains

With few exceptions, most living tissues are colourless. Therefore, it is difficult for the endoscopist to observe them unstained. Methods of staining tissues have been devised that not only make the various tissue components conspicuous, but also permit distinctions to be made between them. Most of the stains or dyes

behave like acidic or basic compounds and have a tendency to form electrostatic linkages with ionizable radicals of the tissues. Basic dyes are more likely to stain basophilic tissue components, while acidic dyes have an affinity for acidophilic tissues. Toluidine blue and methylene blue are examples of basic dyes which react readily with the basophilic components (nucleoproteins, glycosaminoglycans, and acid glycoprotein) of the cell nucleus and RNA-rich portions of the cytoplasm. In contrast, acid dyes, such as orange G, eosin, and acid fuchsin, stain the acidophilic components of tissues, such as mitochondria, secretory granules, and collagen (Junquerira 1998)

In practice, the superficial cells of living tissues can only be visualized using a contact endoscope after they are stained by a vital stain (Richtsmeier 1999). Since the introduction of contact endoscopy, 1% methylene blue solution has been used as the vital staining agent because of its easy accessibility and widespread clinical use (Andrea 1995c, Andrea 1999). Other colorants such as Lugol and Waterman blue ink did not stain the mucosa as well as methylene blue (Andrea 1999).

In our studies of contact rhinoscopy, either 1% or 0.5% methylene blue solution was used (Figure 3-8D). During the procedure of contact endoscopy, the application of the vital stain should always be preceded by careful suction of the mucus on the surface of the nasopharynx. The stain is then applied carefully to the mucosa using a piece of cotton applicator. Any excess stain is wiped off before the introduction of the endoscope. The colour usually lasts for 4 to 5 minutes and gradually disappears. Repeated staining is needed before the examination is completed.

3.4.7. Local anaesthetic agent

Any local anaesthetic agent applicable to nasal endoscopy is suitable to prepare the nasal cavity for contact endoscopy.

10% lignocaine solution has been used by Andrea for contact nasal endoscopy using a contact microlaryngoscope (Andrea 1997). In our centre, 5% cocaine was used as it acts both as a vasoconstrictor and a local anaesthetic. It was applied in the form of a spray, up to a maximum volume of 1ml, to the inferior meatus of the nasal cavity 5-10 minutes before the endoscope was introduced.

Cocaine has been used as a standard local anaesthetic agent in nasal endoscopy for decades (Hashisaki 1987). However, as untoward reactions to cocaine have been reported, alternatives have been sought recently (Latorre 1999). Evidence has showed that the efficacy of co-phenylcaine was as efficacious as that of cocaine (Lennox 1996, Cara 2003).

3.4.8. Miscellaneous items

An anti-fog agent was used to prevent fogging of the endoscope. Blakesley tissue biopsy forceps were used to perform biopsies of the examined tissues.

3.4.9. Maintenance and sterilization of the endoscope

Contact endoscopes should be washed immediately after use in order to prevent contamination and avoid the need for more aggressive cleaning that may

be harmful to the optics. The contact endoscopes can be sterilized with steam or gas, or disinfected in solution.

3.5. Examination by contact rhinoscopy

3.5.1. Contraindications

Contact rhinoscopy is contraindicated in the following patients:

1. patients with a history of allergy to cocaine or other local anaesthetic agents;
2. patients with a history of allergy to vital stains; and
3. pregnant patients; and

3.5.2. Patient preparation

The procedure was performed in a clinic setting under local anaesthesia. Before the procedure, the patient was informed about the details of the procedure and consent was obtained.

The patient was seated on the examination chair. Both sides of the nasal cavities and the nasopharynx were anaesthetized using a maximum of 1ml of a 5% cocaine topical anaesthetic spray. After 10 minutes, the nasal cavities and nasopharynx were gently cleaned of secretions using a nasal suction catheter. The 0^0 contact rhinoscope, which is connected to a xenon light source, a video camera, an S-VHS video recorder, and a video monitor, was introduced into the nasal cavity. A small nasal cotton-ball applicator, passed through the nasal cavities under the guidance of the endoscope, was then used to stain the nasopharyngeal

mucosa with 1% methylene blue. Any excess stain was wiped off with a clean cotton-ball applicator to improve the visualization of the superficial cells of the nasopharynx.

3.5.3. Procedure

The 0^0 contact rhinoscope was slowly advanced through the inferior meatus of the nasal cavity and into the nasopharynx. With the contact rhinoscope close to the mucosal surface, a panoramic view of the nasopharynx was achieved. The gross appearance of the nasopharynx was assessed. The tip of the contact rhinoscope was then advanced until it made gentle contact with the mucosa of the posterior wall of the nasopharynx, and the mucosa was examined at a magnification of x60 by adjusting the zoom switch on the endoscope. Fine adjustment of the focus of the images was attained by turning the zoom switch. At a magnification of x60, the spatial arrangement of individual cells can be shown with a depth of field of 80 µm. To increase the magnification, the zoom switch was then turned anti-clockwise to get a x150 magnified view of the stained cells. Magnification of x150 allows a detailed examination of individual cells in a single superficial layer of epithelium at a depth of field of 30 µm. The procedure was repeated to examine different parts of the nasopharynx. During the examination, all the images were captured by the digital video recorder.

To examine the roof and Eustachian tubes of the nasopharynx, a 30^0 rhinoscope was introduced and the same procedure was repeated. As the methylene blue solution may become faint over time, it has to be reapplied after three minutes.

Using contact rhinoscopes of different angulations of view, one can study the whole surface of the nasopharynx, as shown in Figure 3-9.

During the process of examination, the images of the living cells were stored and examined carefully to study the following parameters:

1. the size and shape of the cells;
2. the spatial pattern of the cells;
3. the size and shape of the nuclei;
4. the nuclear:cytoplasmic ratio;
5. pleomorphism of the cells and nuclei; and
6. the patterns and shapes of the microvasculature.

At sites of abnormal cellular patterns or suspicious appearance, biopsies were taken using Blakesley forceps for histological confirmation.

Under the following circumstances, the procedure was aborted:

1. massive contact bleeding;
2. anatomical obstruction: deviated nasal septum, hypertrophic turbinates, adhesion bands, choanal stenosis, or atresia; or
3. allergic reactions to the stain or local anaesthetic agent.

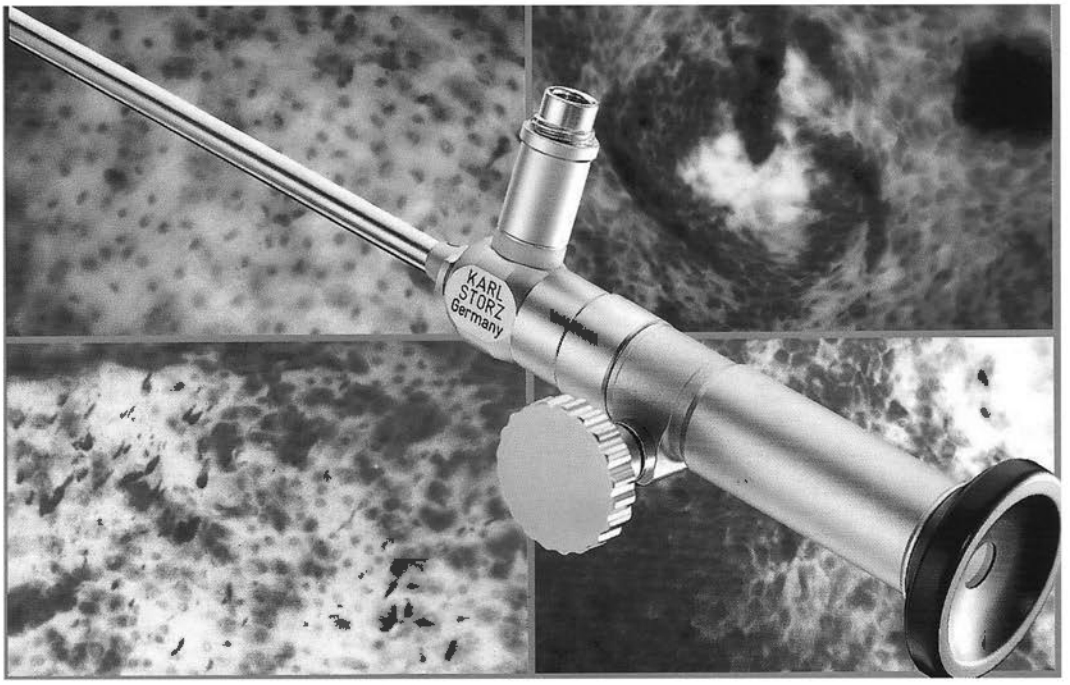
Table 3-1. Equipment for contact rhinoscopy

Endoscopes	4mm in diameter; length 18cm	
	Karl Storz 7215AA	0^0
	Karl Storz 7215BA	30^0
Light source	Karl Storz xenon light source 150W	
Video facilities	Karl Storz Endovision video camera	
	Video monitor	
	S-VHS video recorder	
Anaesthesia	5% cocaine solution	
Stain	1% methylene blue	
	10ml 0.9% normal saline solution	
	Cotton applicators	
	Nasal aspiration tubes	
	Anti-fog solution	
Biopsy instruments	Blakesley forceps (straight bite and 30^0 angled)	
Miscellaneous items	Gauze	
	Cotton balls	

Figure 3-1. Contact rhinoscope.

(A) Contact rhinoscope showing the zoom switch.
(B) 0^0 angle of vision.
(C) 30^0 angle of vision.

A



B **C**

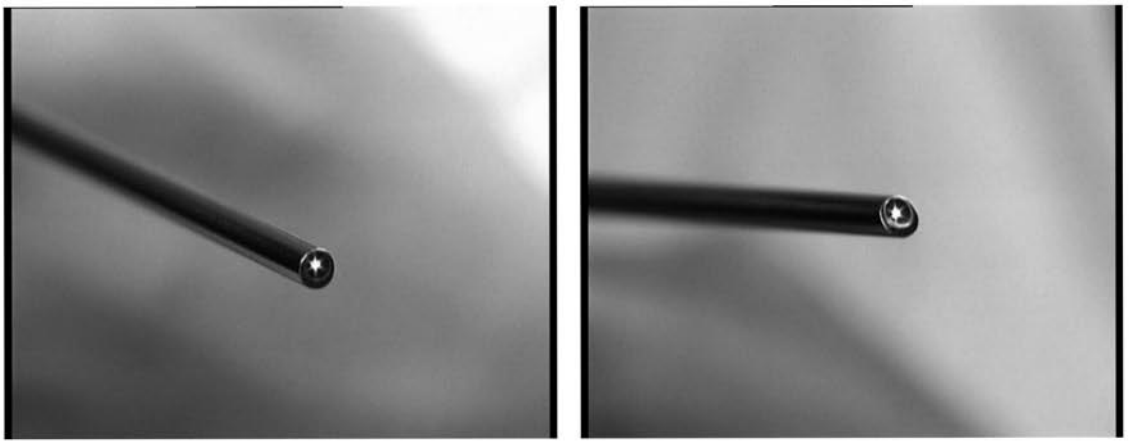


Figure 3-2. Perception of a modified virtual image of a specimen in the microscope.

The objective lens forms a magnified intermediate image of the object in or near the eyepiece; this image is then examined by the eyepiece which projects a real image on the retina. The retina and brain interpret the object as a magnified virtual mage about 25cm in front of the eye.

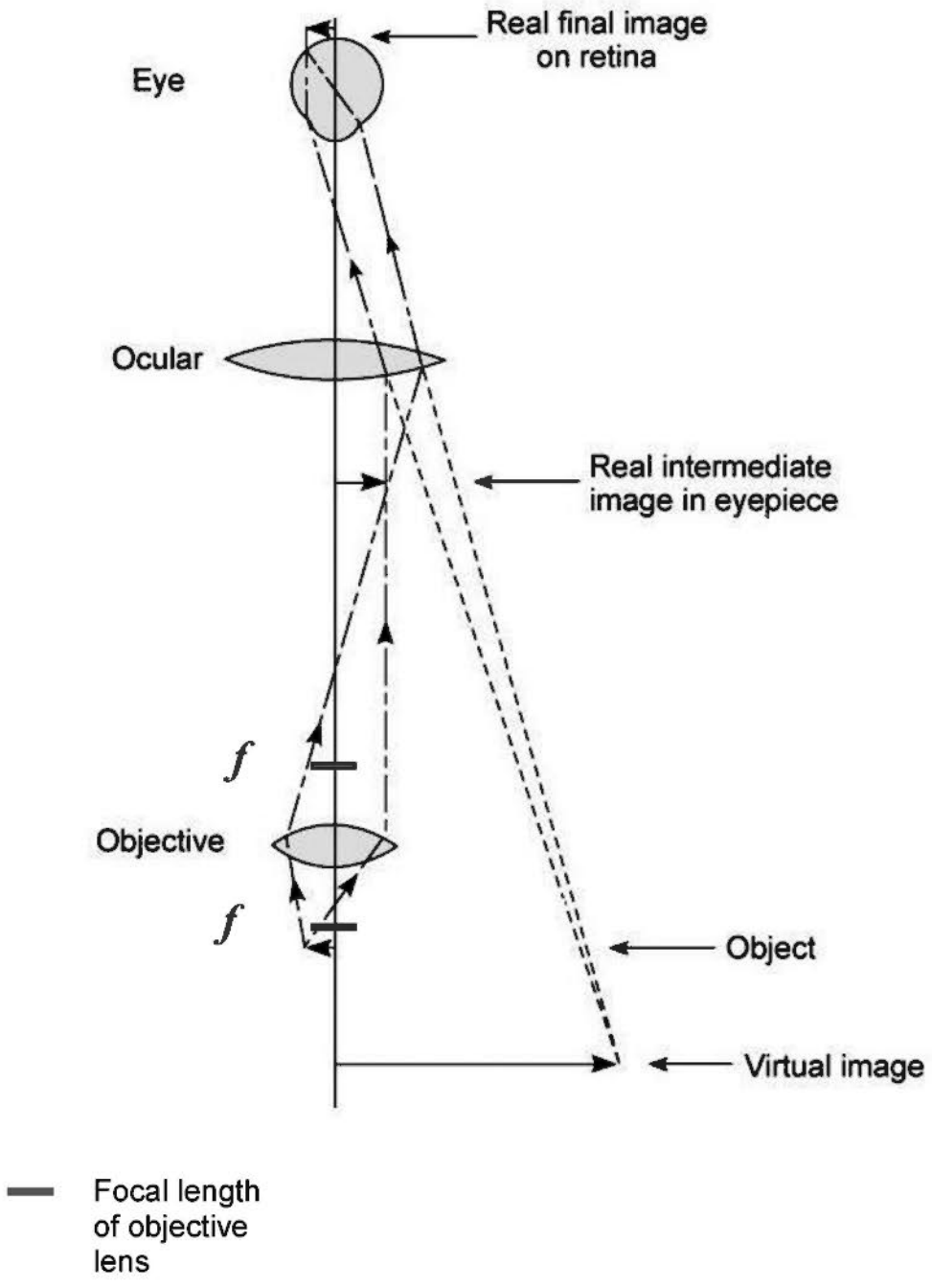


Figure 3-3. Collection of different orders of diffracted light by the objective lens (modified from Murphy 2001).
(A) As diffracted rays are not collected by the objective, the image is not formed.
(B) A minimum of two adjacent diffraction orders are collected, and an image is formed.
(C) Multiple diffracted orders are collected, leading to a high degree of definition in the image.

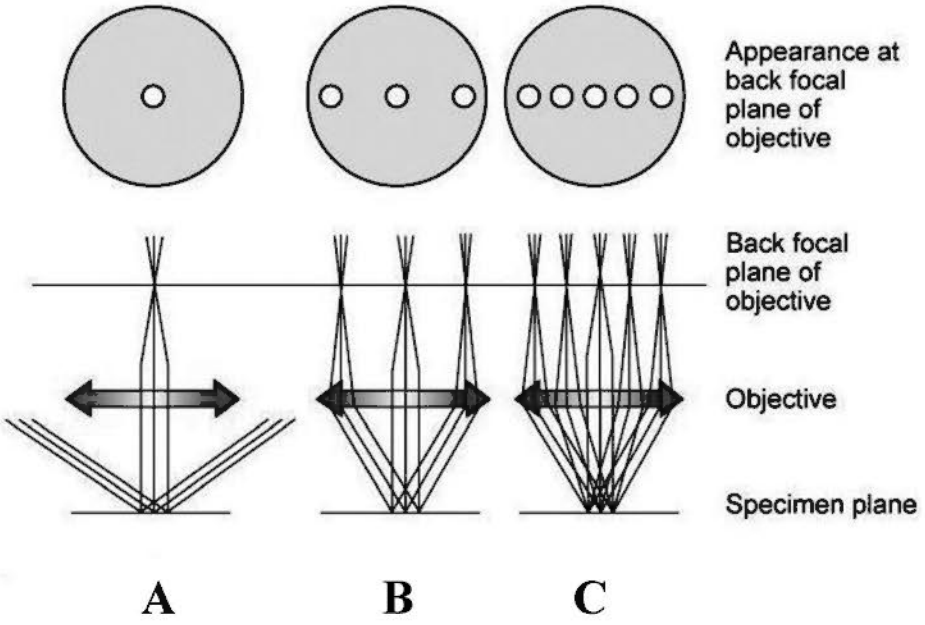


Figure 3-4. Optical layout of a traditional rigid endoscope.

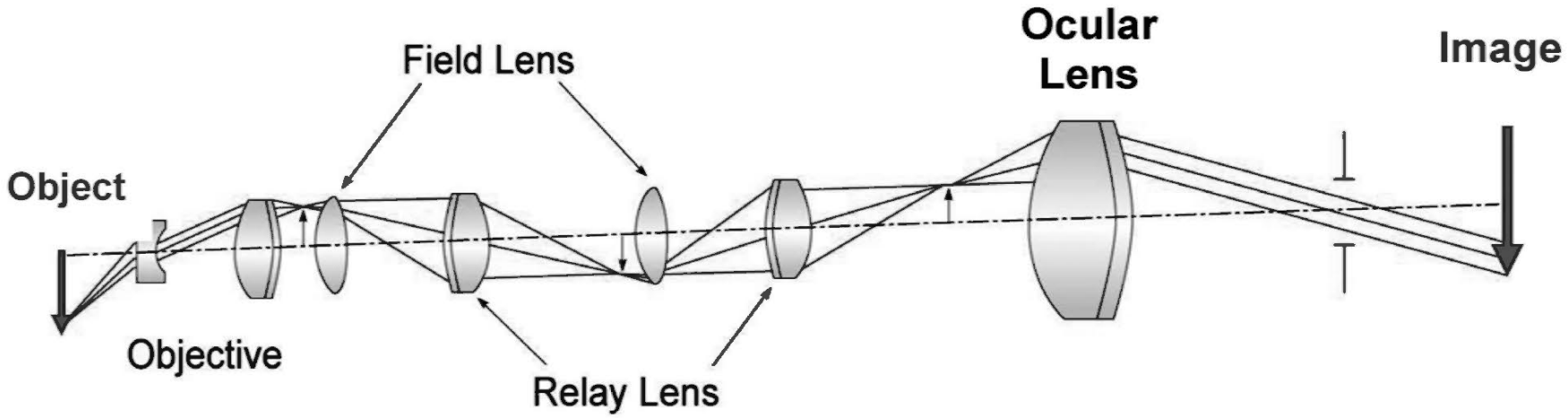


Figure 3-5. Relay lenses in rigid endoscope.

(A) Traditional glass lens system.
(B) Rod lens relay system.

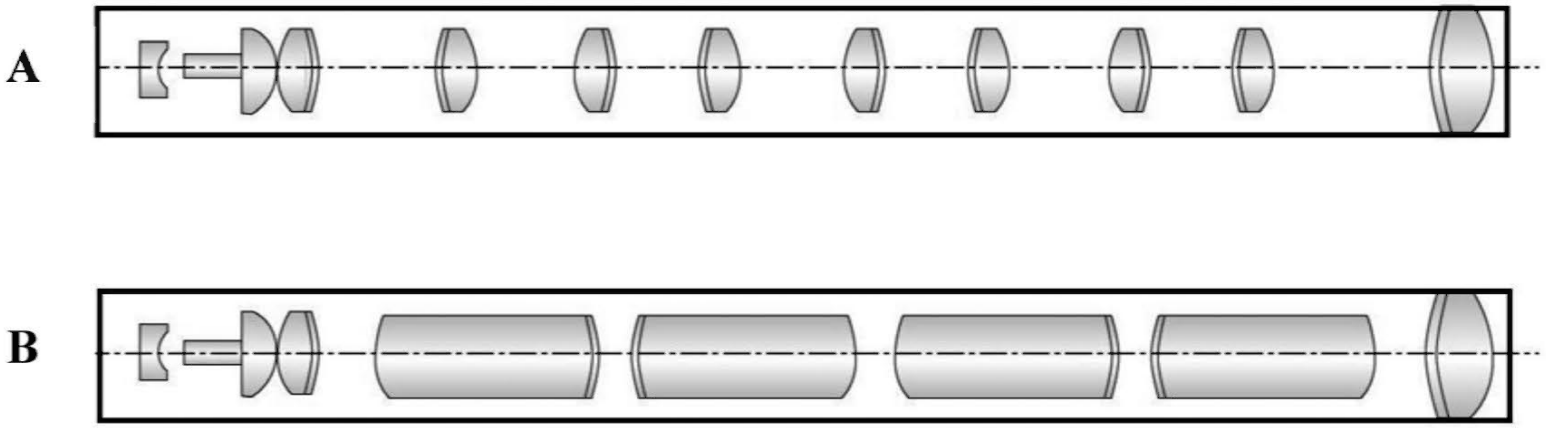


Figure 3-6. The electromagnetic spectrum in a logarithmic distance scale.

