SYSTEM AND METHOD FOR SCREENING OF NASOPHARYNGEAL CARCINOMA

TECHNICAL FIELD

In one of its aspects, the present invention relates to a system for screening nasopharyngeal carcinoma (NPC). In another of its aspects, the present invention relates to a method for screening for NPC.

BACKGROUND ART

Nasopharyngeal carcinoma is often referred to as NPC and these terms will be used interchangeably throughout this specification. NPC originates in an area of the head deep behind both nasal cavities and above the soft palate, in an area known as the nasopharynx, a portion of the pharynx which lies posterior to the nose and is bounded superiorly by the skull base and sphenoid and laterally by the paired tori of the Eustachian tubes and the Rosenmuller's fossae. Anteriorly the posterior choanae form the limit of the space and inferiorly an artificial line drawn at the level of the hard palate delimits the nasopharynx from the oropharynx.

NPC is rare among North American and European Caucasians with age adjusted incidence rates of less than 1 per 100,000. In contrast, the incidence of NPC in Southern China is in the order of 60 to 80 per 100,000. People who have moved out of the endemic regions maintain their high-risk status and their successive generations also maintain a relatively high-risk status. With an increasing number of Asians immigrating to the United States and Canada, NPC has become an important social and medical issue.

Conventionally, NPC has been detected either by visual examination of the nasopharynx or when the patient becomes symptomatic with satellite lesions that are clinically visible. Diagnosis of the lesion is then confirmed by performing a random, mapped or targeted punch biopsy in the nasopharynx after anaesthetizing the patient with a local or general anesthetic. The biopsy samples are then submitted for histologic analysis for the confirmation of diagnosis.

NPC originates in an area that is not routinely examined on general physical examination, due to its difficult access and visualization without proper training and/or the availability of special medical instruments such as an endoscope. Thus, NPC is often left undetected for a long time without any signs and symptoms until the mass effects of the tumor are apparent. Spread to regional lymph nodes is therefore common and occurs in most patients with NPC. Patients often present with nasal stuffiness, discharge, nose bleeds, ear pain or decreasing hearing. Cranial nerve involvement is also common and may cause facial muscle signs, swallowing difficulties, facial pain, and aberrant sense of taste. NPC patients with advanced disease have very poor prognosis and survival.

Not surprisingly, patients with early disease have excellent prognosis and the potential of cure if early treatment is initiated. Conversely, the prognosis is poor for patients who have an advanced stage of NPC. See: (1) "RETROSPECTIVE ANALYSIS OF 5037 PATIENTS WITH NASOPHARYNGEAL CARCINOMA TREATED DURING 1976-1985: OVERALL SURVIVAL AND PATTERNS OF FAILURE", Lee et al., *Int. J. Radiation Oncology Biol. Phys.*, Vol. 23, Number 2, 1992, pp. 261-270; and (2) "CARCINOMA OF THE NASOPHARYNX: FACTORS AFFECTING PROGNOSIS", Perez et al., *Int. J. Radiation Oncology Biol. Phys.*, Vol. 23, Number 2, 1992, pp. 271-280.

Epstein Barr virus ("EBV") is a human DNA tumor virus with an extraordinary diverse oncogenic potential. The association of EBV with NPC was first suggested based on serological evidence. It is well established utilizing Polymerase Chain Reaction ("PCR") technique that EBV encoded deoxyribonucleic acid is present in virtually every biopsied NPC tumor and precancerous epithelial cells, irrespective of histological differentiation. There is widespread acceptance that EBV DNA has not been detected in healthy tissues.

EBV is ubiquitous, being found in every population in which it is sought. EBV is carried by 90% of the world adult population. It is exclusively harbored in a small subset of B-lymphocytes and is excreted in saliva and in the urogenital tract. Considerable concentrations of infectious virus particles are released at

random intervals several times a month. EBV has also been isolated from the cervix and circulates through the blood contained in B-lymphocytes. EBV infections are much more common than the cancers that they produce. Infection is usually asymptomatic and malignancy is a relatively rare outcome.