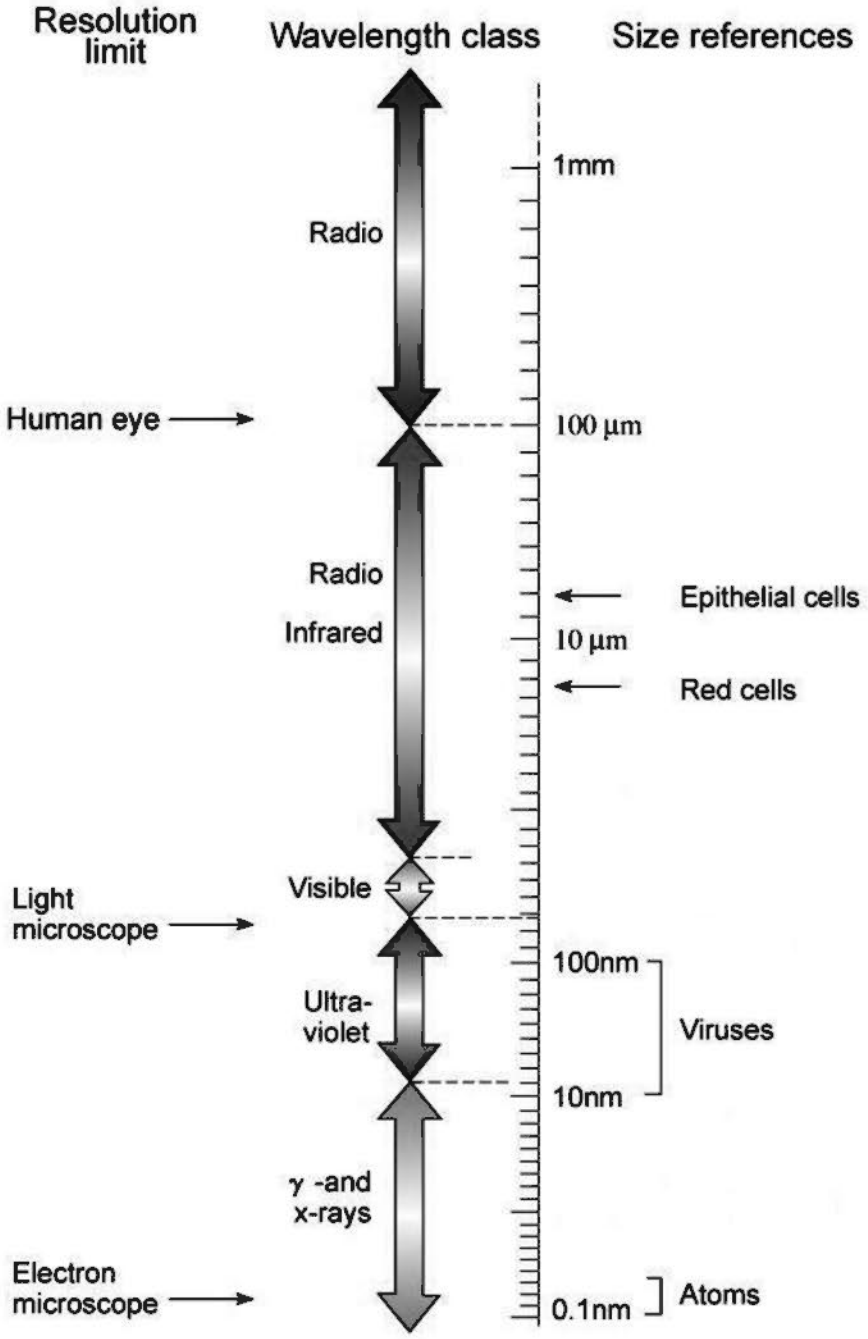


Figure 3-7. Equipment used for contact rhinoscopy.

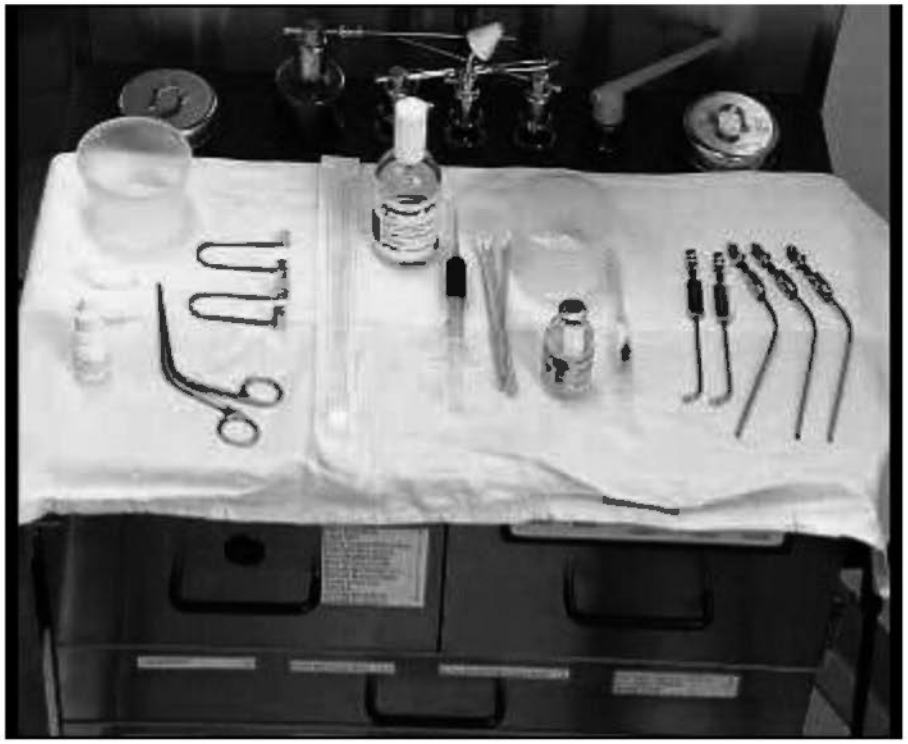


Figure 3-8. Audiovisual equipment for contact rhinoscopy.

(A) Video camera. (B) Xenon light source. (C) Video recorder. (D) Methylene blue solution.

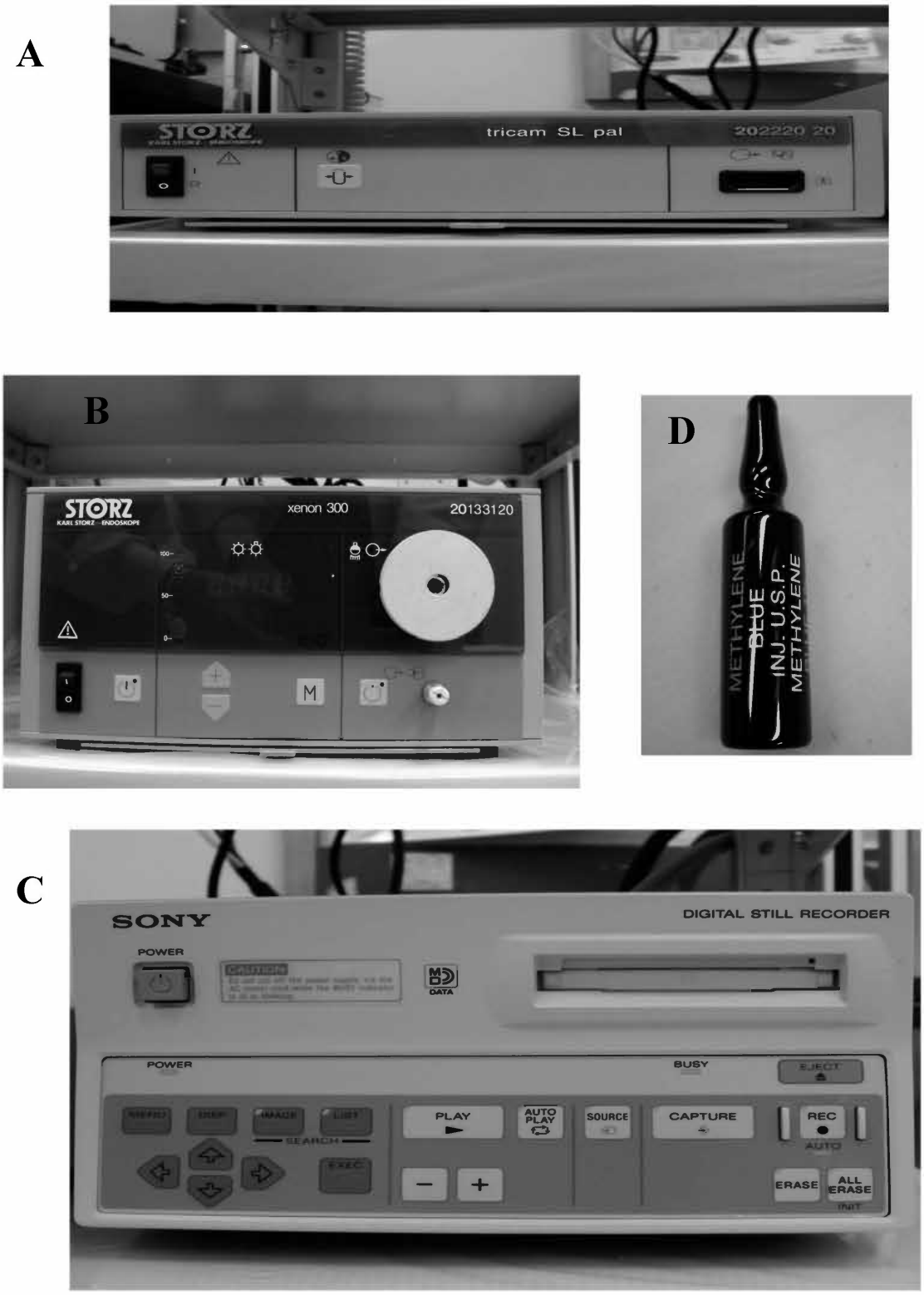
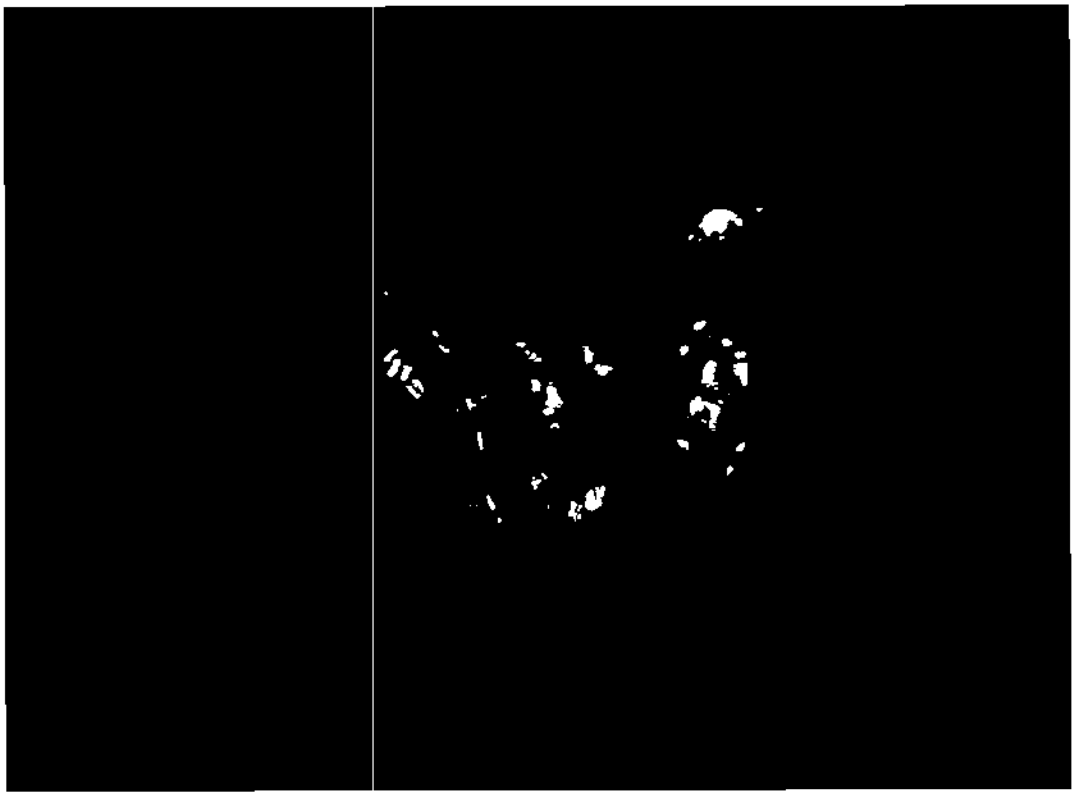


Figure 3-9. The areas of the nasopharynx covered by the endoscope (methylene blue stained region).



PART II

CLINICAL STUDIES

CHAPTER 4

HYPOTHESIS

Diagnosis of nasopharyngeal carcinoma (NPC) at an early stage is critical to improve patients' survival. The development of a more accurate and reliable diagnostic tool to detect subtle foci of primary and early residual or recurrent disease is warranted. Since contact rhinoscopy allows the examination of living tissues in a real-time manner, it emerges as an appealing office-based diagnostic procedure for primary and recurrent nasopharyngeal carcinoma. The validation of its accuracy and reliability in the diagnosis of nasopharyngeal carcinoma may improve our management of these patients.

To explore the potential role of contact rhinoscopy in the diagnosis of nasopharyngeal carcinoma, a series of studies have been performed. The hypotheses of these studies were:

1. Contact rhinoscopy is tolerated and accepted by most patients. There is no significant difference in the pain scores between contact rhinoscopy and conventional rigid endoscopy.

2. Contact rhinoscopy can provide a real-time differentiation between normal nasopharyngeal mucosa and primary NPC. The sensitivity of contact rhinoscopy in the detection of primary NPC is as high as that of conventional nasal endoscopy.

3. Contact rhinoscopy can provide a real-time identification of different mucosal patterns in the irradiated nasopharynx. The sensitivity of contact rhinoscopy

in detecting persistent and recurrent NPC is higher than that of conventional nasal endoscopy.

4. There is a significant difference in visual clarity of cellular details between 0.5% methylene blue and 1% methylene blue when used in contact rhinoscopy.

5. The accuracy of contact rhinoscopy for diagnosing primary and recurrent NPC is reproducible among different assessors.

CHAPTER 5

COMPARISON OF THE PERCEPTION OF PAIN IN PATIENTS UNDERGOING CONTACT RHINOSCOPY AND THOSE UNDERGOING CONVENTIONAL NASAL ENDOSCOPY

5.1. Abstract

Background

Contact rhinoscopy has been designed so that the tip makes direct contact with the surface of the mucosa, allowing a real-time *in vivo* examination of vital-stained superficial cells of target tissues at high magnification. Patients' acceptance of and tolerance to contact rhinoscopy has not been quantified.

Objective

We investigated the pain scores of patients undergoing contact rhinoscopy and compared them with those undergoing conventional rigid endoscopy.

Methods

Between January 1999 and December 2000, 162 patients underwent contact rhinoscopy after topical nasal preparation with 5% cocaine. The pain perceived and the patient's willingness to undergo the procedure again were measured. Independent factors predicting the willingness to undergo contact rhinoscopy again were identified.

To compare the pain scores of patients undergoing contact rhinoscopy with those undergoing conventional rigid endoscopy, a prospective, matched, case-control study was subsequently performed. Among 162 patients who had undergone contact rhinoscopy, the first 50 irradiated and first 50 non-irradiated patients were selected as the study group. Another group of 100 age-matched and

sex-matched patients were recruited to undergo conventional rigid endoscopy as controls. The pain scores of the two procedures were compared.

Results

The mean pain score of 162 patients undergoing contact rhinoscopy was 2.536 (S.D. 1.12). Endoscopy was successful in 157 patients (96.91%), and 140 patients (86.4%) were willing to undergo contact rhinoscopy again. A younger age, a shorter procedure, and a lower pain score were independent variables predicting a patient's willingness to undergo contact rhinoscopy again.

The pain scores of 100 sex- and age-matched patients who underwent either contact rhinoscopy or conventional nasal endoscopy were 2.517±1.073 and 2.282±1.324, respectively. There was no significant difference in the pain scores between the two procedures (p=0.17).

Conclusion

Office-based contact rhinoscopy can be performed with a minimum of discomfort under topical anaesthesia. It is as well tolerated as conventional nasal endoscopy and accepted by most of the patients.

5.2. Introduction

Since the introduction of the rod lens system by Hopkins in the 1960s, rigid nasal endoscopy has remained the standard procedure for examining the nose and the nasopharynx. Following topical administration of one of a number of

different topical anaesthetic and vasoconstrictive agents, rigid nasal endoscopy can be performed with the minimum of discomfort to patients who present with nasal symptoms (Woo 1999, Midwinter 2001, Douglas 2006).

In 1997, Andrea et al. introduced contact rhinoscopy to examine pathologies of the nose and nasopharynx (Andrea 1997). The contact nasal endoscope is designed so that the tip makes direct contact with the surface of the mucosa, allowing a real-time *in vivo* examination of vital-stained superficial cells of target tissues at high magnification. Pak et al. showed that contact rhinoscopy can accurately and reliably identify nasopharyngeal carcinoma (NPC) in patients both before and after radiotherapy (Pak 2001, Pak 2002b). However, the acceptance of and tolerance to contact rhinoscopy by patients has not been quantified. Furthermore, patients' pain perception during contact rhinoscopy has not been compared to that reported during conventional nasal endoscopy.

The vast majority of patients with NPC receive external beam radiotherapy as part of the definitive treatment. Commonly after radiotherapy, mucosal crusting, scarring, adhesions, and stenosis of the nasal cavity make evaluation of the nasopharynx with rigid endoscopy difficult to interpret (Croft 1988). It is also not known how the local condition of the irradiated nasal mucosa affects the efficiency of nasal endoscopy or contact rhinoscopy and influences patients' perception of pain.

This prospective study was designed to quantify the pain perceived by patients undergoing contact rhinoscopy and to compare it to the pain perceived by patients undergoing conventional rigid endoscopy.

5.3. Patients and Methods

Between January 1999 and December 2000, a series of patients were recruited prospectively into the study to undergo contact rhinoscopy in the out-patient clinic of the Prince of Wales Hospital (PWH) in Hong Kong. The patients were aged between 16 and 80 years and had presented to a tertiary-referral clinic of Department of Ear Nose and Throat of PWH either with symptoms of primary NPC, or a positive family history of NPC, or an elevated level of IgA antibodies against antigens of the Epstein-Barr virus, or had been treated with radiotherapy for NPC. The use of contact rhinoscopy was approved by the Hospital Ethics Committee and informed consent was obtained from all recruited patients.

The contact rhinoscopy was performed under topical anaesthesia by a single endoscopist (MWP) in a standardized routine manner. The nasal cavities and nasopharynx were anaesthetised with a maximum of 1ml of a 5% cocaine topical anaesthetic spray. After 10 minutes, the nasal cavities and nasopharynx were gently cleaned of secretions using a nasal suction catheter. A small nasal cotton-ball applicator passed through the nasal cavities was then used to stain the nasopharyngeal mucosa with 1% methylene blue. A contact endoscope (Karl Storz, 7215AA, 0°, 23 cm in length, 4 mm in diameter, Tuttlingen, Germany) was

slowly advanced through the nasal cavity and into the nasopharynx. The tip of the contact endoscope was then advanced until it made gentle contact with the mucosa of the posterior wall of the nasopharynx and the mucosa was examined at magnifications of x60 and x150 by adjusting the zoom switch on the endoscope.

Once the endoscopy was completed, the patient was asked to quantify the pain experienced by scoring the pain on a 10-cm un-scaled visual analogue scale (0 = no pain, 10 = severest pain). The patients' pain scores were recorded by an independent nurse who was blind to the examination. The duration, reasons for failure, and complications of the procedure were recorded. All patients were asked if they would be willing to undergo the same procedure again if required.

The demographic characteristics of the patients were analyzed. The parametric data were expressed as a mean and compared with a two-tailed *t*-test for equality. Non-parametric data were compared using the Pearson Chi-square test or Fisher's exact test as appropriate. A *p* value of less than 0.05 was regarded as statistically significant.

Factors that might predict a patient's willingness to undergo future contact rhinoscopy were first identified using univariate analysis. Categorical data were compared by using the Pearson Chi-square test or Fisher's exact test as appropriate. Yates correction for continuity was used when analyzing 2x2 tables for homogeneity of proportions with the chi-square test. Parametric data were compared by a two-sample *t*-test for equality of means. All factors were then entered into a stepwise multiple logistic regression. Independent factors were

considered significant when the *p* value was less than 0.05. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS version 9.0 for Windows, SPSS Inc., Chicago, USA).

To compare the pain perceived in contact rhinoscopy with that of conventional endoscopy, a prospective, matched, controlled study was performed. The study was approved by the Joint Human Review Ethics Committee of the Chinese University of Hong Kong and the Hong Kong Hospital Authority. Informed consent was obtained from all recruited patients. During sample size analysis, a two-tailed power analysis estimated that at least 47 study cases and the same number of control cases would achieve 80% power at a 5% significance level to detect a bioequivalence within 49% in the pain score from the study group.

Among 162 patients who had undergone contact rhinoscopy between January 1999 and December 2000, the first 50 non-irradiated patients and the first 50 irradiated patients were selected as a study cohort group. The pain scores of this group were measured and compared to the pain scores of a control group of matched patients who underwent conventional nasal endoscopy.

Between March 2008 and July 2008, among all patients presenting to the out-patient clinics with sinonasal symptomatology or with a history of radiotherapy for NPC, 50 non-irradiated patients and 50 previously irradiated patients, who were sex-matched and age-matched (within 5 years) with the patients in the study group, were recruited as the control group. All patients in the control group had their nasopharynges examined under topical anaesthesia by an experienced

endoscopist (JKSW, or DLYL, or MWP) using a rigid 4 mm 0^0 Karl Storz endoscope through the nasal cavity. The preparation and procedure were the same as that for contact rhinoscopy except that the nasopharynx was not painted with a vital stain and the endoscope did not touch the surface of the nasopharynx. After the procedure, the pain was scored by the patient using a 10-cm un-scaled visual analogue scale and recorded by an independent nurse who was blind to the examination.

The mean pain scores of both groups were analyzed and compared using a paired *t*-test. Two-tailed *p* values were calculated and statistical significance was taken when $p < 0.05$.

5.4. Results

Between January 1999 and December 2000, a total of 162 patients were recruited into the study to undergo contact rhinoscopy. There were 60 non-irradiated patients (37%) and 102 irradiated patients (63%). During the study period, 11 eligible patients were excluded, nine who declined to enter the study and two who did not want a tissue biopsy to be taken.

The mean age of the 162 patients was 48.3 years (S.D. 12.35 years) (range 21 to 80 years). The male to female ratio was 3.15:1. The mean duration of contact rhinoscopy was 6.16 minutes (S.D. 2.66 minutes). Contact rhinoscopy was successful in 157 (96.91%) patients but failed in five patients. The causes of the failures were contact bleeding from a primary nasopharyngeal tumour in three

patients, inaccessibility of a small tumour in the roof of the nasopharynx in one patient, and choanal stenosis in one patient.

Despite post-irradiation tissue reactions occurring in 41.17% of 102 irradiated patients (Table 5-1), the procedure was completed in all but one patient who had choanal stenosis.

The pain scores of patients ranged from 0.8 to 6.2 with a mean of 2.536 (S.D. 1.12). The mean and standard deviation of the pain scores for females and males were 2.405±1.046 and 2.578±1.145, respectively. There was no significant difference between the gender groups (p = 0.403). There was no association between pain scores and the age of the patient (r = 0.044, p = 0.577), nor with the duration of the procedure (r = 0.049, p = 0.540). The mean pain score for non-irradiated patients and irradiated patients was 2.387 (S.D. 0.974) and 2.625 (S.D. 1.195), respectively, with no significant difference between them (p = 0.193). The mean and standard deviation of the pain scores for patients with and without complications of radiotherapy were 3.114±1.702 and 2.510± 1.089, respectively. The difference in pain scores between them was not significant (p = 0.160).

Of 162 patients, 140 patients (86.4%) were willing to undergo contact rhinoscopy again. The mean pain score of patients willing to undergo repeated contact rhinoscopy (2.431; S.D.1.056) was statistically lower than those unwilling to undergo repeated contact rhinoscopy (3.174; S.D.1.306; p = 0.003).

In a univariate analysis, an older age ($p < 0.001$) and a higher pain score ($p = 0.003$) were factors related to unwillingness to undergo repeated contact rhinoscopy (Table 5-2). Further analysis using a multiple stepwise logistic regression model showed that an older age ($p = 0.001$; odd ratio (OR) = 0.931; 95% confidence interval (CI) = 0.892-0.972), a longer duration of the procedure ($p = 0.036$; OR = 0.818; 95%CI = 0.678-0.987), and a higher pain score ($p = 0.005$; OR = 0.537; 95%CI = 0.349-0.827) were independent variables predicting a patient's unwillingness to undergo further contact rhinoscopy (Table 5- 3).

In the matched controlled evaluation study, 100 patients who had undergone contact rhinoscopy were selected as the study group. Their mean age was 49.81 years (S.D. 11.35 years) and the male to female ratio was 4:1.

The mean pain score for the contact rhinoscopy study group was 2.517 (S.D. 1.073).

The control group consisted of 50 non-irradiated patients with various sinonasal and related diseases (Table 5- 4), and 50 irradiated patients. Their mean age was 49.31 years (S.D. 11.45 years) and the male to female ratio was 4:1. The control group of patients all underwent and completed conventional nasal endoscopy.

The mean pain score for the conventional endoscopy control group was 2.282 (S.D. 1.324). The mean pain scores of patients examined by MWP, JKSW, and DLYL were 2.200 (S.D. 1.124), 2.323 (S.D. 1.778), and 2.218 (S.D. 1.040),

respectively.

There was no significant difference in pain scores between the two groups of patients and hence between the procedures ($p = 0.17$). There was also no significant difference in pain scores between non-irradiated and radiated patients undergoing either contact rhinoscopy or conventional nasal endoscopy (Table 5-5).

Among 77 pairs of sex-matched and age-matched patients (mean age 47.62 ±12.66 years in the study group; male: female = 3.3:1 in both groups) who had been examined by the same endoscopist (MWP) using contact rhinoscopy or conventional nasal endoscopy, the pain scores of patients who underwent contact rhinoscopy and conventional nasal endoscopy were 2.533 (S.D. 1.008) and 2.200 (S.D. 1.124), respectively. There was no significant difference in the pain scores between the two groups of patients ($p = 0.205$).

5.5. Discussion

Rigid nasal endoscopy is a standard diagnostic tool used to examine the nasopharynx in patients with sinonasal symptomatology. Better visualization of nasal structures, better manipulation of instruments for tissue biopsy, and less patient discomfort are some of the reasons put forward by clinicians for using the rigid endoscope in preference to the flexible endoscope (Midwinter 2001). Previous studies have shown that the mean pain scores experienced by patients vary from 2.0 to 2.3 when using a variety of different topical anaesthetic agents prior to rigid nasal endoscopy with a 2.7 mm endoscope (Midwinter 2001, Douglas

2006). In this series, following preparation of the nose with a cocaine solution, the mean pain score for contact rhinoscopy using a 4 mm endoscope was 2.536, which is similar to that of rigid endoscopy in previous studies. The low pain scores suggest that contact rhinoscopy can be performed as an out-patient procedure without causing significant discomfort to most patients.

In this study, we found that the mean pain scores for contact rhinoscopy and conventional nasal endoscopy were not significantly different. The minor discrepancy in pain scores implied that the manoeuvre of applying the vital dye and the surface contact with the endoscope did not contribute to any additional pain or discomfort for the patients.

The low pain scores for contact rhinoscopy might also explain our observation that 86% of patients were willing to undergo contact rhinoscopy again. Although the results suggest that contact rhinoscopy was readily accepted by most patients, there is still room to improve its general acceptability. Considering that greater age of the patient, higher pain scores, and a longer duration of the procedure were independent factors which predicted unwillingness to undergo a repeat contact rhinoscopy, further improvement in the procedure, including a shorter operation time, a better pre-procedure explanation, and the use of flexible instruments in the future, might enhance the acceptability of the procedure and the willingness of the patient to undergo a repeat procedure.

In this study, we also compared the pain scores of contact rhinoscopy and rigid endoscopy between non-irradiated and irradiated patients. To our knowledge,

this is a unique study as no similar data has previously been reported. We found that there was no statistical difference in the pain scores between these two groups of patients. In practice, although local tissue reactions occur commonly in the nasal cavity and nasopharynx after radiotherapy, they did not adversely affect the process of nasal endoscopy. The dual local anaesthetic and vasoconstrictor effects of cocaine have made it the standard topical agent to use in nasal endoscopy for many years (Hashisaki 1987). We believe that the use of a cocaine solution effectively reduces the patients' pain perception and facilitates the entry of the rigid endoscope into the nasal cavity. These results justify the use of contact rhinoscopy in irradiated patients.

5.6. Conclusions

The pain perceived by patients during contact rhinoscopy is not significantly different to that perceived during conventional nasal endoscopy. Office-based contact rhinoscopy can be performed with a minimum of discomfort under topical anaesthesia and is acceptable to most of the patients.

Table 5-1. Mucosal tissue reactions in nasal cavities of 102 irradiated patients undergoing contact rhinoscopy.

Mucosal crusting	37 (36.27%)
Adhesions	2 (1.96%)
Choanal stenosis	1 #(0.98%)
Hypervascularity	2 (1.96%)
	N = 42 (41.17%)

The procedure was aborted.

Table 5-2. Univariate analysis of factors related to willingness to undergo contact rhinoscopy again.

	Willing (N=140)	**Unwilling (N=22)**	***p* value**
Mean age in years	46.86 (S.D.: 11.656)	57.22 (S.D.: 12.982)	<0.001
Male sex (%)	103 (73.57%)	20 (90.9%)	0.283
Mean duration of procedure in minutes	6.018 (S.D.: 2.642)	6.992 (S.D.: 2.680)	0.104
Mean pain score	2.431 (S.D.: 1.056)	3.174 (S.D.: 1.306)	0.003
No. of irradiated patients (%)	90 (64.28%)	12 (54.54%)	0.254
No. of side effects (%)	6 (4.29%)	1 (4.54%)	0.665

SD = standard deviation

Table 5-3. Logistic regression analysis of independent factors predicting willingness to undergo contact rhinoscopy again.

	Odds ratio (95% CI)	***p* value**
Age	0.931 (0.892-0.972)	0.001
Duration of procedure (min.)	0.818 (0.678-0.987)	0.036
Mean pain score	0.537 (0.349-0.827)	0.005

95%CI = 95% confidence interval

Table 5-4. The principal diagnoses of 50 non-irradiated patients undergoing conventional rigid endoscopy.

Epistaxis	4
Eustachian tube dysfunction	3
Nasal polyposis	3
Deviated nasal septum	3
Primary nasopharyngeal carcinoma	7
Middle ear effusion	6
Rhinitis	15
Septal perforation	1
Sinusitis	5
Idiopathic tinnitus	3

N = 50

Table 5-5 Pain scores of matched non-irradiated and irradiated patients who had either undergone contact rhinoscopy or conventional nasal endoscopy.

	Non-irradiated patients (N = 50)	**Radiated patients (N = 50)**	***p* value**
Contact rhinoscopy	2.402 (S.D.: 1.041)	2.632 (S.D.: 1.103)	0.286
Conventional endoscopy	2.252 (S.D.:0.893)	2.312 (S.D.: 1.656)	0.822

SD = standard deviation

CHAPTER 6

IN VIVO DIAGNOSIS OF NPC AND NORMAL NASOPHARYNGEAL MUCOSA USING CONTACT RHINOSCOPY

6.1. Abstract

Background

Contact rhinoscopy not only allows examination of the gross appearance of the nasopharynx, but also allows real-time and *in vivo* microscopic examination of the epithelial cells of the nasopharynx. However, its accuracy in making a real-time differentiation between normal nasopharyngeal tissues and primary nasopharyngeal carcinoma (NPC) in comparison with conventional endoscopy was unknown.

Objective

We evaluated the accuracy of contact rhinoscopy in the diagnosis of normal nasopharyngeal tissues and primary NPC and tested the hypothesis that the sensitivity of contact rhinoscopy for the detection of primary NPC is similar to that of conventional nasal endoscopy.

Methods

A prospective, single-blind, controlled study was conducted to examine the nasopharynges of 60 patients in a clinic setting using contact rhinoscopes. The gross appearance of the nasopharynx was examined using conventional endoscopy. Examination of the mucosal patterns of the nasopharynx at high magnifications (x60 and x150) was then performed using contact rhinoscopy. The areas under examination were biopsied. The findings of conventional endoscopy and contact rhinoscopy were analysed by an endoscopist and a pathologist,

respectively. The findings of both endoscopies were correlated with the histological sections of the biopsied tissues.

Results

Using the histology of the biopsy as the gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value of conventional endoscopy in diagnosing primary NPC were 97.3%, 100%, 100%, and 95.8%, respectively. Contact rhinoscopy was carried out for 56 of the 60 patients (93.3%). The sensitivity of contact rhinoscopy for the diagnosis of primary NPC was 100%, with a specificity of 95.7%, a positive predictive value of 97.1%, and a negative predictive value of 100%.

Conclusion

Contact rhinoscopy can provide a real-time differentiation between the malignant cells and the normal mucosa of the nasopharynx. Its sensitivity and specificity for the detection of primary NPC were as high as those of conventional endoscopy.

6.2. Introduction

Contact endoscopy was first introduced by Hamou in 1979, as microhysteroscopy, to examine the surfaces of the genital tract at high magnification (Hamou 1980a, Hamou 1980b). The instrument is designed so that its tip can make direct contact with the tissue surface and allow visualization of its superficial cells at high magnifications, thereby allowing *in vivo* and *in situ*

examination of epithelial cellular morphology. Contact laryngoscopy (Karl Storz, BA8715AA, Tuttlingen, Germany, 23 cm long, 5.5 mm in diameter, magnification x1, x60, and x150) has also been applied to visualize superficial cells of various pathologies in the larynx under general anaesthesia. Although attempts have been made with the contact laryngoscope to visualize the epithelial cells in the nose, its size (5.5 mm) made the examination of the nose and the nasopharynx difficult until the advent of the contact rhinoscopes (Karl Storz, Tuttlingen, Germany, 7215AA, 7215 BA) (Andrea 1997). The recently designed contact rhinoscope with a 4 mm diameter shaft allows easy access and examination of the nasopharynx through the nasal cavity.

Nasopharyngeal carcinoma (NPC) is common in southern China. The presenting symptoms are diverse and tend to be vague and non-specific. To facilitate an early diagnosis, detailed examination of the nasopharynx and timely biopsy of suspicious lesions are mandatory in all patients with a suspicious clinical presentation. Nasal endoscopy is a standard procedure in the examination of the nasopharynx and in the diagnosis of NPC. Previous studies showed that the sensitivity of conventional nasal endoscopy in the detection of primary NPC was about 95% (Waldron 1992, Kwong 2001a). However, subclinical disease of NPC occurred in 5% of the high risk patients whose nasopharynx may appear normal during examination using conventional nasal endoscopy.

Contact rhinoscopy not only allows examination of the gross appearance of the nasopharynx, but also allows real-time and *in vivo* microscopic examination of the epithelial cells of the nasopharynx. However, its accuracy in making a

real-time differentiation between normal nasopharyngeal tissues and primary NPC in comparison with conventional endoscopy was unknown.

6.3. Patients and Methods

Between January 1999 and September 2000, a prospective, single-blind, controlled study was conducted in the Prince of Wales Hospital (PWH) to compare the efficacy of contact rhinoscopy and conventional endoscopy in the examination of patients with symptomatology of NPC.

In this study, sample size was estimated based on the assumption that the sensitivity and specificity of contact rhinoscopy in the detection of primary NPC would be less than 20% lower than that of conventional endoscopy. To achieve 80% power to detect NPC with 83% sensitivity and 83% specificity at a 5% level of significance, at least 23 subjects with NPC (case subjects) and 23 subjects with a normal nasopharynx (control subjects) were required.

During the study period, consecutive patients attending a tertiary-referral clinic of PWH, aged between 16 and 80 years, with symptomatology of NPC, or a positive family history of NPC, or elevated Immunoglobulin A (IgA) antibodies against antigens of Epstein-Barr virus were recruited for the study.

For all the recruited patients, conventional nasal endoscopy followed by contact rhinoscopy was performed by a single endoscopist using contact rhinoscopes (Karl Storz 7215AA, 0^0 and 7215BA, 30^0; 23 cm long; 4 mm in

diameter) which were connected to a 150 W xenon light source, a video camera, and an S-VHS video recorder, in the following manner. The nasal cavities and nasopharynx were anaesthetised by a maximum of 1ml of a 5% cocaine topical anaesthetic spray. After 10 minutes, the endoscope was slowly advanced through the nasal cavity and into the nasopharynx. The gross appearance of the nasopharynx was examined using conventional endoscopy. A small nasal cotton-ball applicator was then used to stain the nasopharyngeal mucosa with 1% methylene blue via the nasal cavity. The tip of the contact endoscope was then advanced until it made contact with the mucosa of the posterior wall or tumour of the nasopharynx. The mucosa was then examined at magnifications of x60 and x150 by adjusting the zoom switch on the endoscope. A magnification of 60:1 permits study of cellular arrangements with a depth of field of 80 μm, whereas a magnification of 150:1 allows detailed examination of a single layer of epithelial cells (depth of field 30 μm). Once contact endoscopy was completed, the areas under examination were biopsied and the samples were studied by an independent pathologist who was blind to the findings of the endoscopies. A video recording was made of the entire endoscopic procedure, including the conventional endoscopic examination of the nasopharynx and the contact endoscopic examination of the nasopharyngeal mucosa.

The endoscopist and a consultant pathologist subsequently analyzed the video images before knowing the histology of the corresponding specimens. Independent diagnoses from conventional nasal endoscopy and contact rhinoscopy were made by the endoscopist and pathologist, respectively. The diagnosis of malignancy was made by the endoscopist if there was infiltrative

swelling or if an exophytic mass was seen under conventional nasal endoscopy. During the examination of the nasopharyngeal mucosa under contact rhinoscopy, the diagnosis of malignancy was made by the pathologist if all of the following were present: enlarged cells, cells with variable shapes, enlarged pleomorphic nuclei, an increased nuclear: cytoplasmic ratio, an irregular pattern of cells, and syncytial sheets or clusters of cells. Using the histology of the biopsy specimens as the gold standard, the diagnostic accuracy of both procedures was compared.

6.4. Results

During the study period, 60 consecutive patients (41 men, mean age 48.7+/-13.2 years) were recruited and underwent conventional endoscopy followed by contact rhinoscopy (Figure 6-1). Five eligible patients did not enter the study; among them, two did not consent to tissue biopsy and the remainder did not consent to the study.

Conventional endoscopy was carried out for all of the 60 patients (100%). In 24 of the 60 patients, the nasopharynx looked normal. In 23 of 24 patients, histology of the tissue biopsies showed normal pseudostratified ciliated epithelium. A biopsy from one patient showed undifferentiated carcinoma with syncytial sheets of malignant cells (4.17%).

In the remaining 36 patients, exophytic malignant tumours were seen during conventional endoscopy. All the histological sections of the corresponding

biopsies confirmed the diagnoses of undifferentiated carcinomas of the nasopharynx.

Using the histology of the biopsies as the gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value of conventional endoscopy in diagnosing primary NPC were 97.3%, 100%, 100%, and 95.8%, respectively.

Contact rhinoscopy was completed in 56 of 60 patients (93.3%). In three patients, the procedure was abandoned because of contact bleeding of the tumour with the endoscope. In one patient, the tumour was situated on the roof of the nasopharynx, which could not be accessed by the endoscope.

In 22 of 56 patients, two patterns of normal respiratory epithelium were shown by contact rhinoscopy: pseudostratified ciliated epithelium (86.4%) and squamous epithelium (13.6%).

1. Pseudostratified ciliated epithelium

Normal ciliated epithelium was recognized in 19 of 22 patients (86.4%). Under low magnification, the ciliated respiratory epithelium was characterized by the movement of the stain particles within the blanket of mucus caused by the metachronous beating of the cilia. Under high magnification, the cilia were visualized (Figure 6-2A). However, the presence of the mucus and the active movement of the cilia rendered the cells underneath difficult to stain and visualize with the contact endoscope. All the histological sections of the corresponding

biopsy specimens showed normal respiratory epithelium, and this was consistent with the contact endoscopic findings (Figure 6-2B).

2. Squamous epithelium

Sheets of squamous cells were recognized in 3 of 22 patients (13.6%). Through the low-power view of the contact endoscope, the squamous cells were uniformly arranged with well-defined cytoplasmic boundaries. Under high-power magnification, the squamous cells were polyhedral in shape with small, oval-shaped nuclei. The nuclear:cytoplasmic ratio was low. Nucleoli were not apparent (Figure 6-3A). These endoscopic findings of stratified squamous epithelium were confirmed by the histological sections of the corresponding biopsy specimens (Figure 6-3B).

In 34 of 56 patients, malignant cells were visualized using the contact endoscopes. Although quantification of the vessels was not possible, the presence of numerous tortuous vessels was a common occurrence (Figure 6-4). Under contact rhinoscopy, two patterns of malignant cells were visualized: syncytial sheets of malignant cells (44.1%) and atypical cells underneath the normal respiratory epithelium (55.9%).

1. Syncytial sheets of malignant cells

Fifteen patients exhibited this pattern (44.1%). Under low-power magnification, in contrast to the ciliated respiratory epithelium, ciliary movement was not evident. In comparison with the normal uniform pattern of squamous epithelium, it demonstrated a more variable cellular pattern, composed of large, pleomorphic,

oval-shaped cells with indistinct cytoplasmic boundaries. Under high-power magnification, the pleomorphic cells had darkly stained nuclei with prominent nucleoli and scanty indistinct cytoplasm (Figure 6-5A).

The histology of all the corresponding biopsy specimens showed undifferentiated carcinoma with syncytial sheets of malignant cells with oval nuclei, prominent nucleoli, and dense lymphoplasmacytic infiltrate (Figure 6-5B). Ciliated epithelium was not demonstrated. This cellular pattern corresponds to the Schmincke pattern of NPC (McGuire 1990).

2. Atypical cells underneath the normal respiratory epithelium

This pattern was seen in 19 patients (55.9%). Under low-power magnification, scattered and small clumps of atypical cells were seen among islands of ciliated cells. Under high-power magnification, those atypical cells had darkly stained pleomorphic nuclei, a high nuclear: cytoplasmic ratio, and indistinct cytoplasmic boundaries (Figure 6-6A). Among these 19 patients, 17 histological sections of the corresponding biopsies showed undifferentiated carcinoma arranged in small nests or scattered individual cells among the lymphoplasmacytic infiltrate, which corresponds to the Regaud pattern of NPC (Figure 6-6B) (McGuire 1990). One biopsy specimen demonstrated the coexistence of ciliated epithelium and carcinoma cells. However, one biopsy specimen showed normal pseudostratified ciliated epithelium without malignancy.

Of note, among 33 patients whose malignant mucosal patterns were confirmed by histological sections of the corresponding biopsies, one patient had a normal-looking nasopharynx on conventional endoscopy.

Using the histology of the biopsies as the gold standard, after excluding 4 failed procedures, contact rhinoscopy was used to correctly identify 33 primary NPC patients with 1 case of false positivity. Therefore, the sensitivity of contact rhinoscopy in diagnosing primary NPC was 100%, specificity 95.7%, positive predictive value 97.1%, and negative predictive value 100%.

6.5. Discussion

In an adult, the normal nasopharynx is lined mainly by pseudostratified ciliated columnar epithelium near the choanae and adjacent part of the roof. In the lower and posterior regions of the nasopharynx, the lining may assume stratified squamous characteristics. Areas of the transitional epithelium are encountered at the junctional zone located on the nasopharyngeal roof and the lateral walls (Gibb 1999). In this study, we have shown that these two types of epithelium can be readily visualized and distinguished by the use of contact endoscopes. Nevertheless, in our experience, the epithelial lining of the normal nasopharynx was less readily stained for two reasons. Firstly, the frequent ciliary beats significantly shorten the contact time of the stain with the superficial cells of the ciliated epithelium. Secondly, because the nasopharynx is the final pathway of clearance of secretions in the nasal cavity, the accumulation of nasal secretion in the nasopharynx further hinders the staining and visualisation of the underlying

cells. Owing to these staining properties, the superficial cells of the nasopharynx were less readily visualized than those of the larynx.

The vast majority of NPCs are undifferentiated. They have distinct features of large, round nuclei with well-delineated nuclear membranes and one or more prominent, large, eosinophilic nucleoli (McGuire 1990). Characteristically, the tumour cells are located in the submucosal lymphoid and loose connective tissue, and they exhibit either the Schmincke (syncytial) or the Regaud (trabecular) pattern. Occasionally the tumour cells may merge with or invade the benign epithelium of the overlying mucosa (Allen 1999).

In this study, we have shown that contact rhinoscopy allows the accurate differentiation of normal epithelium of the nasopharynx from malignant cells of NPC. Furthermore, it not only allows an accurate *in vivo* diagnosis of NPC, but also enables its two distinctive cellular patterns to be distinguished. Its sensitivity and specificity in the detection of primary NPC were comparable to those of conventional endoscopy. Nevertheless, we would like to emphasize that it cannot yet be a substitute for diagnosis by histopathology, which still remains the gold standard of diagnosis. In cases in which the nasopharyngeal tumour is obvious, the clinical value of contact rhinoscopy is limited. However, clinicians should be constantly aware of the possible existence of submucosal disease, which is often associated with a normal-looking nasopharynx or a mild degree of asymmetry. This type of patient may require multiple biopsies and even repeated procedures before tumour tissue can be sampled for histological diagnosis. In this study, we have demonstrated that contact rhinoscopy allowed the detection of subclinical

disease, which occurred in one of 24 cases (4.17%). Theoretically, through the real-time identification of the cellular morphology of the nasopharyngeal lesions, contact rhinoscopy can accurately direct biopsies to areas of suspicion and thus help to improve the diagnostic yield of the biopsy and avoid unnecessary multiple-punch biopsies. However, owing to the small number of subclinical cases in this study, the definitive role of contact rhinoscopy in the diagnosis of subclinical disease has to be explored further by administration of a larger scale study.