The strength of immune response to EBV has been studied for use as a possible screening tool to predict NPC. The first serological studies demonstrated higher antibody titers and stronger precipitation bands in cases than controls suggesting an etiologic role in the disease. In most Nasopharyngeal carcinoma patients, an elevated serum IgA titer anti-EBV-VCA has frequently been observed, and the degree of elevation is somewhat parallel to the size of NPC tumor mass. Although most Naeopharyngeal tumors will have a positive IgA titer, the majority of positive titers do not reflect a positive tumor. This screening method suffers from unsatisfactory sensitivity or specificity at different cutoff points. The specificity of this test is questionable as only a few positive test results out of many positive test results will indicate NPC [specificity = (True Negatives)/(True Negative + False Negatives)]. The specificity of a test screen is an indicator of the ability of the test to classify healthy individuals as having no abnormalities.

While the involvement of EBV in the development of NPC is not clearly understood, it is, however, believed that EBV infection is a cofactor and a precursor to NPC. EBV is believed to infect the basal cells, Stratum Basale, of the stratified epithelium through micro lesions of the epithelia. The generation of infected daughter cells begins and continues at the basal layer. These progeny exit the basal layer and by ordinary cell movement migrate to the stratum corneum, which is the uppermost layer of the stratified epithelium. While it may take years for a lesion to be visualized, the EBV infected and cancerous cells are omnipresent in the nasopharynx almost from the first day they begin to migrate from the Stratum Basale. Collection of these cells to screen for the presence of the EBV genome; which is a surrogate marker of malignancy can be performed and the presence of NPC can be predicted.

The nasopharynx is situated deep behind both nasal cavities and samples can be obtained either transnasally or transorally. The transnasal route is

uncomfortable and can be difficult to perform in patients with anatomical abnormalities such as a deviated septum. Furthermore, bleeding can be a problem as the biopsy apparatus transverses the nasal cavities can cause injury to the nasal mucosal surface. The transoral route is ideal as this is a relatively comfortable and a non-traumatic mean of access to the nasopharynx with minimal or no bleeding.

Normally considerable concentrations of infectious virus particles are excreted into saliva at random intervals several times a month and can be residual in the mouth for several days afterwards. Thus, it would be desirable to have a biopsy apparatus designed to obviate the occurrence of contamination with EBV virus from saliva or oral mucosal tissue while in transit to the nasopharynx. NPC cells contain substantially greater numbers of vial genome copies than the aggregate of all possible viral contamination from saliva. Thus, it would also be desirous to have a NPC screen with maximum sensitivity by designing the biopsy apparatus such that it minimizes or excludes contact of the sample collection area with saliva or tissue which is not the nasopharynx.

In copending International patent application PCT/CA00/00242 [Ng et al. (Ng), there is described a novel biopsy apparatus and method of obtaining a biopsy sample. This device shall be referred to herein as the Ng Device.

The Ng Device represents a major advance in the art of biopsy sample collection for screening for NPC.

Prior to the development of the Ng Device, the principal manner by which NPC was detected involved relatively invasive transnasal and transoral approaches to obtain a punch biopsy sample. The punch biopsy sample would then be sent to a pathology lab for diagnosis of NPC in the patient.

The advent of the Ng Device allowed for, in the preferred embodiment, collection of a brush biopsy for screening for NPC. The biopsy sample would be collected and then be assayed for the presence of Epstein Barr virus ("EBV"). Since, during proper us of the Ng device, the collected sample is retained in a relatively sterile form (i.e., substantially free from contamination), the assaying done was simply to detect the presence or absence EBV in the sample. The presence of EBV in the sample was indicative of NPC in the patient.

Given the prior biopsy collection techniques, it is not been known or suggested or even desired in the art to quantify the amount of EBV in the sample. There remains a need for a simple procedure or method that can be performed routinely in an ambulatory setting for mass screening of the presence of NPC, especially early stage disease, in high risk populations.