Despite the effectiveness of contact rhinoscopy in the visualization of nasopharyngeal pathology, there are potential technical shortcomings in its clinical application. Firstly, as seen in this study, NPC is commonly associated with tortuous vasculature. These neo-vessels are fragile and can easily be torn during the manipulation of the endoscope on the surface of the tumour. It is therefore important to maintain gentle contact with the tumour surface to avoid undesirable bleeding. Secondly, the roof and fossa of Rosenmuller of the nasopharynx are regions which are difficult to examine with a 0^0 endoscope. This problem can be overcome by using a 30^0 endoscope. Thirdly, the diagnosis of malignancy using contact endoscopy depends on the recognition of atypical patterns of tumour cells. The presence of false positive results shown in this study is an issue of concern. Clinicians will therefore have to be trained to recognize the tumour cells. It may be essential to work closely with a pathologist in the initial phase of the learning curve.

6.6. Conclusions

This study showed that contact rhinoscopy allows a real-time differentiation between malignant cells of NPC and normal mucosa of the nasopharynx. Its sensitivity and specificity in the detection of primary NPC are as high as those of conventional endoscopy. Furthermore, it is able to detect subclinical disease, which is not readily visualized by conventional endoscopy.

Figure 6-1. Flow chart of endoscopic examination of 60 recruited patients.

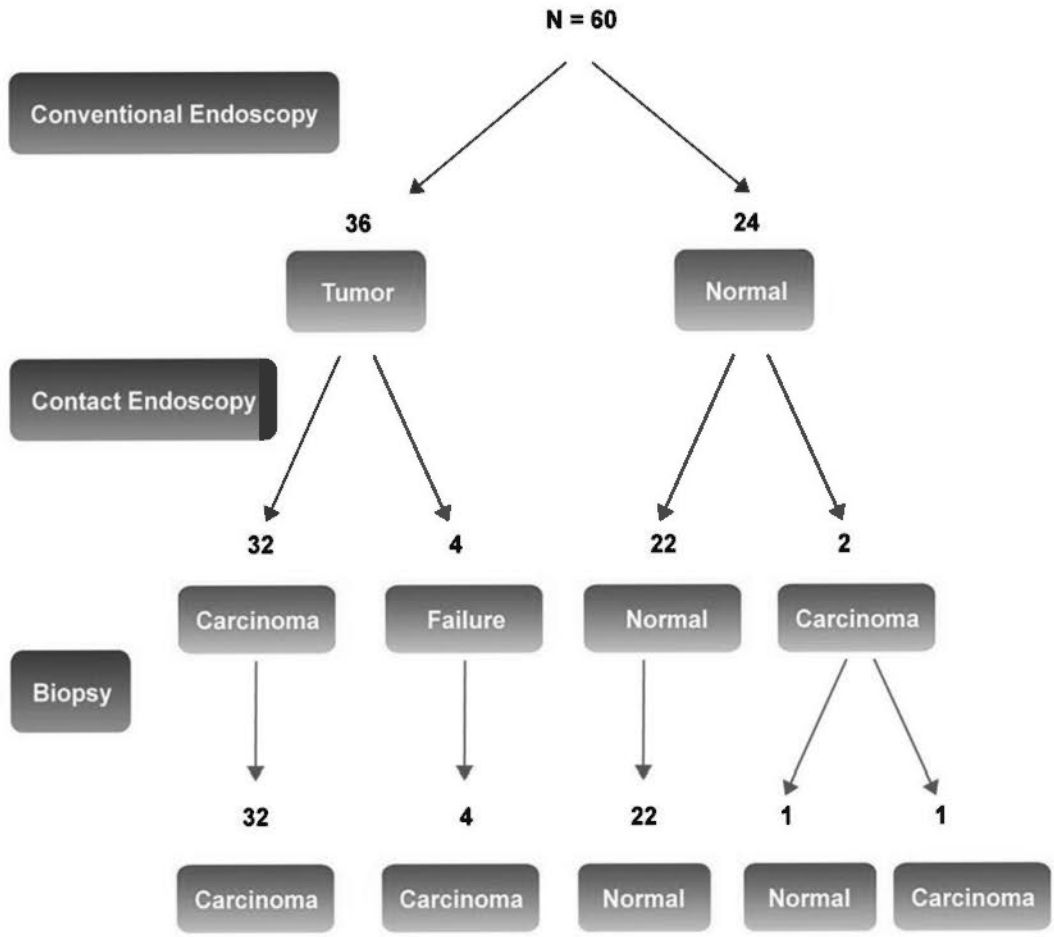
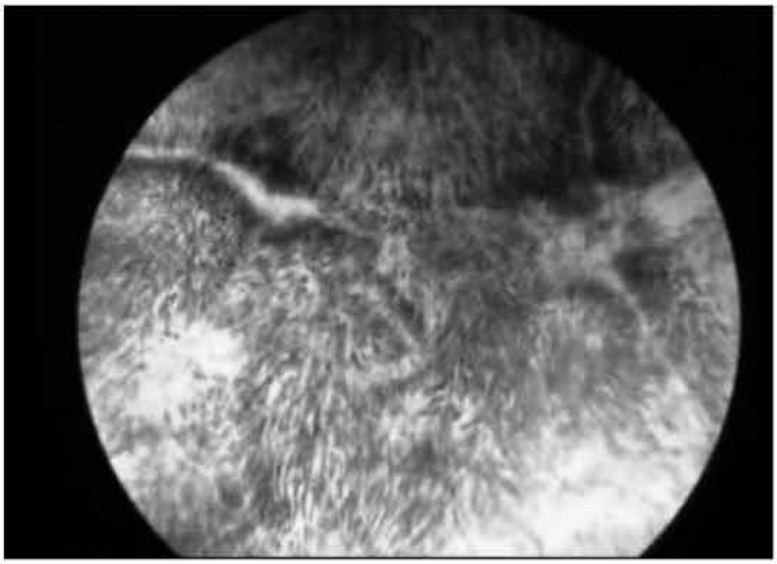


Figure 6-2. Normal nasopharynx. Respiratory epithelium.

(A) Contact endoscopy. Low-power (x60) view of stained cilia of the normal respiratory epithelium.

(B) Contact endoscopy. High-power (x150) view of stained cilia.

A



B

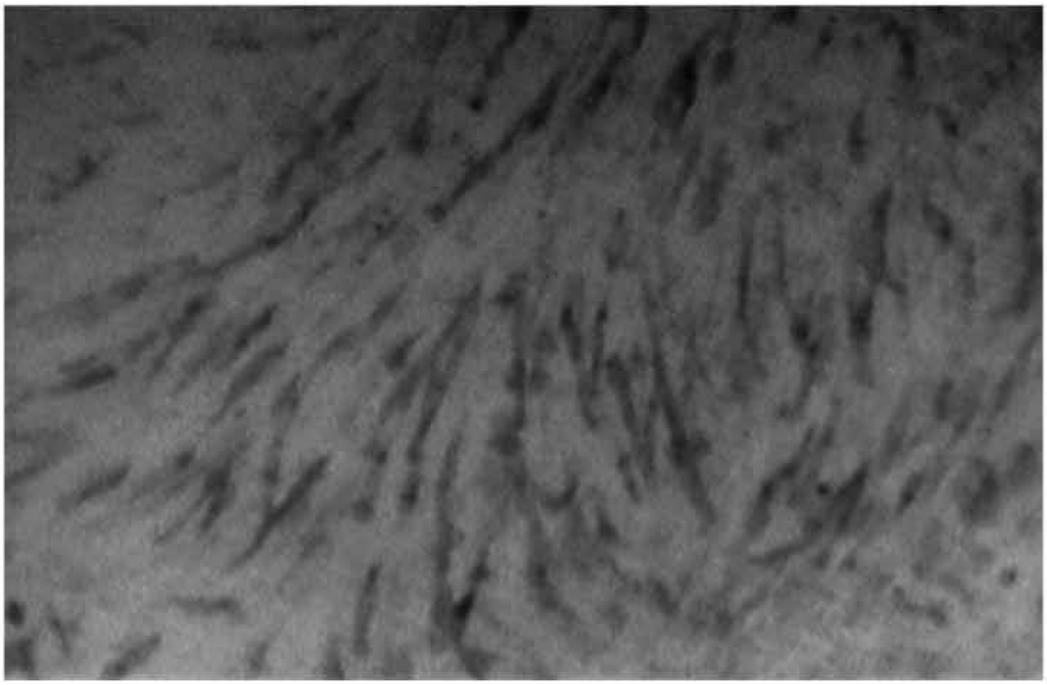


Figure 6-2. Normal nasopharynx. Respiratory epithelium.

(C) Histology of normal respiratory epithelium (Haemotoxylin & eosin stain, original magnification x 350).

C

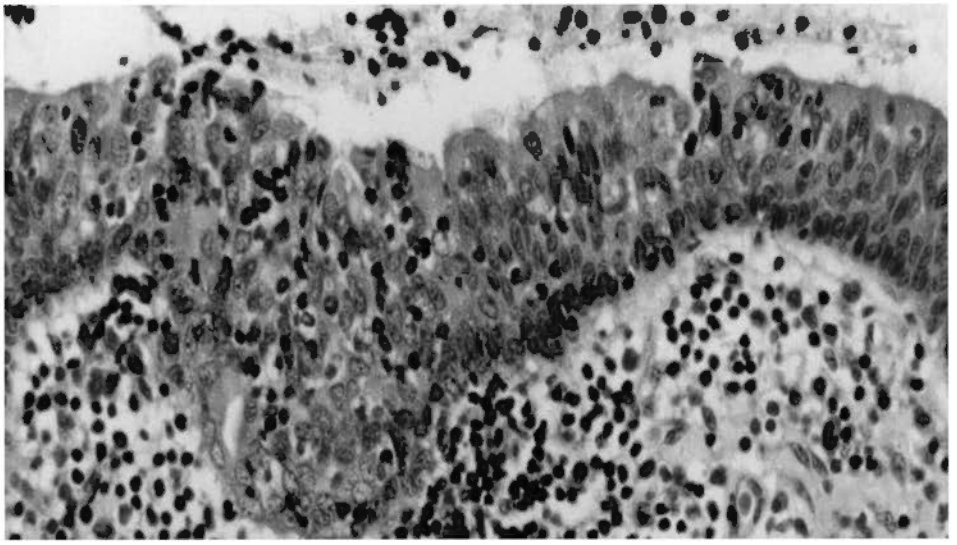
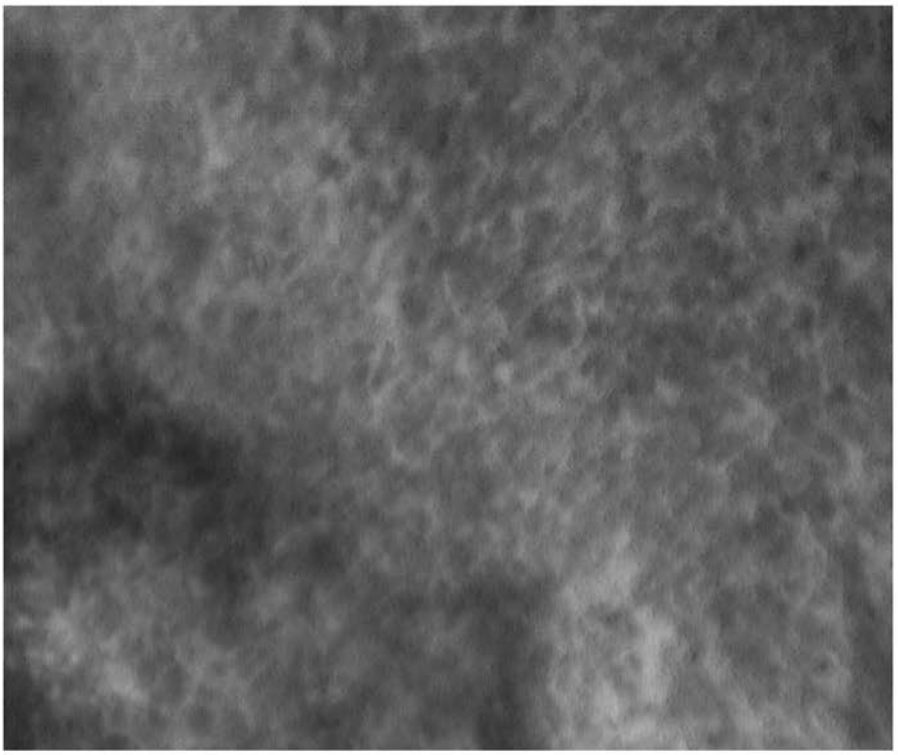


Figure 6-3. Normal nasopharynx. Squamous epithelium.

(A) Contact endoscopy showing uniformly arranged squamous cells in the squamous epithelium. They were of polyhedral in shape with small oval shape nuclei and low nuclear: cytoplasmic ratio. Nucleoli were not apparent (x150).

(B) Histology of the corresponding biopsy specimen. Squamous epithelium (Haemotoxylin & eosin stain, original magnification x250).

A



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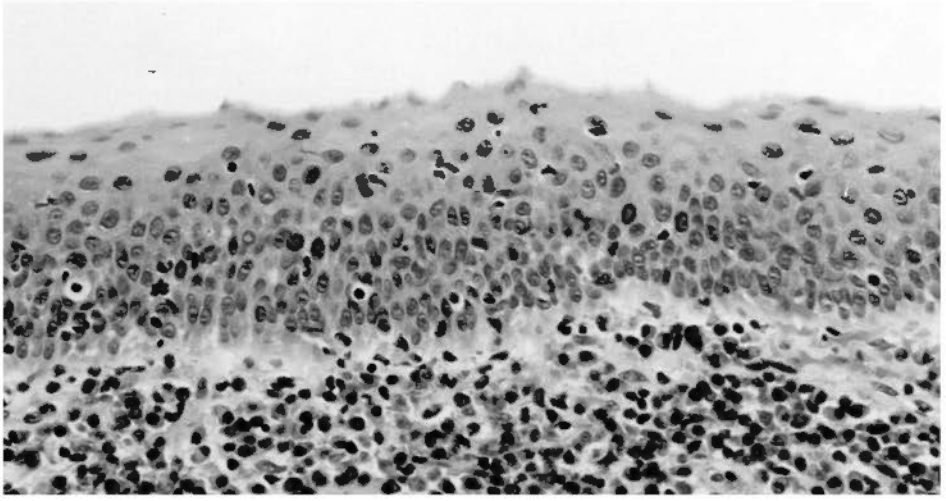


Figure 6-4. Contact endoscopic image of tortuous neo-vessels.

Contact endoscopic high power (x150) view of a nasopharyngeal carcinoma showing numerous tortuous neo-vessels in the background.

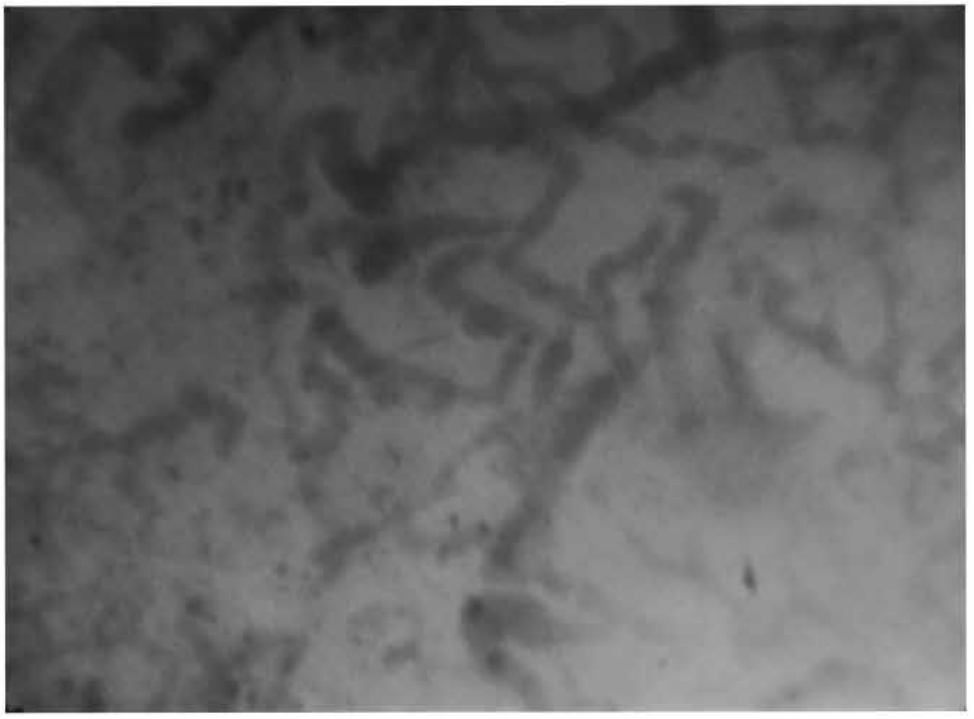
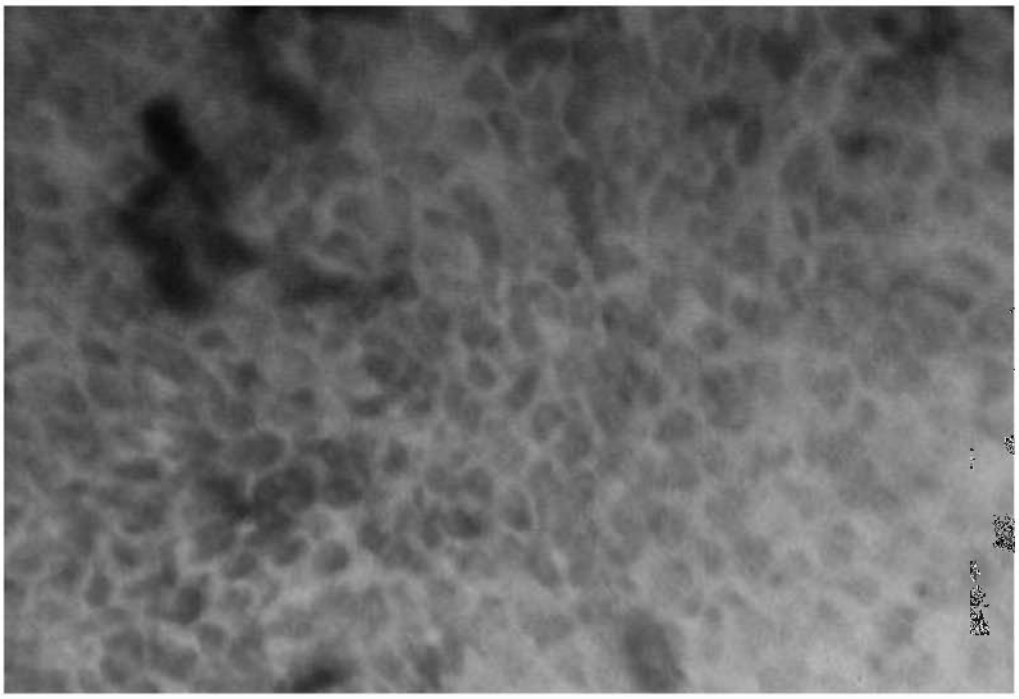


Figure 6-5. Contact endoscopy and histology of primary nasopharyngeal carcinoma (Schmincke pattern).

(A) Contact endoscopic high power (x150) view of a nasopharyngeal carcinoma in a 68-year-old man. Note a large syncytial sheet of malignant cells with enlarged pleomorphic nuclei. Distinct prominent nucleoli can also be demonstrated in some of the tumor cells.

(B) Sections of the corresponding biopsy specimen (Haemotoxylin & eosin stain, original magnification, x250). Syncytial sheets of large oval cells with enlarged pleomorphic nuclei and prominent nucleoli are evident (Schmincke pattern).

A



B

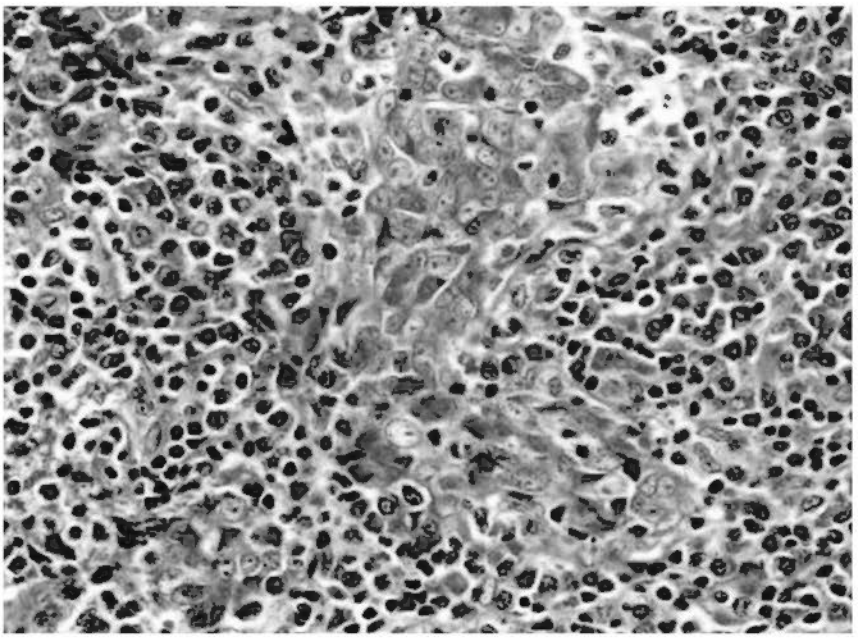
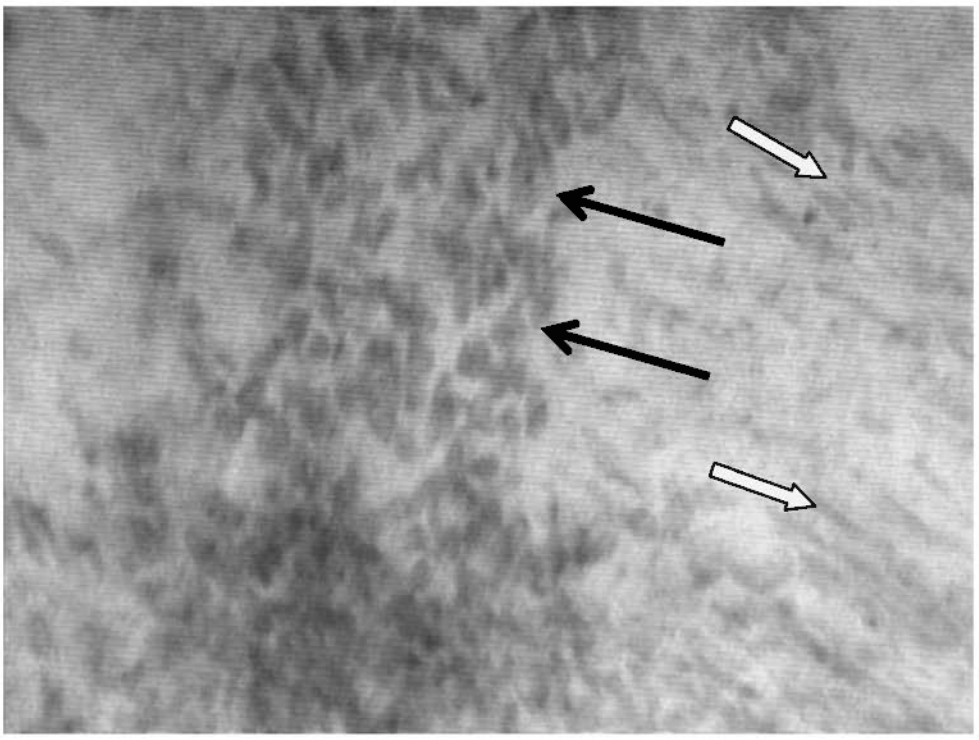


Figure 6-6. Contact endoscopy and histology of primary nasopharyngeal carcinoma (Regaud pattern).

(A) Contact endoscopic high power (x150) view of a nasopharyngeal carcinoma with atypical cellular morphology in a 32-year-old man. The scattered clumps or syncytial sheets of atypical cells with darkly stained pleomorphic nuclei (black arrows) interrupt the cilia of the respiratory epithelium (yellow arrows). Distinct nucleolus of a malignant cell is indicated by the arrow head.

(B) Section of the corresponding biopsy specimen showing the Regaud pattern, characterized by nests or scattered undifferentiated carcinoma cells (arrows) between dense lympho-plasmocytic infiltrates (Haemotoxylin & eosin stain, original magnification, x250).

A



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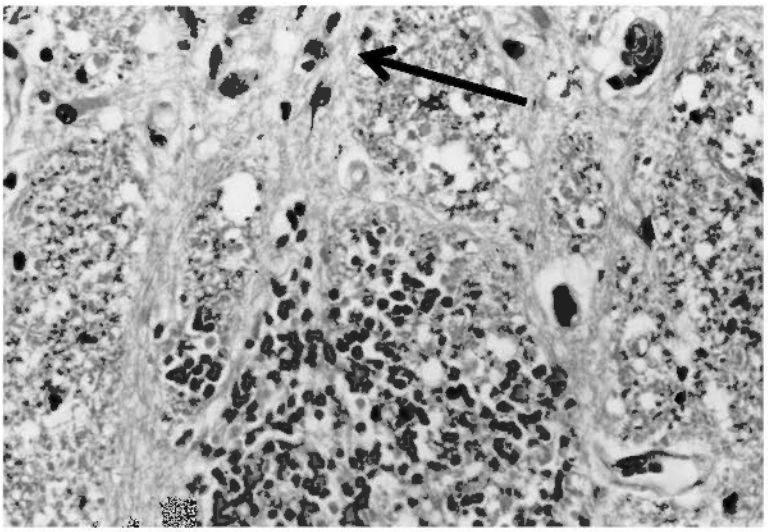


CHAPTER 7

IN VIVO DIAGNOSIS OF PERSISTANT AND RECURRENT NASOPHARYNGEAL CARCINOMA BY CONTACT RHINOSCOPY

7.1. Abstract

Background

Contact rhinoscopy can accurately provide an *in vivo* diagnosis of primary nasopharyngeal carcinoma (NPC). Its efficacy in the diagnosis of residual and recurrent NPC in the irradiated nasopharynx has not been established.

Objective

We tested the hypothesis that contact rhinoscopy can provide real-time identification of different mucosal patterns of an irradiated nasopharynx and that its sensitivity in the detection of persistent and recurrent NPC is higher than that of conventional nasal endoscopy.

Methods

A prospective, single-blind, controlled study was conducted to examine the nasopharynges of 64 consecutive patients who had been irradiated for NPC in a clinic setting using contact rhinoscopes. The gross appearance of the nasopharynx was examined using conventional nasal endoscopy. Examination of the mucosal patterns of the nasopharynx at high magnifications (x50 and x150) was then performed by contact rhinoscopy. The areas under examination were biopsied. The findings of conventional endoscopy and contact rhinoscopy were analysed by an endoscopist and a pathologist, respectively. The findings of both endoscopies were compared and correlated with the histological sections of the biopsied tissues.

Results

During the conventional endoscopy, the appearance of the nasopharynx was assessed as normal and suspicious in 51 and 13 cases, respectively. Using the histology of the biopsy specimens as the gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value of conventional endoscopy in the detection of persistent and recurrent disease were 60%, 83%, 23.1%, and 96.1%, respectively. Contact rhinoscopy was performed in 64 patients (54 men, mean age 42 years). Four patterns of contact endoscopic findings were identified: squamous metaplasia (43 cases), post-irradiation atypia (10), granulation tissue (6), and malignancy (5). In the last group, the nasopharynx appeared normal in two patients (40%). The findings from contact rhinoscopy correlated well with the histological findings (kappa reliability coefficient = 0.847; $p<.001$; diagnostic accuracy, 92.1%). For the prediction of persistent and recurrent disease, the sensitivity and specificity of endoscopic findings were both 100%.

Conclusion

Contact rhinoscopy allows an accurate real-time identification of different mucosal patterns in the irradiated nasopharynx. It has a higher sensitivity and specificity for the detection of persistent and recurrent disease than conventional endoscopy.

7.2. Introduction

Nasopharyngeal carcinoma (NPC) is a radiosensitive tumour. External-beam radiotherapy (ERT) is the mainstay of curative treatment in patients without distant metastasis. A radical tumoricidal dose of approximately 60 to 70 Gy is usually administered to the primary tumour and, if involved, cervical node metastases. Nevertheless, local persistent disease (less than 12 weeks after radiotherapy) and local recurrence (more than 12 weeks after radiotherapy) occur in certain circumstances. Lee et al. reported a cumulative local failure rate of 24% and 5- and 10-year local control rates of 72% and 67%, respectively, in 4128 patients (Lee 1993b). Teo et al. reported a cumulative local failure rate of 20.8% and a 5-year local control rate of 78.8% (Teo 1996c). These series show that the local persistence, which occurs in 8% to 13% of the patients, has been associated with a significantly high rate of ultimate local failure in the advanced-stage tumour (Teo 1996c, Teo 1999). By treating such local diseases with intracavitary brachytherapy (ICT), the local failure rate was significantly reduced where the primary tumour was confined to the nasopharynx or with extension only to the nasal cavity (Teo 1996b). Therefore, early detection of persistent or recurrent tumour by clinical means is vital to achieve better local control and survival in selected patients.

Regular follow-up is essential to detect locoregional recurrence in NPC patients after ERT. During the follow-up, detailed examination of the irradiated nasopharynx using fibre-optic endoscopy is needed. Owing to the propensity of the submucosal pattern in recurrence, most of the endoscopic findings of this disease are described as either normal or non-specific, and up to 50% of

endoscopically-guided or random biopsy findings may be negative in patients who are subsequently proven to have recurrent tumours (Sham 1992). Therefore, clinical examination alone is not reliable in the detection of relapse in NPC.

Imaging including computed tomography (CT) scan, magnetic resonance imaging (MRI), and positron emission tomography (PET) has been employed to detect recurrence of NPC. However, these investigations do have their own limitations. CT scan may not allow the differentiation of recurrence without associated bony erosion from the changes after radiotherapy. Because the hyperintensity, a hallmark of recurrence in MRI, is also seen in oedema, inflammation, and immature fibrosis after radiotherapy, there is an overlap in signal intensity between recurrence and changes after radiotherapy (Gong 1991, Olmi 1995). Although PET scanning has been shown to be superior to CT scanning for the detection of recurrent or persistent NPC, it was recommended that the optimal time for PET scanning in post-irradiated patients should be six months or later (Gong 1991). Therefore, a versatile investigation tool which allows early detection of persistent or recurrent disease before it becomes clinically visible is needed.

Contact endoscopy was first introduced by Hamou as microhysteroscopy to examine the surfaces of the genital tract at high magnification (Hamou 1980b). The advent of recently designed contact rhinoscopes (7215AA and 7215 BA, Karl Storz) with a diameter of 4 mm allows direct access to and examination of the nasopharynx through the nasal cavity using local anaesthesia.

Since January 1999, we have used contact rhinoscopes to examine patients with a clinically normal nasopharynx and NPC in the otorhinolaryngology clinic of the Prince of Wales Hospital. We found that contact rhinoscopy allows accurate differentiation of normal cells of the nasopharynx from malignant cells and allows an *in vivo* diagnosis of primary NPC in a clinical setting (Pak 2001). However, its efficacy in the diagnosis of residual and recurrent NPC in the irradiated nasopharynx has not been established. To evaluate the accuracy of this technique in the diagnosis of persistent and recurrent NPC, we carried out a prospective, single-blind, controlled study in patients with NPC following ERT.

7.3. Patients and Methods

From March 1999 to September 2000, consecutive patients who were attending a tertiary-referral clinic of the Prince of Wales Hospital and had been treated with ERT for NPC were recruited for the study. The study was approved by the Joint Human Review Ethics Committee of the Chinese University of Hong Kong and the Hong Kong Hospital Authority.

In this study, a two-tailed power analysis of sample size estimated that at least 58 patients would achieve 80% power at a 5% significance level to detect a difference of 25% in sensitivity between those examined using conventional nasal endoscopy and those examined using contact endoscopy.

Each recruited patient underwent conventional nasal endoscopy followed by contact rhinoscopy performed by a single endoscopist (MWP) using a contact

rhinoscope in the following manner. The nasal cavities and nasopharynx were anaesthetised using a maximum of 1ml of a 5% cocaine topical anaesthetic spray. After 10 minutes, the rhinoscope was slowly advanced through the nasal cavity and into the nasopharynx. The nasopharynx was examined for abnormalities at zero magnification as in conventional endoscopy. Different degrees of suspicion of local relapse were judged according to the appearance of the nasopharynx, as follows:

1. normal, if the nasopharynx was smooth;
2. suspicious, if the nasopharynx appeared markedly swollen, asymmetrical, or ulcerated, or a prominent growth was present in the nasopharynx.

Afterwards, a small nasal cotton-ball applicator was used to stain the nasopharyngeal mucosa with 1% methylene blue via the nasal cavity. The tip of the contact rhinoscope was then advanced until it made gentle contact with the mucosa of the posterior wall of the nasopharynx. The mucosa was examined at magnifications of x60 and x150 by adjusting the zoom switch on the endoscope. Once the contact rhinoscopy had been completed, the areas under examination were biopsied and the samples were studied by an independent pathologist who was blind to the findings of the endoscopies. A video recording was made of the entire endoscopic procedure including the conventional endoscopic examination of the nasopharynx and the contact endoscopic examination of the nasopharyngeal mucosa.

The endoscopist and a consultant pathologist subsequently analyzed the video images but without prior knowledge of the histology of the corresponding specimens. Independent diagnoses based on the conventional nasal endoscopy and the contact rhinoscopy were made by the endoscopist and the pathologist, respectively. Using the histology of the biopsy specimens as the gold standard, the accuracy of contact rhinoscopy for diagnosing persistent and recurrent NPC was compared with that of conventional nasal endoscopy. The kappa reliability test was employed to evaluate the degree of agreement between the diagnosis made during contact rhinoscopy and the histological examination.

7.4. Results

During the study period, 64 patients with a history of treated undifferentiated carcinoma (54 men, 10 women) were recruited into the study. Their ages ranged from 21 to 77 years (mean age, 42 years). Five of the eligible patients did not consent to participation in the study and so were excluded. The procedures were performed at a time ranging from 4 weeks to 10 years after radiotherapy (median period, seven weeks). The dosage of the radiation ranged from 66 to 86 Gy.

All of the patients recruited (100%) underwent both conventional endoscopy and contact rhinoscopy.

During the conventional endoscopy, the appearance of the nasopharynx was assessed as normal or suspicious in 51 and 13 cases, respectively. The histology of the biopsy specimens showed four types of pathology: squamous metaplasia

(42 cases), radiation changes with cellular atypia (8), granulation tissue (9), and malignancy (5). In those patients with persistent or recurrent disease, the appearance of the nasopharynx was normal in two patients under conventional nasal endoscopy (40%). Using the histology of the biopsy specimens as the gold standard, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of conventional endoscopy in the detection of persistent and recurrent disease were 60%, 83%, 23.1%, and 96.1%, respectively.

During the examination of the mucosal patterns of the nasopharynx using contact rhinoscopy, four patterns were identified: squamous metaplasia (43 cases), post-irradiation atypia (10), radiation changes/granulation tissue (6), and malignancy (5).

1. Squamous metaplasia

The characteristic features of squamous metaplasia were demonstrated under contact rhinoscopy in 43 patients.

The squamous cells were polyhedral, contiguous with each other, and arranged in sheets. The nuclei appeared round and darkly stained, and the cytoplasm had a light blue tone. The nuclear: cytoplasmic ratio was low, and the overall morphological pattern was uniform (Figure 7-2A). Cilia were not seen in these cases. The nasopharynx was regarded as normal in 40 patients and suspicious in three patients. The diagnosis of squamous metaplasia was confirmed in 42 of the 43 corresponding sections (97.7%) from the biopsy

specimens (Figure 7-3A). One specimen showed granulation tissue with denuded epithelium.

2. Radiation changes with cellular atypia

The nasopharynges of 10 patients showed features of cellular atypia under contact rhinoscopy, although the nasopharynx appeared normal in half of the patients and suspicious in half of the patients. In this group of patients, small syncytial sheets of scattered atypical cells were noted. The cells were interpreted as atypical owing to the characteristics of indistinct cell borders and heterogeneity of cellular and nuclear shapes.

However, in most of the cells, the nuclear: cytoplasmic ratio remained low (Figure 7-2B). In this group of patients, seven corresponding paraffin sections (70%) showed atypical changes within the overlying epithelium (Figure 7-3B). Those atypical epithelial cells exhibited increased nuclear size, but the nuclear: cytoplasmic ratio was still low. Degenerative changes with cytoplasmic vacuolation were noted. The changes were consistent with radiation effects. No malignancy was noted. Three histological sections showed granulation tissue without covering epithelium (30%) (Figures 7-3C&D). Radiation changes with scattered atypical stromal cells with enlarged nuclei and a low nuclear: cytoplasmic ratio were frequently seen.

3. Granulation tissue

Under contact rhinoscopy, scattered atypical cells were demonstrated in six patients (Figure 7-2C). The appearance was compatible with that of granulation

tissue. The nasopharynx appeared normal in four out of the six patients. Two nasopharynges appeared suspicious. The corresponding sections confirmed the diagnosis of granulation tissue in five cases (83.3%) (Figure 7-3C&D). One case showed squamous metaplasia in the histological section.

4. Malignancy

Syncytial sheets of malignant cells were identified in five patients under contact rhinoscopy (Figure 7-2D). Among these five cases, two cases were regarded as persistent disease (less than 12 weeks after radiotherapy) because the procedures were performed four and five weeks after radiotherapy, respectively. Local recurrence (more than 12 weeks after radiotherapy) was detected in three patients, in whom the procedures were performed at 11 months and at five and six years after radiotherapy, respectively. In three of the five patients, obvious tumours were observed in the nasopharynx with the naked eye, whereas the nasopharynx appeared normal in two patients who had diagnoses of persistent disease and recurrence, respectively (40%) (Figure 7-4). In all five cases, the findings of malignancy were confirmed by the histological sections (100%) (Figure 7-3E). When the overall findings of contact rhinoscopy were compared and correlated with the histological diagnoses, the diagnostic accuracy of contact rhinoscopy was found to be 92.1% (Table 7-1).

The kappa test also showed that there was excellent agreement between the findings of contact rhinoscopy and histological findings (kappa reliability coefficient = 0.847; $p<0.001$). The sensitivity and specificity of contact rhinoscopy in different pathological conditions are given in Table 7-2. The sensitivity, specificity, PPV, and

NPV of the contact rhinoscopic findings for predicting persistent and recurrent NPC were all 100%.

7.5. Discussion

To our knowledge, the present report is the first study in the English literature to evaluate the role of contact rhinoscopy in identification of the histological changes in the nasopharynx in patients with NPC after ERT. Our results not only demonstrate its usefulness in identification of the various stages of radiation changes, but also demonstrate its high accuracy rate in the detection of persistent or recurrent disease.

The normal nasopharynx is lined mainly by pseudostratified columnar ciliated epithelium near the choanae and adjacent part of the root, whereas in the lower and posterior regions of the nasopharynx the lining assumes a stratified squamous character. Areas of the transitional epithelium are encountered at the junctional zone located between the roof and the lateral walls of the nasopharynx. The mucosa is frequently infiltrated by lymphoid tissues, whereas the submucosal layer contains serous and mucous glands (Gibb 1999). The histological appearance of the mucosa of the nasopharynx after irradiation is characteristic. Following irradiation, the ciliated respiratory epithelium undergoes squamous metaplasia. The cellular changes after irradiation include enlargement of the cells and nuclear enlargement with coarse chromatin, multi-nucleation, prominent nucleoli, and degenerative changes with cytoplasmic vacuolation. An important diagnostic feature is that the nuclear:cytoplasmic ratio remains low. Radiation

changes subside after 12 months, and by two years there is usually little residual evidence of the treatment (Chang 1999).

Because contact endoscopy involves examination of the superficial cells from a tangential axis, the experience of recognizing pathological conditions under contact endoscopy is novel to both the endoscopist and the pathologist. Therefore, in the present study, the diagnosis based on contact rhinoscopy was established jointly by the endoscopist and the pathologist. In our experience, three distinctive patterns of radiation change can be readily recognized under contact rhinoscopy. Among the different patterns of radiation change, the absence of ciliated respiratory epithelium was a common finding. In cases of squamous metaplasia without stromal atypia, which is the most common pattern of radiation change, the cells are homogenous and polyhedral, with small nuclei. Because the cells are easily differentiated from the malignant cells of recurrent NPC, biopsy may not be indicated unless the appearance is clinically suspicious. Nevertheless, the identification of atypical cells under contact rhinoscopy may need more attention. In our experience, atypical cells can be present in two conditions. One features atypical epithelial cells consisting of immature squamous metaplastic cells and epithelial cells with radiation changes. These cells appear more rounded compared with the normal polyhedral squamous cells. Because these atypical cells cannot be easily differentiated from malignant cells under contact rhinoscopy, biopsy of the lesion is indicated to rule out recurrent tumour. Atypical cells can also be present in patients with granulation tissue. The granulation tissue is characterized by the presence of scanty, scattered, atypical stromal cells within

the blanket of acellular exudate. These features are distinctive from those of squamous metaplasia and malignant cells.

As in primary NPC, the appearance of malignant cells in persistent or recurrent disease is distinctive. The cells are round or oval with enlarged nuclei and are arranged in syncytial sheets. The features are readily distinguished from those of benign radiation changes.

It has been recognized that the behaviour of the recurrent tumour may be different from that of the primary tumour. Because the local recurrence has a greater tendency to grow submucosally, the mucosal or surface component of the tumour may be small. It can remain relatively asymptomatic until advanced. Because of the propensity towards submucosal disease in the recurrent cases, early diagnosis of local recurrence based on clinical judgment is not simple. In accordance with other series, we found that clinical examination alone is not adequate to detect local recurrence, especially at an early stage.

In the present study, we found that contact rhinoscopy not only allowed the accurate identification of the malignant cells of persistence and recurrence in all cases, but also facilitated the diagnosis of recurrent disease even in patients with an apparently normal nasopharynx.

The setup cost of the instruments, including the 0° and 30° contact endoscopes, three-chip camera, audiovisual recording system, and xenon light source, is approximately US$5000. The low maintenance and extraordinarily high

sensitivity and specificity in identifying persistent and recurrent diseases support the view that contact rhinoscopy may play two important roles in the routine surveillance of patients who have received radiotherapy (Figure 7-5). First, it allows direct identification of persistent and recurrent disease even in the presence of a normal-looking nasopharynx. Second, it helps to direct the site of biopsy at the suspicious areas with cellular atypia. It not only helps to improve the diagnostic yield of the biopsy but also potentially avoids the need for multiple punch biopsies.

We have used contact rhinoscopy to examine the nasopharynges of normal subjects and patients with primary NPC (Pak 2001). In our experience, the visualization of the superficial cells in the irradiated nasopharynx is easier than in the case of primary NPC or the normal nasopharynx for the following reasons. First, because NPC is commonly associated with tortuous vasculature, these "neo-vessels" are fragile and easily torn during the manipulation of the endoscope on the surface of the tumour. The bleeding renders the visualization of the underlying cells more difficult and confusing. In the present study we found that intra-operative bleeding in post-irradiated patients was not a serious problem. As a matter of fact, we were able to complete the procedure without difficulty. Second, in our experience, part of the lining of a primary tumour and all the superficial lining of the normal nasopharynx is composed of pseudostratified ciliated columnar cells (Pak 2001). The presence of the blanket of mucus and the ciliary beat render the staining and visualization of the underlying cells difficult. In contrast, because most of the lining of the irradiated nasopharynx is composed of

non-ciliated squamous cells, the cellular morphological appearance is more easily visualized.

Although we have demonstrated that contact rhinoscopy allows the detection of local persistence and recurrence even in the presence of a normal-looking nasopharynx, owing to the small number of malignant cases in this study, the definitive role of contact rhinoscopy in the diagnosis of submucosal disease needs to be further confirmed by a larger scale study. Moreover, there are several limitations in the application of contact rhinoscopy in the irradiated nasopharynx:

1. The roof of the nasopharynx and the fossa of Rosenmuller are areas that are not yet accessible to contact rhinoscopy.

2. A small portion of the early recurrence in the nasopharynx remains submucosal. Because the visualization by means of contact rhinoscopy is confined to the first few layers of superficial cells, a small early tumour may not be detected. It is suggested that biopsy is indicated in symptomatic patients with negative findings on contact rhinoscopy.