DISCLOSURE OF THE INVENTION

It is an object of the present invention to provide a novel method for screening nasopharyngeal carcinoma which obviates or mitigates at least one of the disadvantages of the prior art.

It is another object of the present invention to provide a novel method for diagnosis of nasopharyngeal carcinoma which obviates or mitigates at least one of the disadvantages of the prior art.

Accordingly, in one of its aspects, the present invention provides a method for screening for nasopharyngeal carcinoma comprising the steps of:

(i) quantifying the amount of cellular Epstein Barr virus in a sample taken from the nasopharynx of a control patient who is free of nasopharyngeal carcinoma to define a threshold value;

(ii) quantifying the amount of cellular Epstein Barr virus in a sample taken from the nasopharynx of a test patient to define a test value;

(iii) comparing the test value to the threshold value.

In another of its aspects, the present invention provides a diagnostic method for predicting the presence of nasopharyngeal carcinoma in a patient, the method comprising the steps of:

(i) obtaining a sample of epithelial cells from the nasopharynx of the patient;

(ii) quantifying the amount of cellular Epstein Barr virus in the sample to obtain a test value;

(iii) comparing the test value to a predetermined threshold value of the amount of Epstein Barr virus in a subject who is free of nasopharyngeal cancer; and

(iv) predicting the presence of nasopharyngeal cancer in the patient for the test value exceeds the predetermined threshold value.

In yet another of its aspects, the present invention provides a diagnostic method for predicting the presence of nasopharyngeal carcinoma in a patient, the method comprising the steps of:

(i) obtaining a biopsy sample comprising epithelial cells from the nasopharynx of the patient;

(ii) treating the biopsy sample to separate the epithelial cells from non-cellular material in the sample;

(iii) assaying for the presence of cellular Epstein Barr virus in the epithelial cells; and

(iv) predicting the presence of nasopharyngeal cancer in the patient if the presence of cellular Epstein Barr virus is detected in the cells.

Throughout this specification, reference is made to quantifying or assaying for the presence of EBV or Epstein Barr virus in a sample and/or in cells in a sample. It should be understood that this includes DNA, RNA, proteins and any other unique indicators of EBV or Epstein Barr virus. While embodiments of the invention will be described with reference to quantify or assaying for EBV genome or Epstein Barr virus genome, particularly using PCR, this is merely a convenient and preferred embodiment of the invention but should not be used to limit the scope of the invention.

The inventors have discovered a novel approach for screening for NPC for diagnosis of NPC and a system therefor. This approach is applicable with any method which, for a sample from the nasopharynx of a patient, can provide differentiation between cellular EBV genome and all EBV genome (e.g., a method in which the cells in the sample are washed away from non-cellular material such that cellular EBV genome specifically can be assayed or quantified).

One aspect of this novel approach is based on obtaining a sample from the nasopharynx of a patient and quantifying the amount of EBV present in that sample (this could include cellular and non-cellular EBV). Thereafter, the amount of EBV detected in the sample is compared to a predetermined threshold.

Typically, the predetermined threshold will be a measure of the amount of EBV in an NPC-free patient - e.g., the amount of non-cellular EBV in the patient (typically in the saliva, mouth and other regions of the nasopharnyx). If the amount of EBV in the sample is above the threshold, this is indicative of NPC in the patient. If the amount of EBV in the sample is less than the threshold, this is indicative of the absence of NPC in the patient.

By quantifying the presence of EBV in the biopsy sample, the occurrence of false positives and false negatives for NPC in the patient is obviated or mitigated.

Further, the invention allows for use of a simplified or modified form of the Ng Device since contamination of the sample during biopsy collection is rendered moot by establishing the threshold quantity of EBV to be substantially the same as the maximum aggregate amount of EBV present in the mouth and other areas of the patient which are not directly at the nasopharynx (i.e., at the particular site of interest where the amount of EBV should be detected).