3. There is a small chance of bleeding and infection during the application of contact rhinoscopy in irradiated patients. Gentle manipulation of a sterilized endoscope may help to minimize these complications.

4. To master the recognition of the characteristics of the nasopharynx after radiotherapy and of tumour recurrence takes time. During the learning curve,

collaboration between endoscopist and pathologist is of the utmost importance.

Despite the above-mentioned limitations, we have found that contact rhinoscopy is a reliable and valuable technique facilitating *in vivo* and *in situ* diagnosis of local persistence and recurrence in NPC in a clinic setting using local anaesthesia.

7.6. Conclusions

Contact rhinoscopy allows the accurate real-time identification of different mucosal patterns in the irradiated nasopharynx. It has a higher sensitivity and specificity for the detection of persistent and recurrent disease than conventional endoscopy.

Table 7-1. The diagnostic accuracy of contact endoscopy.

Pathology	No. of Patients	
	Contact Endoscopy Positivity	Histopathology Positivity
Squamous metaplasia	43	42
Atypia	10	7
Granulation tissue	6	5
Persistent disease/local recurrence	5	5
Total	64 (100%)	59 (92.1%)

Table 7-2. The sensitivity and specificity of contact endoscopy in different pathologies within the irradiated nasopharynx.

Pathology	Sensitivity (%)	Specificity (%)
Squamous metaplasia	97.7	95.2
Atypia	100	94.7
Granulation tissue	55.6	98.2
Malignancy	100	100

Figure 7-1. Findings of contact endoscopy and the histological appearance of the corresponding paraffin sections.

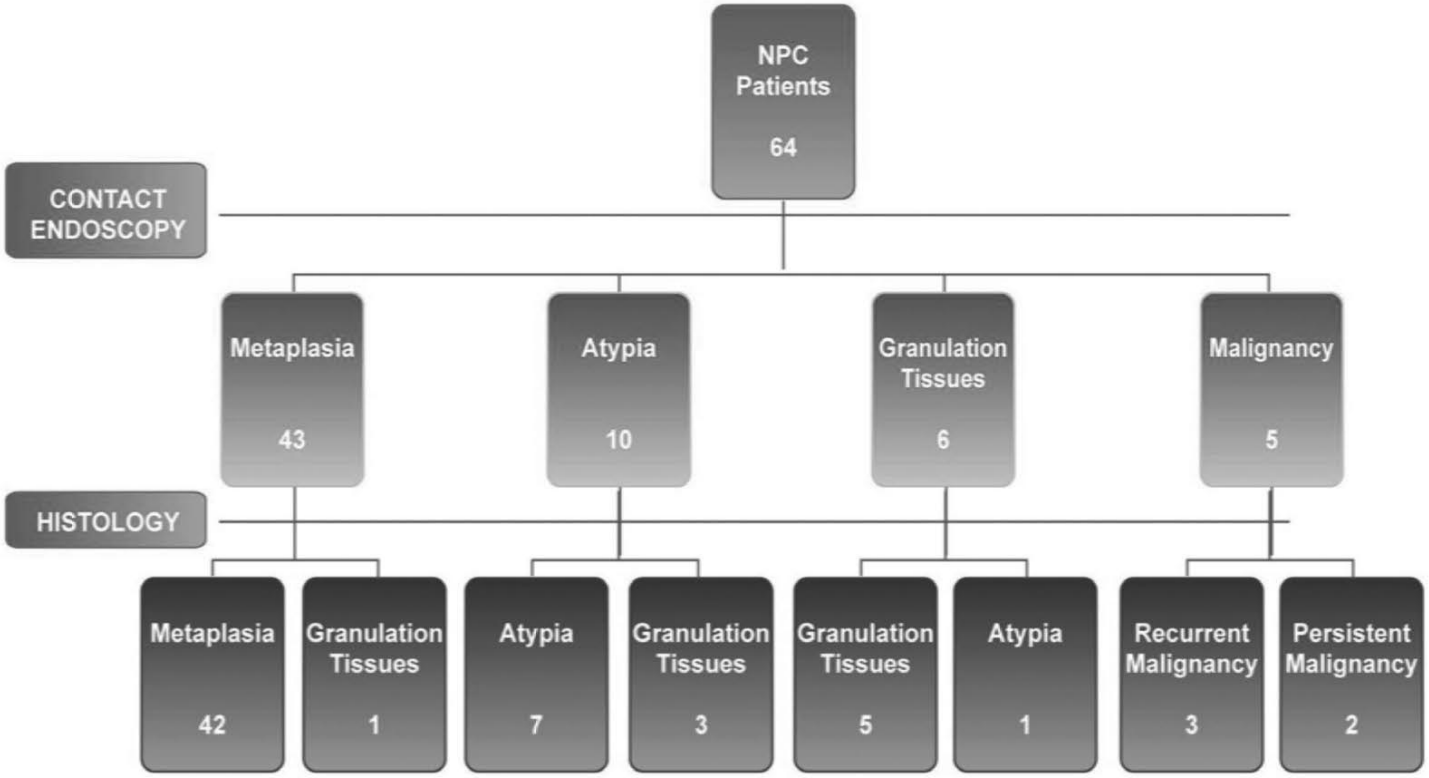


Figure 7-2A Contact endoscopy of squamous metaplasia.

High power view (x150) of squamous metaplasia. The cells are homogenous with round, darkly stained nuclei and light blue cytoplasm. The nuclear: cytoplasmic ratio is low.

Figure 7-2B. Contact endoscopy of cellular atypia.

High power view (x150) of radiation change with cellular atypia. Atypical cells with indistinct cell borders and heterogenous cellular and nuclear content are demonstrated. In most of the cells, the nucleus-to-cytoplasm ratio is normal. These cells appeared more round compared to the normal polyhedral squamous cells.

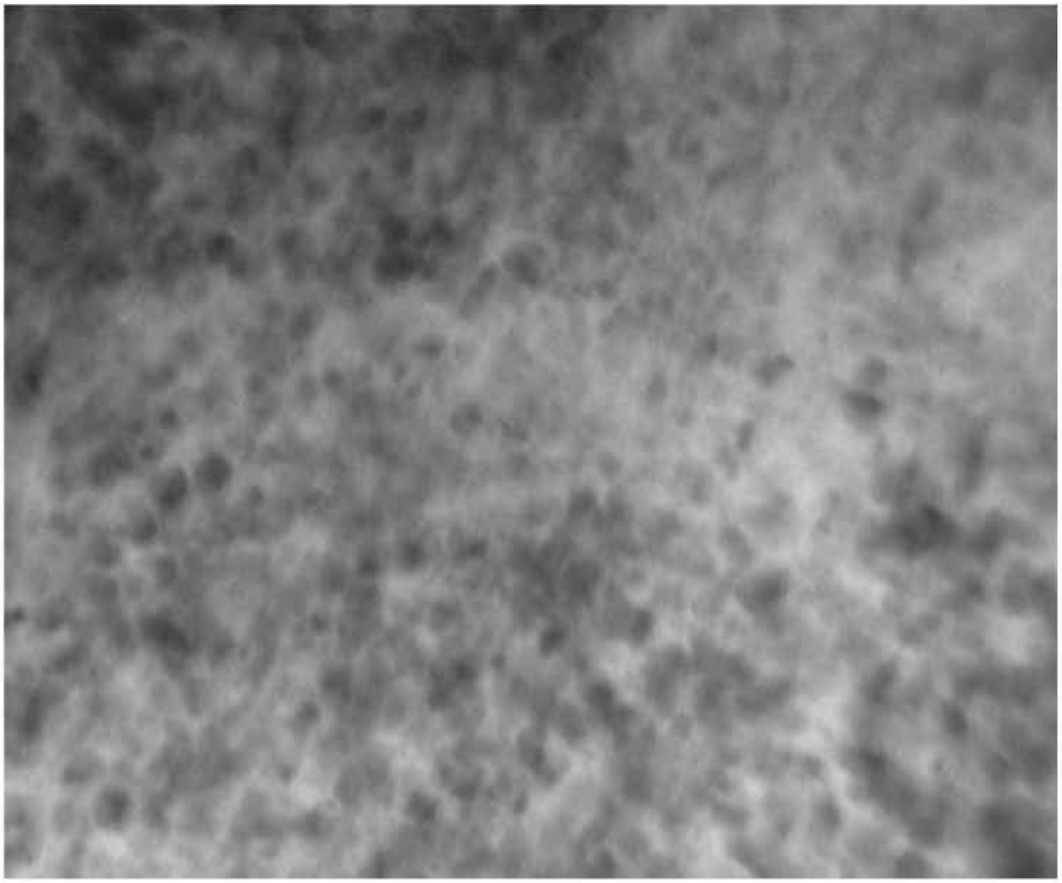


Figure 7-2C. Contact endoscopy of granulation tissue.

High power view (x150) of granulation tissues. The presence of scanty immature cells in the blanket of acellular exudate exhibits the characteristic appearance of scattered atypical cells.

Figure 7-2D. Contact endoscopy of recurrent nasopharyngeal carcinoma.

High power view (x150) of recurrent nasopharyngeal carcinoma with syncytial sheets of malignant cells in a normal-looking nasopharynx. The cells are heterogenous with round and darkly stained nuclei. The nuclear-cytoplasmic ratio is high.

Figure 7-3A&B. Histology of corresponding sections of (A) squamous metaplasia and (B) cellular atypia.

(A) Squamous metaplasia. The epithelium is transformed to squamous epithelium. The squamous metaplastic cells exhibit slightly enlarged nuclei with tiny nucleoli. (Haemotoxylin & eosin stain, original magnification x400).

(B) Radiation change with atypia. The surface is ulcerated and covered by pinkish fibrinous exudate. Scattered atypical stromal cells, as indicated by the arrows, with enlarged atypical nuclei are seen in the ulcer base. Despite the nuclear atypia, the nuclear to cytoplasmic ratio is low. (Haemotoxylin & eosin stain, original magnification x400).

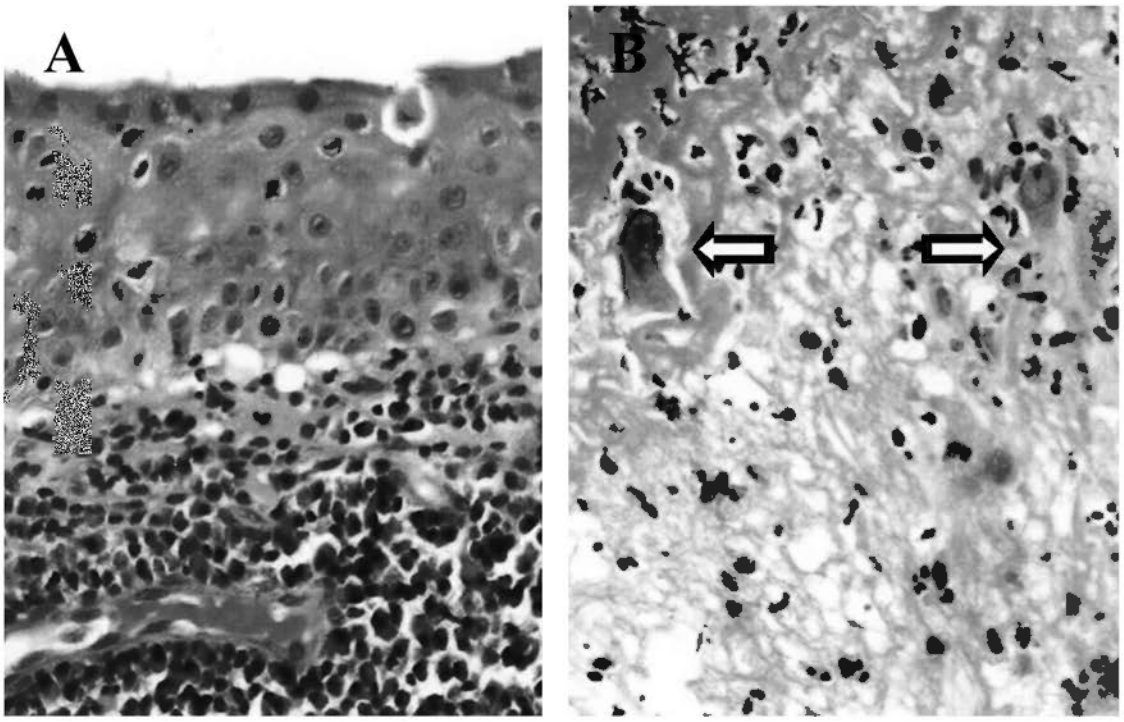


Figure 7-3C&D. Histology of corresponding sections of (C&D) granulation tissue.

(C) Granulation. The low power view showing granulation tissues with a dense mix of inflammatory cells. The surface is ulcerated and covered by fibrous exudate (Haemotoxylin & eosin stain, original magnification x100).

(D) Granulation. The high power view showing granulation tissues with newly formed small vessels among the inflammatory cell infiltrate. (Haemotoxylin & eosin stain, original magnification x400).

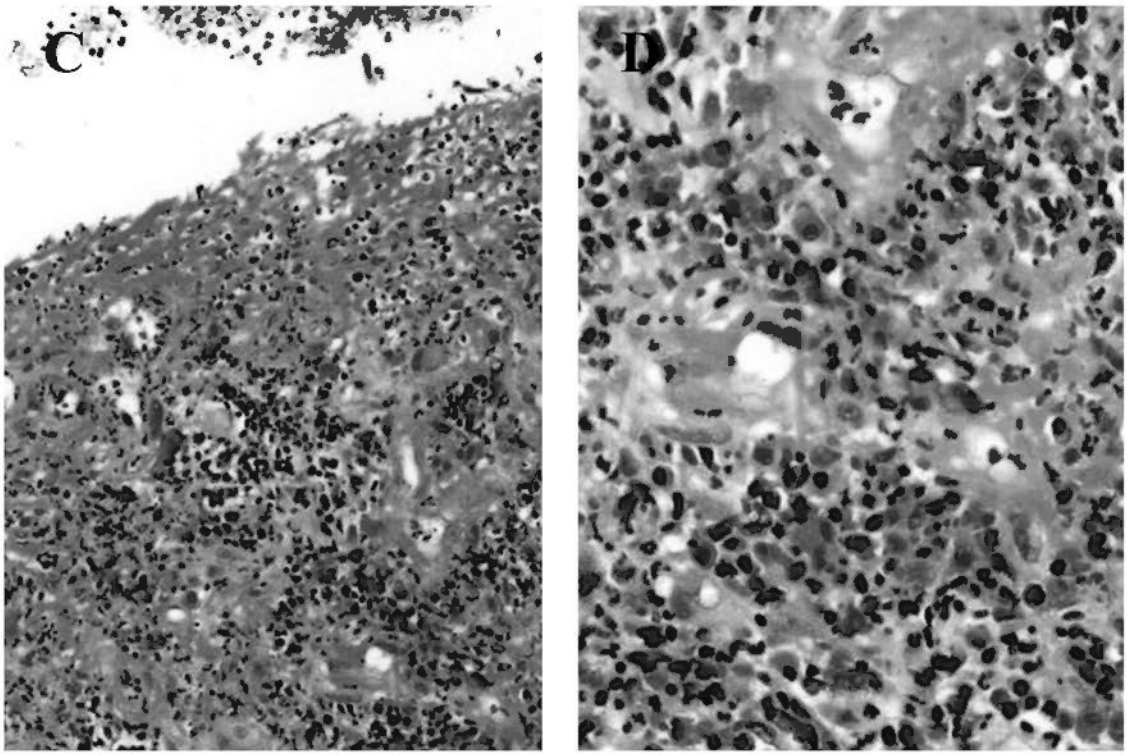


Figure 7-3E. Histology of corresponding sections of recurrent carcinoma.

(E) Local recurrence. The tumor has ulcerated onto the surface and formed syncytial sheets of malignant cells. The carcinoma cells exhibit oval pleomorphic nuclei with prominent nucleoli and scanty indistinct cytoplasm. (Haemotoxylin & eosin stain, original magnification x400).

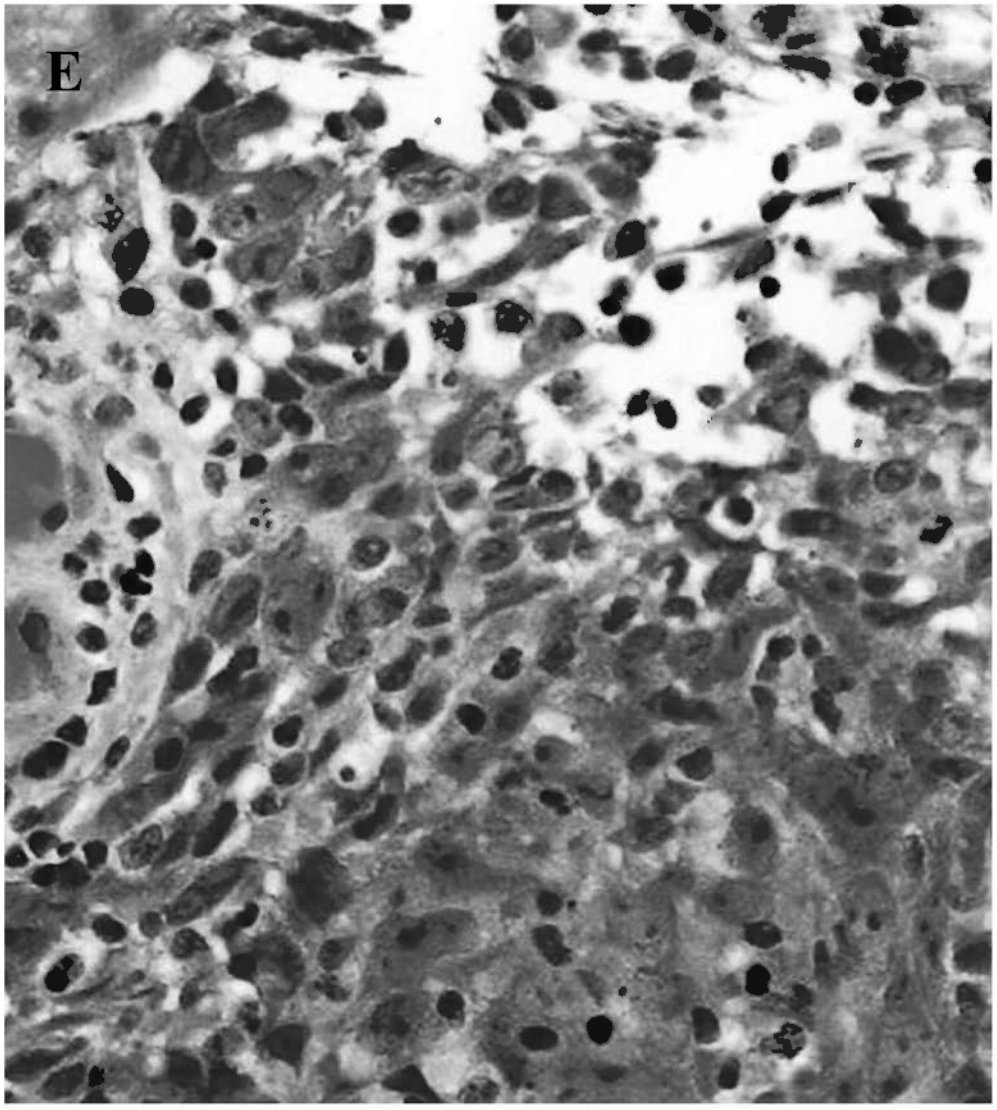


Figure 7-4. Recurrent carcinoma underneath the left normal looking nasopharynx.

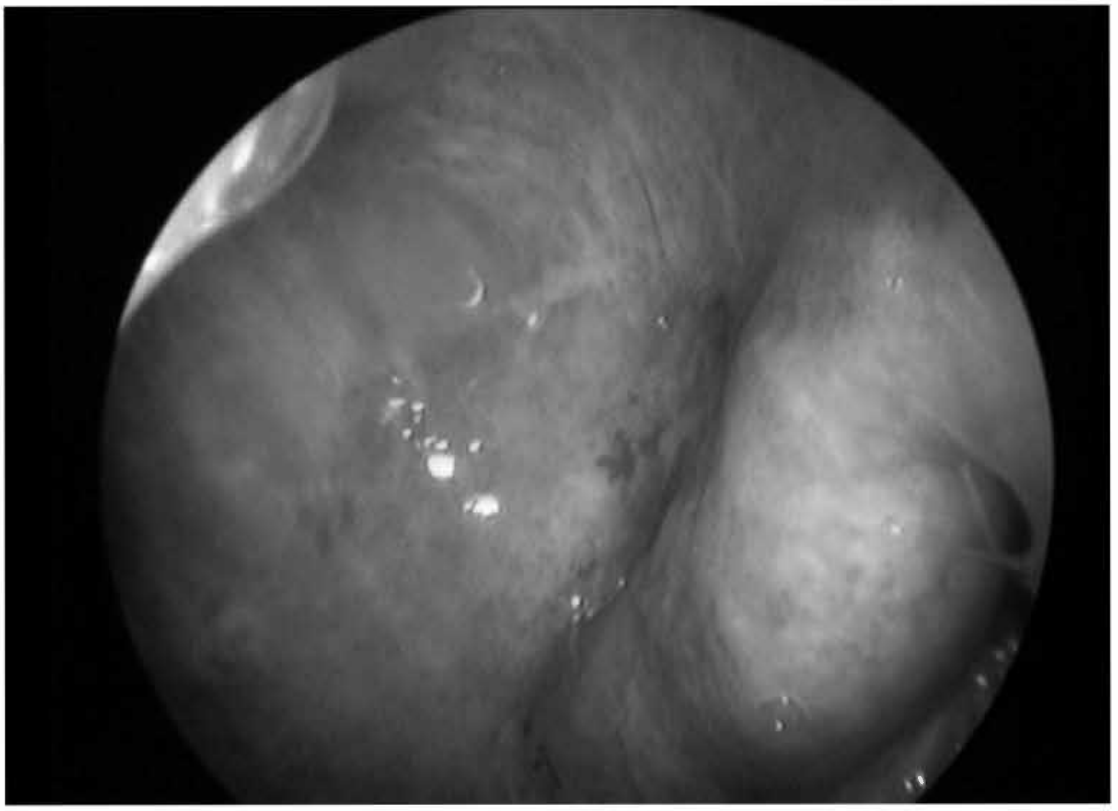
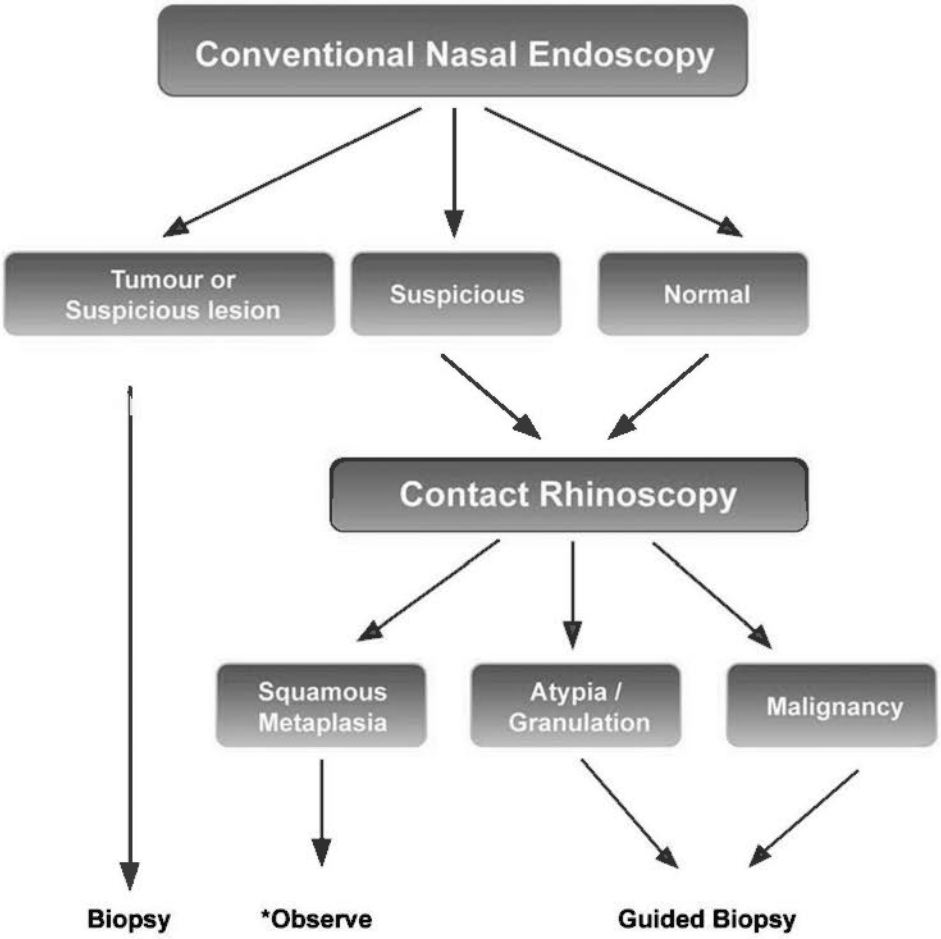


Figure 7-5. Proposed clinical application of contact endoscopy in detection of recurrent nasopharyngeal carcinoma.