Another aspect of this novel approach involves treating (e.g., by washing or similar means) a sample comprising epithelial cells obtained from the nasopharnyx of the patient to separate epithelial cells from non-cellular material and thereafter assaying for the presence of EBV in the epithelial cells (i.e., epithelial EBV). This separation scheme results in removal of non-cellular EBV from the sample thereby obviating the occurrence of a false positive result.

BEST MODE FOR CARRYING OUT THE INVENTION

As mentioned above, the Ng Device represents a significant advance in the art of biopsy sample collection devices, particularly for screening for NPC.

As described in Ng, the Ng Device is in the form of a "sheathed" apparatus. Thus, in use, the sample collecting brush of the Ng Device is sheathed until the device is correctly positioned. At that point, the brush is exposed, a sample is taken and the brush is restowed in the sheath. This sheathing arrangement maintains the integrity of the sample epithelial cells and helps avoid the occurrence of contamination by, inter alia, EBV located in the mouth and other areas of the patient away from the nasopharynx.

Thus, if the Ng Device was improperly used (e.g., the brush was exposed prior to correct positioning of the device), there is a risk of contamination which could lead to a false positive in the screening process. Additionally, if one were to modify the preferred embodiment of Ng Device, for example, to remove, the sheath, the risk of contamination would be greatly enhanced since the brush would be exposed to the entire inner cavity of the patient's mouth and other areas away from the area of interest - i.e., away from the nasopharynx.

Thus, the present inventors have discovered that it is possible to obviate these contamination-related problems by quantifying the amount of EBV in the sample and comparing this to a predetermined threshold. If the amount of EBV in the sample is above the threshold, this is a positive indication for NPC in the patient. If the amount of EBV in the sample is less than the threshold, this a negative indication for the presence of NPC in the patient. The predetermined threshold can be determined from control subjects - i.e., NPC-negative individuals. Thus, the predetermined threshold can be incorporated into a control database which is used for comparative purposes in a screening protocol for NPC - i.e., it is not necessary to quantify the amount of EBV in an NPC-negative patient each time the screen is used.

In one aspects of the invention, the sample is pretreated to separate the epithelial cells from non-cellular material after which the epithelial cells portion of the sample may be assayed for the presence of EBV. In this case, it may not be necessary to quantify the amount of EBV in NPC-negative patients.

The particular mode of quantifying the amount of EBV in the sample is not particularly restricted. Preferably, the amount of EBV in the sample is determined using a Polymerase Chain Reaction ("PCR") technique. While PCR techniques are conventional, the use thereof to quantify the amount of EBV in a sample, particularly a sample comprising epithelial cells, for the purpose of screening for NPC has, heretofore, not been known. If the sample is one comprising epithelial cells, it is preferred that the sample be obtained using a transnasal or transoral approach.

PCR is a biochemical process in which a specific region of DNA (deoxyribonucleic acid) is repeatedly copied (i.e., amplified in a chain reaction

manner) such that a large quantity of the DNA copies of interest can be obtained for biochemical studies. The advantage of the PCR process is that it allows a minute quantity of DNA to be detected and studied.

The following is a very general description of the process. More specific details may be found in United States patent 4,683,195 [Mullis et al. (Mullis)]. More preferably, the form of PCR used is as described in United States patent 5,210,015 [Gelfand et a. (Gelfand)]. The written specifications of both of these references is explicitly incorporated in herein by references for support purposes.

Thus, as it is well known in the art, DNA is a biochemical structure that contains genetic code of human cells. The basic building blocks of DNA are the four nucleotide basis: Adenine (A), cytosine (C), guanine (G) and thymine (T). As is further well known, DNA is a double helical structure with two strands pairing with one another where A pairs with T and C pairs with G. DNA resides in the nucleus of every cell.

In order for the copying process to occur, an enzyme is needed to catalyze the process of pairing A to T and C to G. In essence, the enzyme is Polymerase. Polymerization occurs when the DNA strand is lengthened as the base pairs are added one by one.

A "primer" is a short sequence of DNA containing bases of nucleotides. A primer is needed at the beginning of the PCR process to initiate the amplification. The primer will hybridize or anneal to the beginning of the DNA sequence of interest and the polymerase will then start adding bases to the end of the primer sequence to lengthen the DNA sequence. Thus, as is known in the art, primers are a specific sequence of complementary DNA that can recognize regions of the DNA sequence of interest. Various primers suitable for use in the present invention are set out below in the Sequence Listing section of this specification.

The PCR reaction is carried out in three general parts.

During the first part, the two DNA strands in a double helix are separated (also known as "unzipping" or "denaturing"). This is usually done by heating the DNA sample in the presence of a biochemical.

During the second part of the reaction, the temperature of the sample is usually lowered and the primer is added and allowed to bind or "anneal" to the specific region of the DNA strand of interest.

In the third part, the polymerase and the nucleotides are added, and the temperature of the sample is once again raised to allow the biochemical process of base pairing to occur.

These three parts are described as a single "cycle" and the result is duplication of a new and exact sequence of the DNA in the sample. Each newly synthesized DNA sequence can also serve as a template for copying and therefore, the process is significantly magnified over many cycles in an exponential fashion. Practically, after thirty or forty cycles of amplification, the amount of DNA is usually sufficient for detection and biochemical analysis.

In order to detect and quantify the amount of DNA produced, the primer which is used typically has anchored to it a fluorescent-dye. The final product containing the newly synthesized full sequence of DNA with the fluorescent-labelled primer is then induced to fluoresce using, for example, a laser device. The amount of laser-induced fluorescence signals are then analyzed and compared with a standard control containing a known amount of fluorescent-labelled PCR product as calibration. A calibration curve showing increasing fluorescence signals with increasing amplification cycles serves as a baseline level.

A particularly preferred manner of detecting and quantifying the amount of DNA produced involves the use of a labeled oligonucleotide probe as desribed in Gelfand. As taught by Gelfand, the 5' to 3' nuclease activity of a nucleic acid polymerase is advantageously used to cleave annealed labeled oligonucleotide from hybridized duplexes thereby releasing labeled oligonucleotide fragments for detection. Various such probes suitable for use in the present invention are set out below in the Sequence Listing section of this specification.

Samples containing the cells with DNA of interest are usually treated mechanically (e.g., grinding into powder under liquid-nitrogen frozen conditions) and biochemically (e.g., through the use of a lysis buffer) to release the nuclear content, including the DNA from the cells. The standard acid quanidinium

thiocyanate-phenol method is then used to treat the isolated DNA. For the application of PCR in amplifying and studying EBV, reference is made to the teachings of N.ENGL.J.MED., 1992; 326:17-21.

Since many normal or healthy biopsy samples may also contain low levels of EBV genome (either due to contamination of the sample from reflux of the oral cavity or existing infected healthy cells), the PCR quantification method described herein is particularly advantageous to remove the "background" or contamination level thereby obviated the occurrence of false positives. Since NPC tumor cells have many more copies of EBV genome than the aggregate possible from contamination sources, further amplification of the EBV genome level from NPC will therefore magnify these differences. Thus, a "threshold background contamination" level can be readily established above which, for given a sample, there is a positive indication for the presence of NPC.

Alternatively, it is possible to treat the biopsy sample to separate non-cellular EBV genome (not of interest in screening for NPC) from cellular EBV genome (of interest in screening for NPC). This can be achieved conventionally by using buffers and other known reagents to wash the sample.

Further, the ability to quantify the amount of EBV genome in a sample may lead to establishment of various predictive factors in how NPC may manifest itself in a patient who does not exhibit symptoms of NPC. Thus, a level of EBV genome determined using quantitative PCR can lead to a reference number for screening of NPC in high risk individuals. This allows for relatively early detection of NPC which leads to a much better prognosis for the patient - this is one of the significant advantages of the invention.