* asymptomatic patients without clinical suspicion

CHAPTER 8

THE CHOICE OF CHROMOGEN FOR CONTACT RHINOSCOPY IN THE IRRADIATED NASOPHARYNX

8.1. Abstract

Background

1% methylene blue (methylthionine chloride) has traditionally been used as a chromogen (vital stain) to outline the cellular patterns of the examined tissues. However, the effect of different concentrations of methylene blue on the diagnostic reliability of contact rhinoscopy has not been clearly demonstrated.

Object

A prospective, single-blind study was conducted to assess how concentrations of vital stain and the characteristics of the assessors affect the assessment using contact rhinoscopy.

Methods

Twenty-eight patients who had undergone external radiotherapy (ERT) for nasopharyngeal carcinoma (NPC) were examined by contact rhinoscopy after staining with 0.5% and 1% methylene blue on each side of the nasopharynx. The visual clarity of the endoscopic images was assessed by three independent observers according to a visual analogue scale.

Results

Twenty-four and twenty-one images (x150) stained with 1% and 0.5% methylene blue, respectively, were produced and analyzed by assessors of different clinical backgrounds. All of the images showed a cellular pattern of squamous metaplasia. The intraclass correlation coefficients for intra-observer

reliabilities of assessors were 0.916 to 0.957 and 0.839 to 0.964 in the groups of 0.5% and 1% stains, respectively. The intraclass correlation coefficients for inter-observer reliability of assessors were 0.884 and 0.885 in the groups of 0.5% and 1% stains, respectively. The mean scores for clarity of the cellular details were statistically higher in the group of 1% stain among all assessors.

Conclusion

The assessment of squamous metaplasia by contact rhinoscopy is highly reliable irrespective of the clinical experience and knowledge of histopathology of the assessors. 1% methylene blue should be the vital stain of choice in contact rhinoscopy.

8.2. Introduction

Our experience has shown that contact rhinoscopy is accurate and efficient in allowing the real-time *in vivo* diagnosis of nasopharyngeal carcinoma (NPC) at different stages (Pak 2001, Pak 2002b, Pak 2005). Nevertheless, a few issues remain to be elucidated.

Firstly, since the introduction of contact endoscopy in 1997, 1% methylene blue (methylthionine chloride) has been widely used as a vital stain to outline the cellular patterns of the examined tissues (Hamou 1980b). However, how the concentration of methylene blue affects the diagnostic reliability of contact endoscopy has not been elucidated.

Secondly, as contact endoscopy involves the real-time examination of the superficial cells from a tangential axis, the experience of recognizing the pathologies under contact endoscopy is novel to the endoscopist. We have little knowledge of how the assessors' clinical experience and knowledge of histopathology affect their interpretation of the findings of contact endoscopy.

This study aimed to evaluate how the use of different concentrations of methylene blue and the clinical experience of assessors affect the assessment of contact rhinoscopy in patients with irradiated NPC.

8.3. Patients and Methods

Between November 2000 and March 2001, all consecutive patients who had been treated with external radiotherapy (ERT) for NPC attending the otorhinolaryngology clinic of the Prince of Wales Hospital were recruited into the study. Ethical approvals had been granted by the Human Review Ethics Committee, and informed consents were obtained. Patients who were unwilling or unable to give informed consent were excluded.

A two-tailed power analysis was conducted to calculate the sample size of this study. It was estimated that at least 21 images stained with 1% methylene blue and the same number of images stained with 0.5% methylene blue would achieve 80% power at a 5% significance level to detect a difference of 26% in the mean score of visual clarity of the cellular details between two strengths of stain.

For every patient recruited, contact rhinoscopy was performed by a single endoscopist (MWP) under local anaesthesia (1ml of 5% cocaine spray) in the clinic using a contact rhinoscope (Karl Storz, 7215AA, 0°; 23 cm long; 4mm in diameter) which was connected to a 150-W xenon light source, a video camera, and an S-VHS video recorder.

During the procedure, one side of the posterior wall of the nasopharynx was stained with 1% methylene blue and the contra-lateral side was stained with 0.5% methylene blue using a small nasal cotton-ball applicator. The endoscope was then advanced slowly until its tip was in gentle contact with the surface of the posterior nasopharyngeal wall. The stained cells of the superficial layer of the nasopharynx were carefully examined under magnifications of x60 and x150. The images of the stained cells were recorded using a video camera.

All the images obtained by contact rhinoscopy were randomized and examined by three independent assessors who had no knowledge of the staining information of each individual image. The three assessors consisted of a consultant otorhinolaryngologist, a junior trainee in radiology, and a consultant pathologist. Each assessor was instructed to gauge the visual clarity of the cellular details of each image on a 10-cm un-scaled visual analogue scale based on his or her own perception. Zero represented poor details and 10 represented excellent details. The assessment was repeated two weeks later after the order of the images was reshuffled. The paired visual scores were compared and the intra-observer and inter-observer variations were then analyzed. An independent-samples *t*-test was performed to compare the mean score of clarity

of images at different concentrations of stain among the assessors. The level of significance was set to $p \leq 0.05$. Statistical analysis was performed using SPSS 14.0 for Windows (SPSS, Chicago, IL, USA).

8.4. Results

During the period, 28 patients with a history of NPC who had undergone ERT were recruited. Two patients could not complete the procedure because of contact bleeding and difficulty in examining the pharynx because of choanal stenosis secondary to ERT. They were excluded from the study. Among the 26 subjects, the male to female ratio was 18:8. The mean age and standard deviation was 46.1±11.4 years. The median time interval after completion of ERT was five years. The gross appearance of the nasopharynx was normal in all of the patients without suspicion of recurrence of malignancy.

Twenty-four and 21 images (x150) stained with 1% and 0.5% methylene blue, respectively, were successfully produced and analyzed by the assessors. All the images showed the cellular pattern of squamous metaplasia without recurrence and this was recognized by all assessors. The cells were homogenous with round, darkly stained nuclei and light blue cytoplasm. The nuclear: cytoplasmic ratio was low (Figure 8-1).

The intraclass correlation coefficients were calculated to examine the intra-observer and inter-observer reliabilities of the assessors. The intraclass correlation coefficients for the intra-observer reliabilities of assessors were 0.916

to 0.957 and 0.839 to 0.964 in the groups of 0.5% and 1% stains, respectively (Table 8-1). The intraclass correlation coefficients for the inter-observer reliability of assessors were 0.884 and 0.885 in the groups of 0.5% and 1% stains, respectively (Table 8-2).

The independent-samples *t*-test showed that the mean scores of clarity of the cellular details were statistically higher in the group of the 1% stain than that of the 0.5% stain with respect to each individual assessor and all assessors (Table 8-3).

8.5. Discussion

Chromoscopy is a term which denotes the use of stains or chromogens to enhance the accuracy of endoscopic examination. The classical example is the use of Lugol's solution, an iodine-based adsorptive stain with an affinity for the glycogen in non-keratinized squamous epithelium, in oesophagoscopy to detect early oesophageal cancer and to evaluate Barrett's oesophagus (Shiozaki 1990).

To date, chromoscopy has been applied mostly in gastrointestinal endoscopy to enhance diagnosis, detect diseased lesions, guide endoscopic biopsies, and obtain better visual diagnoses of small abnormalities in the gastrointestinal tract (Shiozaki 1990, Canto 1996). The classification of stains used for endoscopic purposes differs from the dyes used in histochemistry. Three classes of stains have been commonly utilized in endoscopy: 1. contrast stains, e.g., India ink, which enter mucosal depressions and crevices to highlight the tissue topography; 2. reactive stains, e.g., Congo red, which identify cellular products by a colour

change; and 3. vital stains, e.g., methylene blue, which identify specific epithelial cells or cellular constituents by preferential colouring.

Contact endoscopy is an excellent example of chromoscopy to visualize the morphology of epithelial cells using a vital stain. Methylene blue is an absorptive or vital stain which selectively adsorbs in the squamous epithelium and metaplastic tissue of the organs. Since the advent of contact endoscopy, it has been widely used to enhance the visualization of the squamous epithelium of the cervix and larynx (Hamou 1980b, Andrea 1995b). In contact endoscopy, the stain differentially enters the cytoplasm of the adsorptive epithelium or metaplastic tissues and outlines the cellular details of the individual cells against the illuminated background. Traditionally, only 1% methylene blue was used in contact endoscopy. The value of the stain at different concentrations was unknown.

In this study, we confirmed that 1% methylene blue gives images of clearer cellular detail than 0.5% methylene blue in contact rhinoscopy. It is postulated that the more dilute 0.5% methylene blue provides an inadequate amount of stain to enter the cytoplasm of the examined epithelium or metaplastic tissues and therefore provides relatively poorer visibility of the cells. We advocate that 1% methylene blue should be used to visualize the metaplastic cells of the irradiated nasopharynx.

Although we have found that methylene blue is a good companion to contact rhinoscopy, the use of other vital stains including Lugol's iodine and toluidine blue have not been studied. Lugol's staining has been used to evaluate the

squamo-columnar junction of the upper gastrointestinal tract for detection of early cancer of the oesophagus, while toluidine blue differentially stains the nuclear material of malignant epithelial cells and has been used to stain oropharyngeal and oesophageal neoplastic lesions (Misumi 1990, Seitz 1990). The roles of these vital stains in contact endoscopy have to be elucidated by further studies.

In this study, we found that there were excellent inter-observer and intra-observer reliabilities in the assessment of contact endoscopic images among assessors of different backgrounds. The reason for the good agreement of assessments may be related to the morphology of the irradiated tissues. The histology of nasopharyngeal mucosa after irradiation is characteristic. Following irradiation, the ciliated respiratory epithelium undergoes squamous metaplasia. The cellular changes following irradiation include enlargement of the cells and nucleus with coarse chromatin. The nuclear: cytoplasmic ratio remains low (Chang 1999). The selective staining property of methylene blue makes the irradiated cells more recognizable in the illuminated background under contact endoscopy. This may explain why a junior clinician can identify the properly stained metaplastic cells as reliably as an experienced pathologist in contact endoscopy.

8.6. Conclusion

It is concluded that 1% methylene blue provides a better clarity of cellular detail in contact rhinoscopy and thus enhances the histological diagnosis of an irradiated nasopharynx compared with its 0.5% counterpart.

Figure 8-1A. Contact endoscopy using 1% methylene blue.

High power view (x150) of contact endoscopy using 1% methylene blue reveals pattern of squamous metaplasia. The cells are homogenous with round, darkly stained nuclei and blue cytoplasm. The nuclear: cytoplasmic ratio is low.

Figure 8-1B. Contact endoscopy using 0.5% methylene blue.

High power view (x150) of contact endoscopy using 0.5% methylene blue. The cells of the squamous metaplasia are homogenous with round, darkly stained nuclei and light blue cytoplasm.

Table 8-1. Intra-observer reliabilities of assessors.

	0.5% stain		1% stain	
	Intraclass correlation coefficient	**95% CI**	**Intraclass correlation coefficient**	**95% CI**
Consultant Pathologist	0.916	0.793-0.966	0.944	0.872-0.976
Junior Trainee in Radiology	0.928	0.823-0.971	0.839	0.629-0.930
Consultant Otorhinolaryngologist	0.957	0.894-0.983	0.964	0.917-0.984

CI = confidence interval

Table 8-2. Inter-observer reliabilities of assessors.

	Intraclass correlation coefficient	95% confidence interval
0.5% stain	0.884	0.759-0.949
1% stain	0.885	0.773-0.946

Table 8-3. The mean scores of visual clarity of the cellular details between two stains.

	0.5% stain	1% stain	
	Mean ± S.D.	**Mean ± S.D.**	***p* value**
Consultant Pathologist	4.871 ± 2.405	6.583 ± 1.656	0.007
Junior Trainee in Radiology	3.933 ± 1.267	5.954 ± 1.049	<0.001
Consultant Otorhinolaryngologist	3.695 ± 2.370	6.729 ± 1.895	<0.001
All assessors	4.167 ± 2.111	6.422 ± 1.588	<0.001

SD = standard deviation

CHAPTER 9

RELIABILITY OF CONTACT RHINOSCOPY IN THE DIAGNOSIS OF PRIMARY AND RECURRENT NASOPHARYNGEAL CARCINOMA

9.1. Abstract

Background

Contact rhinoscopy can effectively identify premalignant mucosa and primary and recurrent nasopharyngeal carcinoma (NPC). How the clinical experience and histopathological knowledge of the endoscopist affect the diagnostic reliability of contact endoscopy is unknown.

Objective

We evaluate the reliability of the findings of contact rhinoscopy in patients with NPC diagnosed by different assessors.

Methods

Random images from contact rhinoscopy of 157 subjects (56 non-irradiated patients and 101 irradiated patients) were available for analysis. Five independent observers of varying clinical experience and histopathological knowledge viewed the images and made a diagnosis for each one. The diagnosis of each image was correlated with the histology of the biopsy from the corresponding patient.

Results

In the non-irradiated group, the overall Kappa values for inter-observer and intra-observer reliability in the differentiation of normal epithelium from primary NPC by five assessors were 0.856 (95%CI = 0.753-0.958) and 0.736 (95%CI = 0.590-0.882), respectively. There were no significant differences in the inter-observer and intra-observer reliabilities between the assessors. In the

irradiated group, the overall Kappa values for inter-observer and intra-observer reliability in the diagnosis of recurrent carcinoma, atypia, squamous metaplasia, and radiation changes by five assessors were 0.681 (95%CI = 0.594-0.768) and 0.687 (95%CI = 0.601-0.773), respectively. There were no significant differences in the inter-observer and intra-observer reliabilities between the assessors.

Conclusions

These results show that the clinical diagnosis of NPC by contact rhinoscopy is highly reliable and is not dependent on the clinical experience or knowledge of histopathology of the observer.

9.2. Introduction

Radiation therapy (RT) is the mainstay of treatment for primary NPC, achieving local control rates of 80% to 90% at five years (Lee 1993b). However, residual or recurrent NPC tends to grow submucosally, making the diagnostic yield of a biopsy indicated by clinical surveillance using conventional endoscopy of an irradiated nasopharynx less than 50% (Sham 1992). A more reliable clinical and diagnostic tool is needed to detect subtle primary and residual or recurrent disease.

Since 1999, we have performed a series of qualitative and quantitative studies to evaluate the value of contact rhinoscopy in the diagnosis of primary and residual or recurrent NPC using a contact rhinoscope. We have previously shown

that contact rhinoscopy can be used to effectively identify premalignant mucosa and primary and recurrent NPC (Pak 2001, Pak 2002b, Pak 2005).

The diagnostic accuracy of contact endoscopy depends on the endoscopist's ability to identify the cellular pattern of the tissue examined. There is some concern that the clinical experience and histopathological knowledge of the endoscopist may affect the diagnostic reliability of contact endoscopy. As there is currently no published data on the inter- and intra-observer reliability of contact endoscopy, this study was undertaken to address this point.

9.3. Patients and Methods

The medical records and video recording of all patients who underwent contact rhinoscopy of the nasopharynx in an out-patient clinic at the Prince of Wales Hospital in Hong Kong from January 1999 to December 2000 were retrieved.

The use of contact rhinoscopy was approved by the Joint Human Review Ethics Committee of the Chinese University of Hong Kong and the Hong Kong Hospital Authority. During the study period, the patients were recruited from an out-patient clinic of the Prince of Wales Hospital. Informed consent was obtained from all patients. Patients who declined to give informed consent or did not want tissue biopsy to be performed were excluded from the study.

During the study period, two groups of patients were recruited. The non-irradiated (non-RT) group consisted of patients with untreated NPC and patients with a normal nasopharynx but a positive family history of NPC, elevated IgA antibodies against antigens of Epstein-Barr virus, or other ear, nose, and throat diseases. The post-irradiated (post-RT) group consisted of consecutive patients who had undergone radiation therapy for primary NPC.

Contact rhinoscopy and biopsy of the nasopharynx were all performed by a single endoscopist with a contact endoscope (Karl Storz, 7215AA, 0°, 23 cm in length, 4 mm in diameter, Tuttlingen, Germany) as an out-patient procedure. A standardised technique was used in each patient. The nasal cavities and nasopharynx were anaesthetised with a maximum of 1ml of a 5% cocaine topical anaesthetic spray. After 10 minutes, the nasal cavity and nasopharynx were cleaned of secretions using a nasal suction catheter. A small nasal cotton-ball applicator was then used to stain the nasopharyngeal mucosa with 1% methylene blue via the nasal cavity. Excess methylene blue was gently dabbed or wiped off the mucosa with a clean cotton-ball applicator. A contact endoscope was connected to a 150W xenon light source, a video camera, and an S-VHS video recorder. The endoscope was slowly advanced through the nasal cavity into the nasopharynx, which was inspected for any abnormality. The tip of the contact endoscope was then advanced slowly until it made gentle contact with the mucosa of the posterior wall of the nasopharynx, and the mucosa was examined at magnifications of x60 and x150 by adjusting the zoom switch on the endoscope. Once contact rhinoscopy was completed, an endoscopically guided biopsy of the mucosa was taken of the exact site of contact rhinoscopy. A video recording was

made of the entire endoscopic procedure. The biopsy was sent to a pathologist for histological examination.

The most representative video frame from the contact rhinoscopy of each patient was captured and printed as a colour print. An independent pathologist reviewed the histology of all biopsies. The contact rhinoscopy colour prints were categorised according to the histology of the nasopharyngeal biopsy. There were five histological categories: normal epithelium, NPC (from both untreated and treated patients), atypical epithelium, squamous metaplasia, and post-irradiation epithelial changes. If any histological category had more than 10 patients, then 10 patients from that particular histological category were randomly selected. Randomisation was always done using a computer to generate the randomised selection. In this way, colour prints of contact rhinoscopy of 20 patients from the non-RT group and 34 patients from the post-RT group were randomly selected.

Five independent observers with a wide range of clinical experience and knowledge of histopathology, a consultant otorhinolaryngologist, a trainee in otorhinolaryngology, a surgical house-officer, a consultant radiologist, and a consultant pathologist, assessed the colour prints. None of them had any prior knowledge of the patients' diagnoses.

The observers were briefed on the histological characteristics of normal and irradiated nasopharyngeal mucosa and NPC prior to their assessment of the photographs. Specifically, the following criteria were emphasized: the size and

shape of the cells, the regularity of the cells, pleomorphism of the cytoplasm and nucleus, the nuclear: cytoplasmic ratio, and the spatial distribution of the cells.

Thirty minutes after the briefing presentation, the colour photos were presented to the observers in a random order and they were asked to make a histological diagnosis based on their interpretation of the endoscopic pictures. Two weeks later, the assessment was repeated with the same assessors but with the order of the pictures randomly changed.

The diagnosis of each endoscopic picture made by each assessor was correlated with the actual histology. The scores of the correct diagnoses of each assessor were compared. Kappa (*k*) statistics were used to assess the inter-observer and intra-observer reliabilities. The agreement or reliability of the assessment was graded as follows: poor, k 0.20; fair, $0.21 \leq k \leq 0.40$; moderate, $0.41 \leqq k \leqq 0.60$; substantial, $0.61 \leqq k \leqq 0.80$; good, $k > 0.80$, and perfect, $k = 1$ (Landis 1977).

SPSS for Windows (Version 14.0, Chicago, IL, USA) was used to produce descriptive statistics for continuous and discrete variables. An independent *t*-test identified the significant differences between two groups of continuous variables. Kappa statistics were calculated to assess inter- and intra-observer reliabilities. A p value of < 0.05 was used to indicate statistical significance. All statistical tests were two sided.

9.4. Results

During the period, 162 consecutive patients consented to undergo contact rhinoscopy and biopsy of the nasopharynx under topical anaesthesia in the outpatient clinic. Of the 162 patients, contact rhinoscopy was feasible in 157 (96.91%) patients, but failed in five patients.

Of the 157 patients studied, 37 were women and 120 were men. Their mean age was 48.7 years (S.D. 12.093; range 21-80 years). They consisted of two groups of patients: 56 patients without radiotherapy (non-RT group) and 101 patients after radiotherapy (post-RT group).