While this invention has been described with reference to illustrative embodiments, this description is not intended to be construed in a limiting sense. Thus, various modifications of the illustrative embodiments, as well as other embodiments of the invention, will be apparent to persons skilled in the art upon reference to this description. It is therefore contemplated that the appended claims will cover any such modifications or embodiments.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

SEQUENCE LISTING

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<120> System and Method For Screening of Nasopharyngeal Carcinoma

<130> T8464951WO

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<151> 1999-04-30

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What is claimed is:

1. A method for screening for nasopharyngeal carcinoma comprising the steps of:

(i) quantifying the amount of cellular Epstein Barr virus in a sample taken from the nasopharynx of a control patient who is free of nasopharyngeal carcinoma to define a threshold value;

(ii) quantifying the amount of cellular Epstein Barr virus in a sample taken from the nasopharynx of a test patient to define a test value;

(iii) comparing the test value to the threshold value.

2. The method defined in claim 1, wherein Step (i) comprises using a PCR technique.

3. The method defined in claim 1, wherein Step (ii) comprises using a PCR technique.

4. The method defined in claim 1, wherein each of Step (i) and Step (ii) comprise a PCR technique.

5. The method defined in any one of claim s 1-4, wherein the PCR technique comprises the use of a pair of primers capable of annealing to opposed strands of denatured DNA oligonucleotides in Epstein Barr virus.

6. The method defined in claim 5, wherein the pair of primers is selected from the following group of sequences:

SEQ ID NO:1/SEQ. ID 2,
SEQ ID NO:4/SEQ. ID 5,
SEQ ID NO:7/SEQ. ID 8,
SEQ ID NO:10/SEQ. ID 11,
SEQ ID NO:13/SEQ. ID 14,

* C L M S *

SEQ ID NO:16/SEQ. ID 17,
SEQ ID NO:19/SEQ. ID 20,
SEQ ID NO:22/SEQ. ID 23,
SEQ ID NO:25/SEQ. ID 26,
SEQ ID NO:28/SEQ. ID 29
SEQ ID NO:31/SEQ. ID 32, and
SEQ ID NO:34/SEQ. ID 35.

7. The method defined in any one of claims 1-4, wherein the PCR technique comprises the use of: (i) a pair of primers, each primer capable of annealing to one of a pair of opposed strands of denatured DNA oligonucleotides in Epstein Barr virus; and (ii) a labeled probe capable of annealing to each of the pair of opposed strands of denatured DNA oligonucleotides in Epstein Barr virus and releasing detectable labeled fragments during nucleic acid polymerase activity.

8. The method defined in claim 7, wherein the pair of primers and the labeled probe are selected from the following group of sequences:

PAIR OF PRIMERS	PROBE
SEQ ID NO:1/SEQ. ID 2	SEQ ID NO:3
SEQ ID NO:4/SEQ. ID 5	SEQ ID NO:6
SEQ ID NO:7/SEQ. ID 8	SEQ ID NO:9
SEQ ID NO:10/SEQ. ID 11	SEQ ID NO:12
SEQ ID NO:13/SEQ. ID 14	SEQ ID NO:15
SEQ ID NO:16/SEQ. ID 17	SEQ ID NO:18
SEQ ID NO:19/SEQ. ID 20	SEQ ID NO:21
SEQ ID NO:22/SEQ. ID 23	SEQ ID NO:24
SEQ ID NO:25/SEQ. ID 26	SEQ ID NO:27
SEQ ID NO:28/SEQ. ID 29	SEQ ID NO:30
SEQ ID NO:31/SEQ. ID 32	SEQ ID NO:33
SEQ ID NO:34/SEQ. ID 35	SEQ ID NO:36.