In the non-RT group, 20 of the 56 patients were randomly selected for assessment. There were two histological diagnoses in this group: normal nasopharyngeal epithelium (Figure 9-1) and NPC (Figure 9-2).

The proportion of correct diagnoses of primary carcinoma made by the consultant otorhinolaryngologist, trainee in otorhinolaryngology, house officer, radiologist, and pathologist was 95%, 95%, 95%, 85%, and 90%, respectively, and there were no significant differences in the proportions between them (Chi-square test, $p = 0.704$).

Inter-observer reliability analyses were performed to correlate the diagnosis made by the assessor with the histology of the corresponding biopsy. Kappa values to differentiate normal epithelium from primary NPC for five observers are

shown in Table 9-1. The overall Kappa value for the inter-observer reliability between the five assessors was 0.856 (95%CI = 0.753-0.958) and no significant difference was detected between them (Chi-square test, $p = 0.851$).

Intra-observer reliability analyses were performed to compare the assessment of the contact rhinoscopic findings of each assessor at time zero and after two weeks. The overall Kappa value for the intra-observer reliability between assessors was 0.736 (95%CI = 0.590-0.882) (Table 9-2). There was no significant difference in intra-observer reliability between them (Chi-square test, $p = 0.966$).

In the post-RT group, 34 of the 101 patients were randomly selected. There were four histological diagnoses in this group: NPC, atypical epithelium (Figure 9-3), squamous metaplasia (Figure 9-4), and post-radiation epithelium / granulation tissue (Figure 9-5).

The proportion of correct diagnoses of carcinoma and atypia made by the consultant otorhinolaryngologist, trainee in otorhinolaryngology, house officer, radiologist, and pathologist was 88%, 88%, 88%, 82%, and 85%, respectively, and there were no significant differences in the proportions between them (Chi-square test, $p = 0.938$).

In this group, the Kappa values for the inter-observer reliability of five assessors in diagnosing NPC, atypical epithelium, squamous metaplasia, and post-radiation changes are shown in Table 9-1. The overall Kappa value of the inter-observer reliability between the five assessors was 0.681 (95%CI =

0.594-0.768). There was no significant difference in the inter-observer reliability between them (Chi-square test, $p = 0.851$).

The overall Kappa value for the intra-observer reliability between the five assessors was 0.687 (95%CI = 0.601-0.773) (Table 9-2). There were no significant differences between them (Chi-square test, $p = 0.365$).

9.5. Discussion

The appearance of nasopharyngeal mucosa on contact rhinoscopy can be reliably assessed for different pathologies by clinicians with a wide range of clinical experience and histopathological knowledge. The ability to differentiate normal from malignant epithelium in untreated patients ranged from 85% to 95%, while the ability to diagnose recurrent or residual cancer or atypical epithelium in treated patients ranged from 82% to 88%.

Although there are considerable variations in the appearance of primary NPC, most tumours are easily identifiable by an experienced endoscopist using conventional nasal endoscopy. However, due to the wide variation in the configuration of the fossa of Rosenmuller, the site from where most tumours arise, and the rare occurrence of a submucosal tumour, an early or submucosal tumour may not easily be identified with conventional endoscopy (Woo 1999). On the other hand, benign lymphoid hyperplasia of the nasopharynx may sometimes be mistaken for a tumour. In the non-RT group of this study, all assessors accurately and reliably differentiated carcinoma from normal nasopharyngeal epithelium on

contact rhinoscopy irrespective of their experience or knowledge of histopathology. Contact rhinoscopy of the nasopharynx may therefore complement conventional endoscopy and provided a diagnosis when conventional endoscopy is inconclusive.

In contrast to untreated primary NPC, post-irradiation changes in the epithelium and excessive crust formation on the mucosa of the irradiated nasopharynx make the recognition of residual tumour or an early recurrence difficult. Residual carcinoma in an irradiated nasopharynx at 8-10 weeks after radiation therapy is an indicator of local failure. The timely detection of early persistent disease after radiation therapy is crucial to achieve local control of the disease (Teo 1996b). However, the profound difficulty in identifying early disease by direct visualization leads to a positive pick-up rate of persistent and recurrent lesions by conventional endoscopy of less than 50% (Sham 1992). In circumstances where conventional endoscopy fails to identify the lesion, contact rhinoscopy can help to target the biopsy in areas containing suspicious cells. However, to improve the diagnostic yield of a biopsy of the nasopharynx indicated by contact rhinoscopy, a high degree of repeatability and reproducibility of the contact rhinoscopic findings is desirable.

In a previous study of ours, we showed that contact rhinoscopy was highly reliable for the diagnosis of squamous metaplasia in an irradiated nasopharynx (Pak 2008). We hypothesized that the highly selective affinity of the cytoplasm of squamous metaplastic cells for methylene blue makes them more recognizable in the illuminated background of immature cells of irradiated tissue on contact

rhinoscopy. In this study, we took a step forward and found that, apart from squamous metaplasia, contact rhinoscopy is able to demonstrate different types of cells in an irradiated nasopharynx which allowed assessors from different backgrounds to accurately make a diagnosis. The evidence suggests that contact rhinoscopy can be used reliably to diagnose early persistent or recurrent disease in an irradiated nasopharynx, which may be suitable for salvage treatment.

Although there is a learning curve for an inexperienced endoscopist to identify the cellular morphology and various conditions of the mucosa of the nasopharynx with real-time contact rhinoscopy, we showed that after a brief period of training, clinicians from diverse clinical backgrounds are able to reliably interpret the findings of contact rhinoscopy.

9.6. Conclusions

We have shown in this study that using contact rhinoscopy, primary and recurrent NPC can be accurately and reliably assessed by clinicians with a wide range of clinical experience and histopathological knowledge. Contact rhinoscopy is a useful diagnostic tool with a high degree of reproducibility and repeatability for NPC patients.

Figure 9-1.Contact endoscopy. Normal pseudostratified ciliated epithelium.

Contact endoscopic high-power (x150) view of stained cilia of healthy respiratory epithelium.

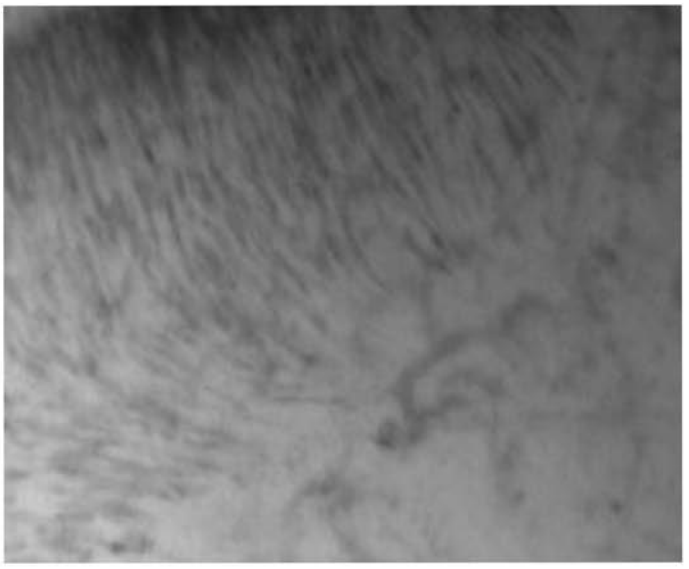


Figure 9-2. Contact endoscopy. Nasopharyngeal carcinoma.

Primary or recurrent nasopharyngeal carcinoma. High-power view (x150) showing a large syncytial sheet of malignant cells with enlarged, pleomorphic and darkly stained nuclei. Distinct prominent nucleoli can also be seen.

Figure 9-3. Contact endoscopy. Atypia after radiation.

High-power view (x150) showing scattered atypical cells with enlarged pleomorphic nuclei. The atypical cells represent either immature regenerating cells or genuine carcinoma cells in the irradiated tissues.

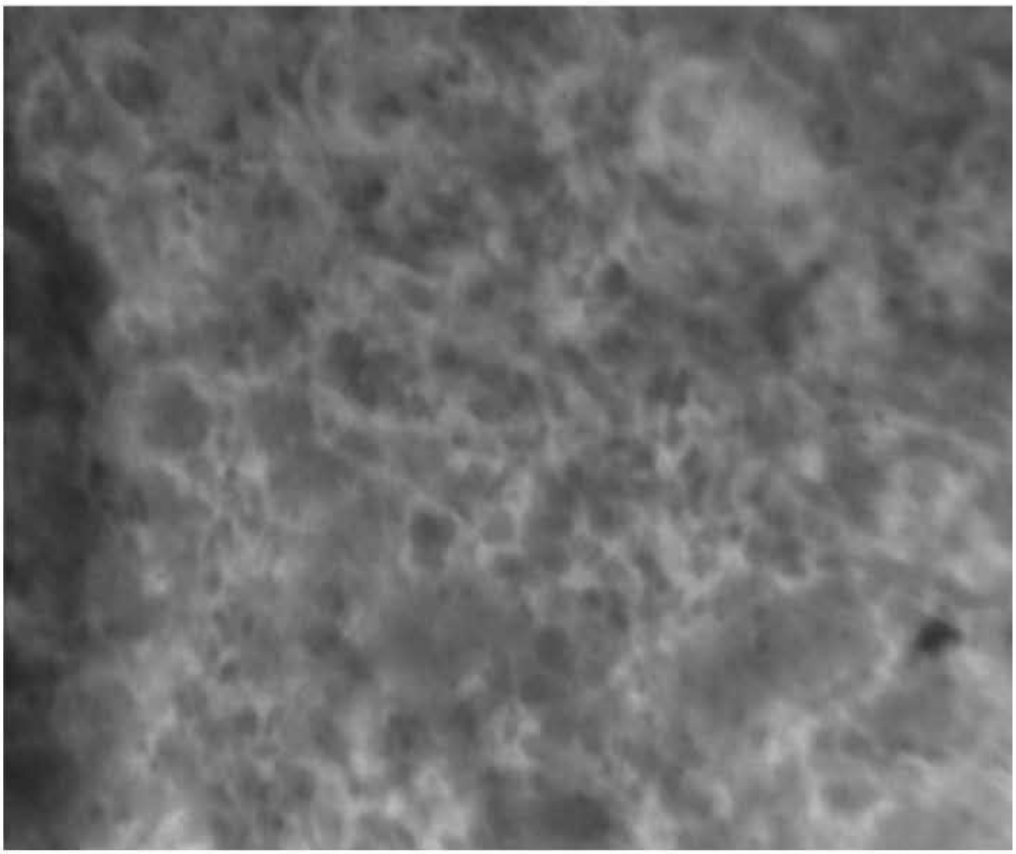


Figure 9-4. Contact endoscopy. Squamous metaplasia.

The high power view (x150) shows polyhedral squamous cells which are homogenous with round, darkly stained nuclei and light blue cytoplasm. The nuclear-to-cytoplasm ratio is low.

Figure 9-5. Contact endoscopy. Radiation changes in irradiated epithelium. The high power view (x150) demonstrates scanty poorly-stained immature cells in a blanket of acellular exudate.

Table 9-1: Inter-rater agreement (kappa) to interpret different mucosal patterns in contact rhinoscopy among five assessors.

	Consultant Otorhinolaryngologist	Trainee in Otorhinolaryngology	House Officer	Radiologist	Pathologist	Overall Kappa value	*p* value (Chi Square)
Non-RT group	0.886 (CI: 0.669-1.103)	0.894 (CI:0.690-1.097)	0.894 (CI:0.690-1.097)	0.721 (CI:0.430-1.012)	0.794 (CI:0.523-1.065)	0.856 (CI:0.753-0.958)	0.851
Post-RT group	0.719 (CI:0.534-0.905)	0.716 (CI:0.528-0.903)	0.642 (CI:0.441-0.842)	0.583 (CI:0.366-0.800)	0.717 (CI:0.531-0.904)	0.681 (CI:0.594-0.768)	0.851

CI: 95% Confidence Interval

Table 9-2: Intra-rater agreement (kappa) to interpret different mucosal patterns in contact rhinoscopy among five assessors.

	Consultant Otorhinolaryngologist	Trainee in Otorhinolaryngology	House Officer	Radiologist	Pathologist	Overall Kappa value	*p* value (Chi Square)
Non-RT group	1.000 (CI:1.000-1.000)	0.792 (CI:0.518-1.066)	0.694 (CI:0.375-1.013)	0.735 (CI:0.458-1.011)	0.704 (CI:0.396-1.013)	0.736 (CI:0.590-0.882)	0.966
Post-RT group	0.800 (CI:0.637-0.962)	0.596 (CI:0.386-0.806)	0.680 (CI:0.486-0.874)	0.542 (CI:0.319-0.764)	0.719 (CI:0.533-0.904)	0.687 (CI:0.601-0.773)	0.365

CI: 95% Confidence Interval

CHAPTER 10

SUMMARY

Contact endoscopy was introduced as early as the 1970s. The contact rhinoscope, specifically designed for examination of the nose and nasopharynx, was introduced in 1997. Contact rhinoscopy not only allows examination of the gross appearance of the nasopharynx, but also enables the visualisation of the epithelial cells of the nasopharynx in real time and *in vivo* when it is placed in contact with the vitally stained surface of the nasopharynx.

The first study showed that contact rhinoscopy is acceptable to most patients as an office-based procedure under topical anaesthesia. The procedure can be administered safely to the patients in this setting. The pain perceived by patients is minimal and not significantly different to that occurring during conventional nasal endoscopy.

NPC is common in southern China, including Hong Kong. Delay in the diagnosis of primary NPC is not uncommon. Early diagnosis is critical to improve the overall survival of the patients. Early examination by nasal endoscopy is essential in patients with a suspicious clinical presentation, and the sensitivity of conventional nasal endoscopy for the diagnosis of primary NPC is as high as 95%. Other investigations, including Epstein Barr virus serology, EBV DNA assay, computed tomography or magnetic resonance imaging of the nasopharynx and ultrasound of the neck may help to diagnose NPC. However, early or submucosal disease may escape the direct surveillance of conventional nasal endoscopy. In the second study, we found that contact rhinoscopy can be used to accurately identify different mucosal patterns of primary NPC and contribute to its diagnosis. The sensitivity and specificity of contact rhinoscopy for the diagnosis of primary

NPC were 100% and 95.7%, respectively, which is similar to those of conventional endoscopy.

The mainstay of treatment of NPC is radiotherapy, with a five-year local control rate of 70% to 80%. Early diagnosis of locally persistent and recurrent disease after radiotherapy is associated with improved survival. Nevertheless, conventional nasal endoscopy is not sensitive enough to allow detection of early relapses. The shortcomings and false negative findings of other investigations urge the need for a better diagnostic tool. In the third study, we showed that contact rhinoscopy facilitates an accurate real-time identification of different mucosal patterns in the irradiated nasopharynx. It can be used to detect recurrent disease even in a normal-looking nasopharynx. It has a higher sensitivity and specificity for the detection of persistent and recurrent disease than conventional endoscopy.

In the fourth study, we compared 1% and 0.5% methylene blue (methylthionine chloride) and found that the 1% stain provides better clarity of the cellular detail under contact rhinoscopy and thus enhances the histological diagnosis of an irradiated nasopharynx. It should be the chromogen of choice for contact rhinoscopy.

Our final study showed that the accuracy in the diagnosis of malignancy and different mucosal patterns of the nasopharynx using contact rhinoscopy is highly reproducible by different assessors irrespective of their clinical experience and knowledge of histopathology.

This series of qualitative and quantitative studies have broadened our understanding of the applicability of contact rhinoscopy in the management of NPC. The results of these studies have suggested that contact rhinoscopy is a safe, well-tolerated, accurate, and reliable office-based procedure for real-time diagnosis of primary and recurrent NPC. It is advocated that contact rhinoscopy should be incorporated in the management of NPC patients before and after radiotherapy. It can be used as an adjunct to conventional nasal endoscopy to locate suspicious areas in the nasopharynx and hence direct the biopsy.

Nevertheless, there are some concerns regarding the routine use of contact rhinoscopy in the management of NPC. Firstly, the duration of the procedure is longer than that of conventional nasal endoscopy. Secondly, contact bleeding from vascular tumours makes the real-time recognition of the mucosal patterns of nasopharyngeal tumours difficult. Thirdly, the lack of flexibility of the rigid instrument precludes its use in hidden areas of the nasal cavity and nasopharynx. These shortcomings can be solved by better training of the endoscopists and further improvement of the design of the tool.

It has to be emphasized that contact endoscopy cannot replace the role of histological study of tissue biopsies. The principle of contact endoscopy is different from that of histology in that the former merely illustrates the tangential morphology of the epithelial cells, whereas the latter also demonstrates the cross-sectional characteristics of the cells. A histological study not only allows for the identification of tissue invasion by the tumour but also makes

immunohistochemical staining possible to determine the nature and origin of the cells, which is not possible with contact endoscopy. For this reason, contact rhinoscopy cannot replace histology, which remains the gold standard in the diagnosis of NPC.

CHAPTER 11

FUTURE DIRECTIONS

11.1. Future studies

The present studies have concluded that contact rhinoscopy is a safe, well-tolerated, accurate, and reliable office-based procedure for the real-time diagnosis of primary and recurrent nasopharyngeal carcinoma (NPC). Nevertheless, there are a few issues that deserve further attention:

1. The present studies have suggested that contact rhinoscopy can be used as an adjunct to conventional nasal endoscopy to direct the biopsy at suspicious areas in the nasopharynx and minimize unnecessary random biopsies. However, this benefit remains to be proven. Future studies should focus on the advantages of contact rhinoscopy in the diagnosis of early primary and recurrent NPC over the conventional method of multiple random nasopharyngeal biopsies in populations with a high risk of developing NPC.

2. As previously illustrated (Chapter 2), among those patients who had raised IgA anti-VCA titres without evidence of a malignant nasopharyngeal tumour, 2% subsequently developed NPC within follow-up of a median of 54 months (Lo 2004). To those patients with persistently elevated EBV serology, it is usual practice to perform multiple random biopsies of the nasopharynx under general anaesthesia. By comparing the results of biopsies which were guided by contact rhinoscopy with those of random biopsies in this group of patients, we may be able to assess the value of contact rhinoscopy in screening for NPC among high risk patients.

To take a step forward, we can also assess the role of contact rhinoscopy as a universal screening tool for NPC in the general population.

3. The diagnostic value of contact rhinoscopy for primary and recurrent NPC in comparison with other diagnostic tools, including MRI and ^{18}F-FDG PET, was unknown. It would be worthwhile to perform a randomized study to compare the sensitivity and specificity of contact rhinoscopy with those of MRI in patients with primary or recurrent NPC.

4. Although it is generally believed that vital stains other than methylene blue cannot make the superficial cells visible under contact endoscopy (Andrea 1999), no study has yet been published to justify this statement. Further randomized studies should be performed to compare the value of different vital stains in contact endoscopy.

11.2. Contact rhinoscopy in the future

To further improve the acceptance and accuracy rates of contact rhinoscopy, I would advocate and anticipate the following innovative developments:

1. Development of a slimmer rigid contact rhinoscope with different angulations.

Further developments in the manufacturing technology of mini-lenses could make a slimmer contact rhinoscope possible. This would not only improve the patients' acceptance of the procedure but also maximize the area of the nasopharynx which could be viewed.

2. Development of a flexible contact rhinoscope.

The incorporation of a rigid zoom lens system with a flexible optic endoscope may make a flexible contact rhinoscope possible, which would allow easy and comfortable access to the nasopharynx.

3. Development of an automated device to analyze images from contact rhinoscopy.

A device has been introduced to provide automatic analysis of Papanicolaou (Pap) smears for the diagnosis of cervical carcinoma (Koss 1994). A similar device and software could be developed to produce a real-time diagnosis of NPC from the video output of the contact rhinoscope. This would eliminate the error of subjective interpretation of assessors and improve the reliability of the device.

4. Incorporation of narrow band imaging (NBI) with contact rhinoscopy.

As illustrated in the previous and present studies, conventional white light nasal endoscopy is not sensitive in the detection of early and minor malignant lesions in the nasopharynx, especially after radiotherapy.

Recently, a narrow band imaging upper gastrointestinal endoscopy has been introduced to identify early malignant disease in the upper gastrointestinal tract (GIT). Growing evidence has disclosed several characteristic mucosal capillary and pit patterns in premalignant lesions of the upper GIT (Liu 2003, Huang 2004, Kudo 2005, East 2008). Upper GI endoscopes with enhanced resolution and

visualization of these surface microstructures, enabling early diagnosis and eradication of premalignant diseases, are therefore eagerly awaited.

Conventional white light endoscopic imaging is generated by the frame sequential image pickup method. This involves a light source consisting of a xenon lamp and rotation disk with three optic filters. The rotation disk and monochrome charge-coupled device are synchronized and sequentially generate images in three colours (red, blue, and green; RBG). By using all three band images, a single colour endoscopic image is synthesized by the video processor.

The narrow band imaging (NBI) system is a novel technology based on narrowing of the bandwidth of the spectral transmittance of the optical filters used in the frame sequential image method for creating video-endoscopy images. The central wavelengths of the trichromatic optical filters are 500 nm, 445 nm, and 415 nm, and the bandwidth of each wavelength is 30 nm. By using these narrow band spectra, surface microstructures, capillary and pit patterns of the GIT mucosa, and other structures at different depths can be selectively visualized with enhanced contrast. To date, NBI has been applied in the evaluation of Barrett's oesophagus, early gastric carcinoma, colonic polyps, and even various head and neck tumours (Nakayoshi 2004, East 2008, Sikka 2008, Singh 2008, Watanabe 2008).

It is hypothesized that by incorporating narrow band technology with the contact rhinoscope, early malignant lesions in the nasopharynx can be picked up more easily.

5. Incorporation of the autofluorescence technique with contact rhinoscopy.

Tissue-specific endogenous autofluorescence and exogenous fluorophores have been found in different types of malignant cells (Haringsma 1999). Autofluorescence and light-induced fluorescence in dysplastic and malignant cells of various cancers can be detected by autofluorescence endoscopy (Takehana 1999). It has been found that autofluorescence endoscopy is also effective in the differentiation of NPC from normal mucosa (Qu 2000). Although the clinical application of fluorescence detection in dysplasia and early cancer is still in its infancy, more and more studies have indicated that fluorescence imaging can provide an accurate, real-time, non-invasive diagnosis of early cancers of the oesophagus, stomach, colon, lung, and head and neck regions (Malzahn 2002, Mayinger 2003, Niepsuj 2003, Mayinger 2004, Kara 2005). Theoretically, incorporating a spectrometer with a contact rhinoscope may increase the diagnostic accuracy of the latter.

With the advance in technology of optical and electronic devices, I believe that contact rhinoscopy can become an accurate and popular diagnostic device for all nasopharyngeal pathologies in the near future.

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