9. A diagnostic method for predicting the presence of nasopharyngeal carcinoma in a patient, the method comprising the steps of:

(i) obtaining a sample of epithelial cells from the nasopharynx of the patient;

(ii) quantifying the amount of cellular Epstein Barr virus in the sample to obtain a test value;

(iii) comparing the test value to a predetermined threshold value of the amount of Epstein Barr virus in a subject who is free of nasopharyngeal cancer; and

(iv) predicting the presence of nasopharyngeal cancer in the patient for the test value exceeds the predetermined threshold value.

10. The method defined in claim 9, wherein Step (i) comprises obtaining the sample of epithelial cells transorally from the patient.

11. The method defined in claim 9, wherein Step (i) comprises obtaining the sample of epithelial cells transnasally from the patient.

12. The method defined in any one of claims 9-11, wherein Step (ii) comprises using a PCR technique.

13. The method defined in claim 12, wherein the PCR technique comprises the use of a pair of primers capable of annealing to opposed strands of denatured DNA oligonucleotides in Epstein Barr virus.

14. The method defined in claim 13, wherein the pair of primers is selected from the following group of sequences:

SEQ ID NO:1/SEQ. ID 2,
SEQ ID NO:4/SEQ. ID 5,
SEQ ID NO:7/SEQ. ID 8,
SEQ ID NO:10/SEQ. ID 11,

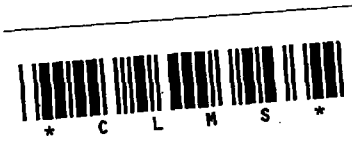
* C L M S *

SEQ ID NO:13/SEQ. ID 14,
SEQ ID NO:16/SEQ. ID 17,
SEQ ID NO:19/SEQ. ID 20,
SEQ ID NO:22/SEQ. ID 23,
SEQ ID NO:25/SEQ. ID 26,
SEQ ID NO:28/SEQ. ID 29
SEQ ID NO:31/SEQ. ID 32, and
SEQ ID NO:34/SEQ. ID 35.

15. The method defined in claim 12, wherein the PCR technique comprises the use of: (i) a pair of primers, each primer capable of annealing to one of a pair of opposed strands of denatured DNA oligonucleotides in Epstein Barr virus; and (ii) a labeled probe capable of annealing to each of the pair of opposed strands of denatured DNA oligonucleotides in Epstein Barr virus and releasing detectable labeled fragments during nucleic acid polymerase activity.

16. The method defined in claim 15, wherein the pair of primers and the labeled probe are selected from the following group of sequences:

PAIR OF PRIMERS	PROBE
SEQ ID NO:1/SEQ. ID 2	SEQ ID NO:3
SEQ ID NO:4/SEQ. ID 5	SEQ ID NO:6
SEQ ID NO:7/SEQ. ID 8	SEQ ID NO:9
SEQ ID NO:10/SEQ. ID 11	SEQ ID NO:12
SEQ ID NO:13/SEQ. ID 14	SEQ ID NO:15
SEQ ID NO:16/SEQ. ID 17	SEQ ID NO:18
SEQ ID NO:19/SEQ. ID 20	SEQ ID NO:21
SEQ ID NO:22/SEQ. ID 23	SEQ ID NO:24
SEQ ID NO:25/SEQ. ID 26	SEQ ID NO:27
SEQ ID NO:28/SEQ. ID 29	SEQ ID NO:30
SEQ ID NO:31/SEQ. ID 32	SEQ ID NO:33



SEQ ID NO:34/SEQ. ID 35 SEQ ID NO:36.

17. A diagnostic method for predicting the presence of nasopharyngeal carcinoma in a patient, the method comprising the steps of:

(i) obtaining a biopsy sample comprising epithelial cells from the nasopharynx of the patient;

(ii) treating the biopsy sample to separate the epithelial cells from non-cellular material in the sample;

(iii) assaying for the presence of cellular Epstein Barr virus in the epithelial cells; and

(iv) predicting the presence of nasopharyngeal cancer in the patient if the presence of cellular Epstein Barr virus is detected in the cells.

18. The method defined in claim 17, wherein Step (i) comprises obtaining the sample of epithelial cells transorally from the patient.

19. The method defined in claim 17, wherein Step (i) comprises obtaining the sample of epithelial cells transnasally from the patient.

20. The method defined in any one of claims 17-19, wherein Step (iii) comprises using a PCR technique.

21. The method defined in claim 20, wherein the PCR technique comprises the use of a pair of primers capable of annealing to opposed strands of denatured DNA oligonucleotides in Epstein Barr virus.

22. The method defined in claim 21, wherein the pair of primers is selected from the following group of sequences:

SEQ ID NO:1/SEQ. ID 2,
SEQ ID NO:4/SEQ. ID 5,
SEQ ID NO:7/SEQ. ID 8,



SEQ ID NO:10/SEQ. ID 11,
SEQ ID NO:13/SEQ. ID 14,
SEQ ID NO:16/SEQ. ID 17,
SEQ ID NO:19/SEQ. ID 20,
SEQ ID NO:22/SEQ. ID 23,
SEQ ID NO:25/SEQ. ID 26,
SEQ ID NO:28/SEQ. ID 29
SEQ ID NO:31/SEQ. ID 32, and
SEQ ID NO:34/SEQ. ID 35.

23. The method defined in claim 20, wherein the PCR technique comprises the use of: (i) a pair of primers, each primer capable of annealing to one of a pair of opposed strands of denatured DNA oligonucleotides in Epstein Barr virus; and (ii) a labeled probe capable of annealing to each of the pair of opposed strands of denatured DNA oligonucleotides in Epstein Barr virus and releasing detectable labeled fragments during nucleic acid polymerase activity.

24. The method defined in claim 23, wherein the pair of primers and the labeled probe are selected from the following group of sequences:

PAIR OF PRIMERS	PROBE
SEQ ID NO:1/SEQ. ID 2	SEQ ID NO:3
SEQ ID NO:4/SEQ. ID 5	SEQ ID NO:6
SEQ ID NO:7/SEQ. ID 8	SEQ ID NO:9
SEQ ID NO:10/SEQ. ID 11	SEQ ID NO:12
SEQ ID NO:13/SEQ. ID 14	SEQ ID NO:15
SEQ ID NO:16/SEQ. ID 17	SEQ ID NO:18
SEQ ID NO:19/SEQ. ID 20	SEQ ID NO:21
SEQ ID NO:22/SEQ. ID 23	SEQ ID NO:24
SEQ ID NO:25/SEQ. ID 26	SEQ ID NO:27
SEQ ID NO:28/SEQ. ID 29	SEQ ID NO:30

* C L M S *

SEQ ID NO:31/SEQ. ID 32 SEQ ID NO:33

SEQ ID NO:34/SEQ. ID 35 SEQ ID NO:36.

25. A DNA primer having a sequence selected from the group comprising:

SEQ ID NO:1 SEQ. ID 2

SEQ ID NO:4 SEQ. ID 5

SEQ ID NO:7 SEQ. ID 8

SEQ ID NO:10 SEQ. ID 11

SEQ ID NO:13 SEQ. ID 14

SEQ ID NO:16 SEQ. ID 17

SEQ ID NO:19 SEQ. ID 20

SEQ ID NO:22 SEQ. ID 23

SEQ ID NO:25 SEQ. ID 26

SEQ ID NO:28 SEQ. ID 29

SEQ ID NO:31 SEQ. ID 32

SEQ ID NO:34 SEQ. ID 35.

26. A DNA probe having sequence selected from the group comprising:

SEQ ID NO:3

SEQ ID NO:6

SEQ ID NO:9

SEQ ID NO:12

SEQ ID NO:15

SEQ ID NO:18

SEQ ID NO:21

SEQ ID NO:24

SEQ ID NO:27

SEQ ID NO:30

SEQ ID NO:33, and

SEQ ID NO:36.